Mohammad Shahid
Anuradha Singh
Hiba Sami *Editors*

Beta-Lactam Resistance in Gram-Negative Bacteria

Threats and Challenges

Springer

Beta-Lactam Resistance in Gram-Negative Bacteria

Mohammad Shahid • Anuradha Singh • Hiba Sami
Editors

Beta-Lactam Resistance in Gram-Negative Bacteria

Threats and Challenges

Springer

Editors
Mohammad Shahid
Department of Microbiology,
Immunology & Infectious Diseases
Arabian Gulf University
Manama, Bahrain

Anuradha Singh
Department of Microbiology, Faculty of
Medicine, Jawaharlal Nehru Medical College
Aligarh Muslim University
Aligarh, Uttar Pradesh, India

Hiba Sami
Department of Microbiology, Faculty of
Medicine, Jawaharlal Nehru Medical
College
Aligarh Muslim University
Aligarh, Uttar Pradesh, India

ISBN 978-981-16-9096-9 ISBN 978-981-16-9097-6 (eBook)
https://doi.org/10.1007/978-981-16-9097-6

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to our Parents and Family Members

Foreword I

This book summarizes different mechanisms of drug resistance in Gram-negative bacteria and associated antibiotic resistance genes which is a cause of growing worldwide concern. The ability of different bacterial species to resist antimicrobial agents has become a global problem. As a result, the book offers a detailed review of developments in the knowledge of the root and mechanism of resistance, addresses the modern definition of antibacterial resistance, their biochemical and genetic basis, and emphasizes the clinical consequences of the increased prevalence of antimicrobial resistant pathogens and their ecotoxic impact. It also reviews various new drugs in pipeline for the treatment of these dreaded pathogens. Antibiotic resistance spreads internationally because of overuse and abuse of these drugs; thus, attempts must be made to educate people and introduce new policies and management systems about how to use and dispense antibiotics. Gram-negative resistant bacteria are the most dangerous type of MDR bacteria, and all of these new therapies and methods are needed to keep antimicrobial resistance to a minimum. Readers, especially students, will be benefitted by this concise book in which they can get all the different mechanisms of Antibiotic Resistance in Gram-Negative Bacteria at a single place. I wish students a happy learning and best wishes to authors and editors.

College of Medicine and Medical Sciences,
Arabian Gulf University
Manama, Bahrain

Abdelhalim Deifalla

Foreword II

I congratulate the editors for selecting such a timely topic for their book. Antimicrobial resistance is a major global health problem and one of humanity's most severe challenges today. Antibiotic resistance has been developed by certain bacterial strains. As a result, new antibacterial agents are urgently needed to combat resistant bacteria. *Beta-Lactam Resistance in Gram-Negative Bacteria: Threats and Challenges* serves as a refresher for doctors, a starting point for postgraduate students interested in solving the resistance crisis, and a text for a course on antibiotic resistance. The biological basis of drug resistance in Gram-negative bacteria and their various strategies to acquire drug resistance are the focus of this book. With the development of more drug-resistant species, the approach to combating drug resistance must include research into various aspects of bacterial resistance mechanisms and resistance gene dissemination, as well as research using new genomic information on the development of newer antimicrobials. Readers who are not acquainted with microbiology will gain a better understanding of a medical problem that promises to be one of the most pressing issues of our time.

Department of Microbiology,
JNMCH, AMU,
Aligarh, India

Haris M. Khan

Disclosure Statement

The views expressed in the contributed chapters are solely the views of the contributing authors, and the editors bear no responsibility in relation to its contents. The primary author/corresponding author also takes the responsibility for the originality of the contribution and for any form of copyright/plagiarism issues.

Preface

Every year in November the World Health Organization celebrates "the World Antimicrobial Awareness Week" to increase global awareness about antimicrobial resistance among general public, health care workers, policy makers, and other stakeholders to curb its emergence and further spread of drug-resistant infections. This encouraged us to design and work on a book proposal which can also help to generate awareness in people about this pressing issue of antimicrobial resistance.

Antibacterial agents are in use for decades to treat bacterial infections. However, use of these therapeutic agents also developed some drastic changes in the genes of bacteria. Beta-lactams are the commonly used antibiotics to treat bacterial infections worldwide. Most of the people use these antibiotics to treat minor and major infections with or without any prescription. The misuse of antibiotics is continuously increasing which leads to changes in resistance pattern in the bacterial population, due to which these resistant infections become difficult to treat. This resistant pattern in bacteria can disseminate clinically as well as at the environmental level. In the present era it is difficult to understand precisely which reservoir is exactly responsible for disseminating these resistance markers, i.e., clinical misuse, increasing use/misuse in animal sector, or at the environmental level, including improper medical/therapeutic waste disposal. Bacteria primarily generate beta-lactamase enzymes to destroy the therapeutic activity of beta-lactams, and this causes a big hindrance to treatment.

As titled, the book focuses on beta-lactam resistance in Gram-negative bacteria. We incorporated relevant possible topics to understand the drug resistance in Gram-negative bacteria and the potential threats and challenges towards this threatening issue of antibiotic resistance. This book facilitates the reader to understand the mechanism of beta-lactamase production, their classification systems; older and newer classification systems, detailed description of different classes of beta-lactamases, and genetic environment of bacteria that is responsible for the migration of beta-lactamase genes in Gram-negative bacteria. This book will provide complete knowledge of phenotypic and molecular detection methods to detect resistant bacteria. A brief description of newer antibiotics is also included in this book to understand the current therapeutic scenario. Moreover, addressing the issue of antibiotic resistance at the environmental level is also attempted.

In the era of the COVID-19 pandemic, this book proposal was a difficult task to compile because every Microbiologist was busy in COVID diagnostics providing medical services to mankind. Eminent Scientists and Microbiologists working in the field of Medicine, Microbiology, Biotechnology, and Agriculture/Environmental Sciences were requested to contribute their contributions. The editors are thankful to all the authors for providing their valuable contributions and for their continuous support during the compilation process. We hope from this book readers in the field of Medical Microbiology, other relevant branches of science, health care workers, and policy makers can benefit towards this relevant topic. This book will also serve students in the medical field to gain a complete knowledge of beta-lactamases in Gram-negative bacteria.

We hope the book will provide material in academics particularly for those who are working in the field of medical science and Microbiology and particularly on antibiotic resistance in Gram-negative bacteria.

Manama, Bahrain — Mohammad Shahid
Uttar Pradesh, India — Anuradha Singh
Uttar Pradesh, India — Hiba Sami

Acknowledgments

First and foremost, we submit our work and endeavors to the Almighty for blessing us with this great opportunity. Sincere acknowledgments to our teachers, peers and colleagues, departmental staff, family members, and others for their continuous support and encouragement. Special thanks to Dr. Khalid Abdulrahman Al Ohaly, the honorable President of Arabian Gulf University, Bahrain, and Prof. Tariq Mansoor, the honorable Vice Chancellor of Aligarh Muslim University, India, for their encouragement and continuous support during this publication process. Thanks are due to Prof. Abdel Halim Deifalla, the Dean of College of Medicine & Medical Sciences, AGU, Bahrain, and Prof. Rakesh Bhargava, the Dean of J N Medical College, AMU, Aligarh, India and Prof. Haris M Khan, Chairman, Department of Microbiology, J N Medical College, AMU, Aligarh, India for their support. We are thankful to Dr. Parvez Anwar Khan, Assistant Professor, Department of Microbiology, for his support in figure preparations for our book. We are also grateful to Mr. Tomy Kaitharath for his technical and administrative support in organizing the chapters and other book contents. We are also thankful to our institutes (Arabian Gulf University, Bahrain, and Aligarh Muslim University, India) for supporting us in every way to fulfill this task. Special thanks are due to the Springer publication team, especially Madhurima Kahali, Lenold Christ Raj, Naren Aggarwal, and Ashok Kumar, for their continuous help and guidance during the publication process.

Last but not least, we would like to acknowledge that without the support of our parents and immediate family members, this work would not have been possible, so a special thanks to all of them.

Contents

Editors and Contributors

About the Editors

Mohammad Shahid is a Professor and Chairman in the Department of Microbiology, Immunology & Infectious Diseases of the College of Medicine & Medical Sciences of Arabian Gulf University, Kingdom of Bahrain. Before joining the Medical College in Bahrain, he held the Chair of Department of Microbiology, JN Medical College & Hospital of Aligarh Muslim University, Aligarh, India, during 2017–2019. He did his MBBS, MD (Microbiology), and PhD (Microbiology) from JN Medical College & Hospital of Aligarh Muslim University, Aligarh, India, in years 1995, 2000, and 2008, respectively. He holds the professional registration of Medical Sciences Council of New Zealand and is a Fellow of New Zealand Institute of Medical Laboratory Sciences. He is a Commonwealth Academic Fellow (UK) and has affiliate membership of Royal College of Pathologists of Australasia (RCPA). He also worked as Visiting Scholar (Research) in West Midlands Public Health & Birmingham Heartlands NHS Hospital, Birmingham, UK, and as an Honorary Research Fellow in Antimicrobial Agents Research Group, Institute of Biomedical Research, The Medical School, University of Birmingham, UK. He has authored 6 books, 19 book chapters, and more than 100 scientific papers in various national and international journals of high repute. He holds membership of various professional bodies including American Society for Microbiology (ASM), Infectious Diseases Society of America (IDSA), New Zealand Institute of Medical Laboratory Sciences (NZIMLS), and Royal College of Pathologists of

Australasia (RCPA). He sits on the international advisory panel and editorial boards of numerous international journals of high repute.

Anuradha Singh is a research fellow in the ICMR Project and currently affiliated to JNMC, AMU. She was awarded Young Scientist project by Scientific and Engineering Research Board (SERB), a three-year duration project at JNMC, AMU, in the year 2013. She was awarded a PhD degree in Microbiology in the year 2012. After completing her PhD, she worked as Junior Research Fellow in a DST-funded project on "Screening of Antimalarial drug resistance genes in *Plasmodium falciparum*" at Parasitology lab, Department of Microbiology, JNMC, AMU. She has published several scientific publications in national and international journals in her field of research.

Hiba Sami is an Assistant Professor in the Department of Microbiology, JNMCH, AMU, Aligarh. She completed her MBBS in 2010 and MD Microbiology in 2013. She has a teaching and research experience of more than 9 years. She has 32 papers published in indexed journals (25 international/7 national). She underwent a molecular training in the virology lab at the Institute of Liver and Biliary Sciences, New Delhi. She is a life member of Indian Association of Medical Microbiologist (IAMM) and Hospital Infection Society, India.

Contributors

Iqbal Ahmad Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

Shariq Ahmed Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Anees Akhtar Department of Microbiology, JNMCH, AMU, Aligarh, Uttar Pradesh, India

Ali Al-Mahmeed Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Hanan Abdullah Almaimani Department of Health Education and Promotion, Faculty of Public Health and Tropical Medicine, Jazan University, Jizan, Saudi Arabia

Amani Awwadh Alotaibi Department of Health Education and Promotion, Faculty of Public Health and Tropical Medicine, Jazan University, Jizan, Saudi Arabia

Khalid M. Bindayna Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Bhanu Chaudhary Microbiology Department, Faculty of Medicine, Aligarh Muslim University, Aligarh, India

Fazal K. Dar Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Nabeela Farhat Medical Microbiology and Molecular Biology Lab, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

Nazish Fatima Department of Microbiology, JNMCH, AMU, Aligarh, Uttar Pradesh, India

Sana Jamali Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India

Ronni Mol Joji Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Asad U. Khan Medical Microbiology and Molecular Biology Lab, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

Fatima Khan Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Haris M. Khan Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Irfan Ahmad Khan Department of Pharmacology, J.N.M.C., AMU, Aligarh, Uttar Pradesh, India

Parvez Anwar Khan Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Shahazad Niwazi Department of Health Education and Promotion, Faculty of Public Health and Tropical Medicine, Jazan University, Jizan, Saudi Arabia

Nusrat Perween Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Faizan Abul Qais Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

P. Ronni Mol Division of Medical Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India
Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Hiba Sami Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Samreen Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Mohd. Shadab Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Mohammad Shahid Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain

Ganesan Shanthi Division of Medical Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India

Shirjeel Ahmad Siddiqui Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Anuradha Singh Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Gourav Pratap Singh Holy Family Hospital, New Delhi, India

Farrukh Sobia Department of Health Education and Promotion, Faculty of Public Health and Tropical Medicine, Jazan University, Jizan, Saudi Arabia

Asfia Sultan Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Kirti Suman Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Uzma Tayyaba Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Part I

Understanding Antimicrobial Resistance in Gram-Negative Bacteria

An Overview on Antibiotic Resistance in Gram-Negative Bacteria

1

Anuradha Singh, Mohammad Shahid, Parvez Anwar Khan, Haris M. Khan, and Hiba Sami

Abstract

Antimicrobial agents have been in use since ages in various forms in curing bacterial infections. Gram-negative bacteria cause a wide range of infections in human as well as in animals. The continuous use of antibacterial agents in treating humans and animals may generate resistance towards antibiotics. Antibiotic resistance in bacteria occurs due to various mechanisms adopted by bacteria to make them safe from therapeutic activity of antibiotics. With the beginning of historical perspective of antibiotics, this chapter will describe structure and nomenclature of beta-lactams and the phenomenon how beta-lactams work on bacterial cell to diminish their activity. The four most common mechanisms adopted by bacteria for evading lethal effect of antibiotics, viz. modification of target site of antibiotics, modification of penicillin binding proteins, permeability-based resistance, and efflux pump, are also described briefly. This chapter will describe various mechanisms of bacteria which are responsible for dissemination of antibiotic resistance among bacterial population.

Keywords

Resistance mechanisms · β-Lactams nomenclature · β-Lactamase · PBPs · Efflux pump

A. Singh (✉) · P. A. Khan · H. M. Khan · H. Sami
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid
Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: mohammeds@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_1

1.1 Introduction: Historical Perspective

Antimicrobials have been in use for decades in various setups. In the early twentieth century, Selman Waksman defined the term antibiotic as chemical substance produced from microorganisms that can kill other microbes (Hopwood 2007; Davies and Davies 2010). Most of the antibiotics that are in use today have been derived from the phylum *Actinobacteria*. Approximately 80% of antibiotics obtained from actinobacteria were made from soil dwelling bacteria of genus *Streptomyces* (Barka et al. 2016). The initial discovery of antibiotics is generally attributed to Alexander Fleming in 1929, who observed a diffusible bacteriolytic substance produced by a mould strain, contaminant of an agar plate inoculated with *Staphylococcus aureus*. Some synthetic chemicals include salvarsan, sulpha drugs and quinolones used as chemotherapeutic medicines prior to the discovery of natural antibiotics (Aminov 2010). Alexander Fleming in 1928 discovered the first natural antibiotic penicillin accidentally on culture plate in his laboratory. Because the contaminant mould was identified as a *Penicillium* sp., he named this bacteriolytic substance penicillin (Fleming 1929). Using the filtrate of liquid cultures of *Penicillin notatum*, he determined the antibacterial activity of this antibiotic in vitro, as well as its non-toxicity when injected into mice and rabbits. Nevertheless, since no studies with bacterial infected animals were performed, Fleming failed to demonstrate the penicillin's ability to overcome these types of infections (Rolinson 1998). With yet weak techniques, attempts to obtain purified penicillin in the 1930s were mostly ineffective, and interest in penicillin had almost vanished. However, a study in 1940 by Florey and his associates at Oxford University isolated the active ingredients and used the crude material clinically. A decade later, penicillin became a medicinal semisynthetic agent and found as an effective antibacterial agent against a streptococcal infection in mice (Abraham and Chain 1940).

Penicillins are a class of β-lactam antibiotics of related structure with slightly different properties and activities. The subsequent introduction of penicillin into clinical use in humans motivated the discovery of other antibiotics (Shahid et al. 2009). In 1948, the Sardinian scientist Giuseppe Brotzu identified cephalosporin chemicals from marine fungal crops, *Cephalosporium acremonium* from a sewer (Podolsky and Lawrence 1998). He realized that these crops produced chemicals beneficial for β-lactamase-causing typhoid fever causing bacteria *Salmonella enterica* serovar typhi. The cultivation of the fungus contained three distinct antibiotics named as Cephalosporin P, N and C. These antibiotics were effective against Gram-positive and Gram-negative bacteria and had similar properties as semisynthetic penicillins. The expansion of β-lactams started only in the early 1960s with semisynthetic penicillins and semisynthetic cephalosporins, followed by β-lactam antibiotics (Rolinson 1998). Hermann Staudinger produced the first synthetic β-lactam by the reaction of the aniline and benzaldehyde base Schiff with diphenylketene in 1907 (Staudinger 1907; Tidwell and Thomas 2008; https://upload.wikimedia.org/wikipedia/commons/5/5b/StaudingerLactam.svg) in a [2+2] cyclo addition (Fig. 1.1).

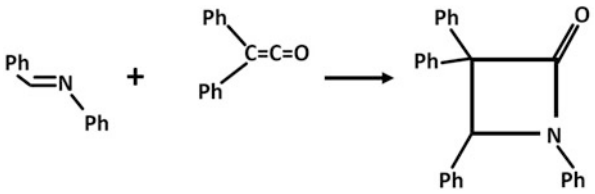


Fig. 1.1 Schiff base reaction of aniline and benzaldehyde with diphenylketene to form synthetic β-lactam

1.2 β-Lactam Antibiotics

1.2.1 Structure of β-Lactams

The composition of β-lactam antibiotics is of either isolated ring, as in monobactams, or bicyclic ring structures as seen in other classes. Penicillins are natural or semisynthetic antibiotics where the β-lactam ring is fused with a thiazolidine ring. Cephalosporins have β-lactam ring which is merged with a dihydrothiazine ring (Fig. 1.2). In the carbapenems, the β-lactam ring is combined with a hydroxyethyl side chain, lacking an oxygen or sulphur atom in the bicyclic nucleus. Overall, modifications of the R and R′ groups (Fig. 1.3) alter the pharmacokinetic and antibacterial properties in different β-lactam antibiotics. For example, modifications at position 7 of cephalosporins increase the penetration into the periplasmatic space and the stability against β-lactamases, but may reduce antibiotic activity (Donowitz and Mandell 1988). In contrast to the antibiotic, the clavulanic acid, a β-lactamase inhibitor, is composed of a β-lactam ring fused with an oxazolidine ring and does not possess an amide function.

1.2.2 Nomenclature of β-Lactams

β-lactam antibiotics are classified according to their core ring structure (Ana and Faisca Phillips 2021) depending on β-lactams fused to specific ring structures (Fig. 1.2) as discussed below:

- Fused to five-membered rings (saturated), such as:
  - Containing thiazolidine rings which are named as penams, i.e. penicillin.
  - Containing pyrrolidine rings which are named as carbapenams, i.e. imipenem.
  - Fused to oxazolidine rings which are named oxapenams or clavams, i.e. clavulanic acid.
- Fused to five-membered rings (unsaturated), such as:
  - Containing 2,3-dihydrothiazole rings which are named penems, i.e. faropenem.
  - Containing 2,3-dihydro-1H-pyrrole rings which are named carbapenems, i.e. meropenem.

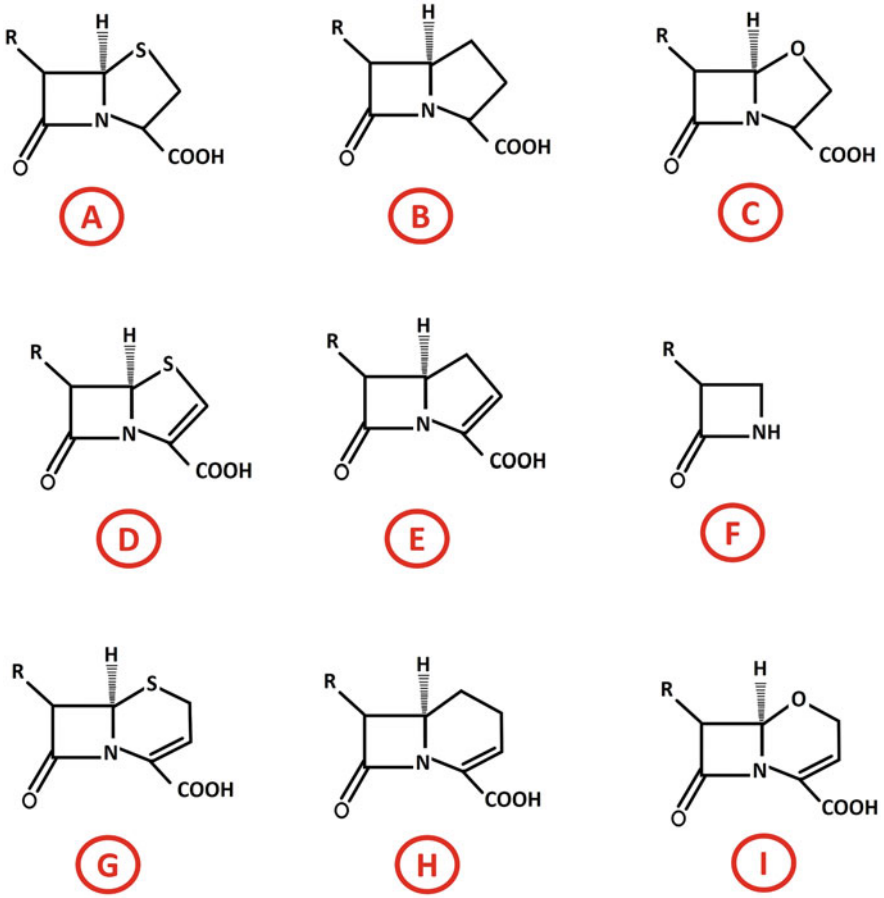
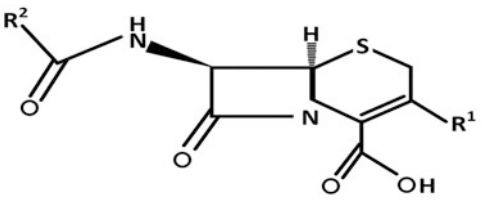


Fig. 1.2 The beta-lactam core structures: (**a**) penam, (**b**) carbapenam, (**c**) oxapenam, (**d**) penem, (**e**) carbapenem, (**f**) monobactam, (**g**) cephem, (**h**) carbacephem, (**i**) oxacephem (adapted from Wikipedia (https://en.wikipedia.org/wiki/β-lactam_antibiotic))

Fig. 1.3 Structure of cephalosporins



- Fused to six-membered rings (unsaturated), such as:
  - Containing 3,6-dihydro-2H-1,3-thiazine rings which are named cephems, i.e. cephalosporins and cephamycins.

  - Containing 1,2,3,4-tetrahydropyridine rings which are named carbacephems, i.e. lobacarbef.
  - Containing 3,6-dihydro-2H-1,3-oxazine rings which are named oxacephems, i.e. moxalactam and flomoxef.
- Not fused to any other ring which are named monobactams, i.e. aztreonam.

1.3 Cephalosporin Chemistry

Cephalosporin C (CC) comprises a side chain which is obtained from D-α-aminoadipic acid. It is condensed with a dihydrothiazine β-lactam ring system, i.e. 7-aminocephalosporanic acid. CC may be decomposed by acid leading to 7-aminocephalosporanic acid (Fig. 1.3). Discovering the nucleus (7-aminocephalosporanic acid) made possible, the introduction of semisynthetic compounds possessing antibacterial activity more than the parent material; this was achievable by addition of side chains (Abraham 1962; Flynn 1972). Subsequent modifications were made by the insertion of different side chains to develop a whole family of cephalosporin antibiotics (Mandell et al. 1996). The cephamycin is structurally identical to the cephalosporin, but it has an aminocephalosporanic acid nucleus methoxy group at position 7 of the β-lactam rings (Shahid et al. 2009).

1.4 Mechanism of Action of β-Lactam Antibiotics on Bacteria

β-Lactam antibiotics act on bacteria by inactivating the enzyme located in the cytoplasmic membrane which catalyses synthesis of the cross-linked peptidoglycan, which is an essential component of the bacterial cell wall. For their normal growth and development, the cell walls of bacteria are crucial. Peptidoglycan is a heteropolymeric component that forms the cell wall, and its highly interconnected grid structure gives it robust mechanical stability. The wall structure of Gram-positive and Gram-negative bacteria is different; the peptidogylcan in Gram-positive bacteria is 50–100 layers thick, while in Gram-negative bacteria it is only 1 or 2 layers thick (Fig. 1.4).

The peptidoglycan is composed of glycan chains, which are linear strands of two alternating amino sugars: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) linked by β-(1,4)-glycoside units (Fig. 1.5).

Biosynthesis of peptidoglycan involves numerous enzymes (~30) and completes in at least three stages. The primary stage occurs in cytoplasm leading to accumulation of uridine diphosphate (UDP)-acetylmuramyl-pentapeptide, accumulates in cells. The final reaction in the synthesis of this compound is by addition of a dipeptide D-alanyl-D-alanine synthetase. D-Cycloserine is a structural analogue of D-alanine and acts as a competitive inhibitor of both the racemase and the synthetase. In the reactions of the second stage, UDP-acetylmuramyl-pentapeptide and UDP-acetylglucosamine are linked to form a long polymer with the release of uridine nucleotides. In the final stage for the completion of cross-link, a

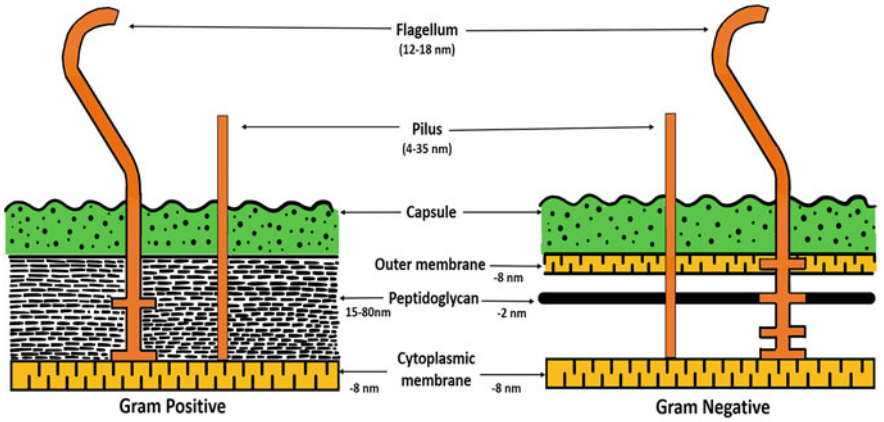


Fig. 1.4 Structure of cell wall of Gram-positive and Gram-negative bacteria

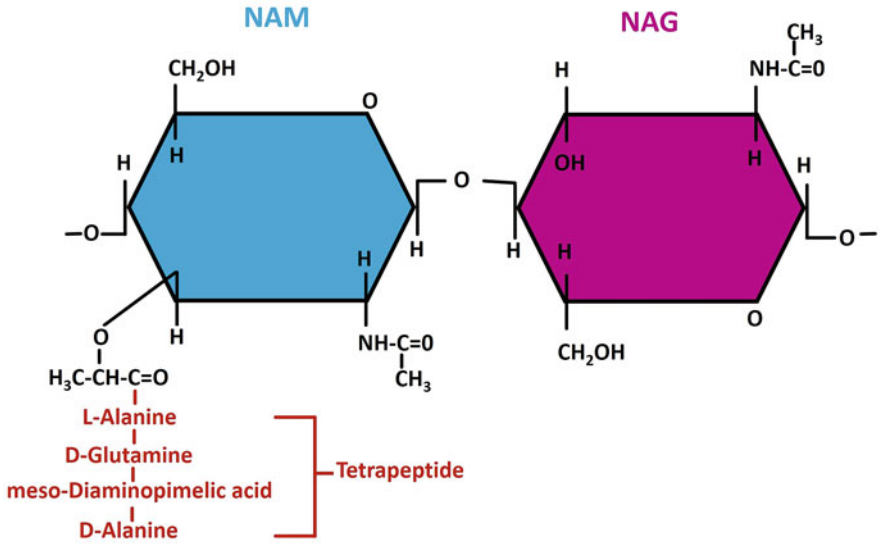


Fig. 1.5 Structure of peptidoglycan monomer

transpeptidation reaction occurs outside the cell membrane, with the help of membrane bound transpeptidase. The terminal glycine residue of the pentaglycine bridge is linked to the fourth residue of the pentapeptide (D-alanine), releasing the fifth residue (also D-alanine). D-Carboxypeptidases are responsible for the removal of the terminal D-alanine from the peptide chain, which could become a donor in the transpeptidation reaction. This is the last step of peptidoglycan synthesis that is inhibited by the β-lactam antibiotics. The penicillin-binding proteins (PBPs), transpeptidase and carboxypeptidase, are involved in the final stages of the synthesis

of peptidoglycan. These PBPs are subject to inhibition by β-lactam antibiotics, because penicillins act as an analogue of D-alanyl-D-alanine (Tipper and Strominger 1965). These transpeptidase and carboxypeptidases react with acyl-D-alanyl-D-alanine. β-Lactam-enzyme complex, formed after interaction between the enzyme and the β-lactam-enzyme complex, would act as participant to the formation of the normal acylated enzyme. The β-lactam-enzyme complex is very stable and terminates with the inactivation of the PBP's functions (Ghuysen 1988), which results in cellular lysis by interference in normal cross-linking of cell wall synthesis. In addition of transpeptidase and carboxypeptidase functions, PBPs also have transglycosylase function, which is responsible for the polymerization of glycan chain in peptidoglycan, which is not sensitive to β-lactams (Waxman and Strominger 1983). Various bacterial strains have diverse PBPs, which are polypeptides with 40–120 kDa. In *Enterobacteriaceae,* the number of PBPs varies from six to eight, *Escherichia coli* shows seven enzymes while *Klebsiella pneumoniae* shows six (Georgopapadakou and Lin 1980; Spratt 1983). In *E. coli*, the inactivation of PBPs with higher molecular weight, normally with transpeptidase or transglycosylase domains, leads in bacterial death. The inhibition of PBP1 results to cellular lysis (Spratt 1983), inhibition of PBP2 effects the formation of spherical cells while PBP3 effects the formation of filaments (Spencer et al. 1987). The inactivation of low molecular weight PBPs with lower molecular weight, normally with carboxypeptidase functions, does not found lethal to the cell (Spratt 1983).

PBPs have different affinities towards β-lactams, on the bases of substitutions present in the β-lactam ring. In *E. coli*, penicillin-derived β-lactams show higher affinities to PBP1 (amoxicillin), PBP2 (mecillinam and piperacillin), PBP3 (mezlocillin and piperacillin) and to PBP4, all cephalosporins show higher affinities to PBP1, while some of them like cefotaxime shows affinity to PBP2 and PBP3 and ceftazidime to PBP3 (Bryan and Godfrey 1991).

1.5 Mechanism of Resistance Against β-Lactam Antibiotics

After a successful decade of antibiotic therapy, the accelerating increase in antibiotic resistance among significant human pathogens and the paucity of the developing novel new anti-infective drug families now confronts us with a troublesome condition. With the beginning of semisynthetic penicillins in 1950–1960, followed by cephalosporins and by the other β-lactam antibiotics in 1970–1980, bacterial cells continuously evolved due to some mechanisms of mutations, genetic transference and natural selection (Frere et al. 1991; Jacoby and Archer 1991). There are usually four mechanisms which are responsible for bacterial resistance to antibiotics (Babic et al. 2006). These are briefly described below and further elaborated in Fig. 1.6).

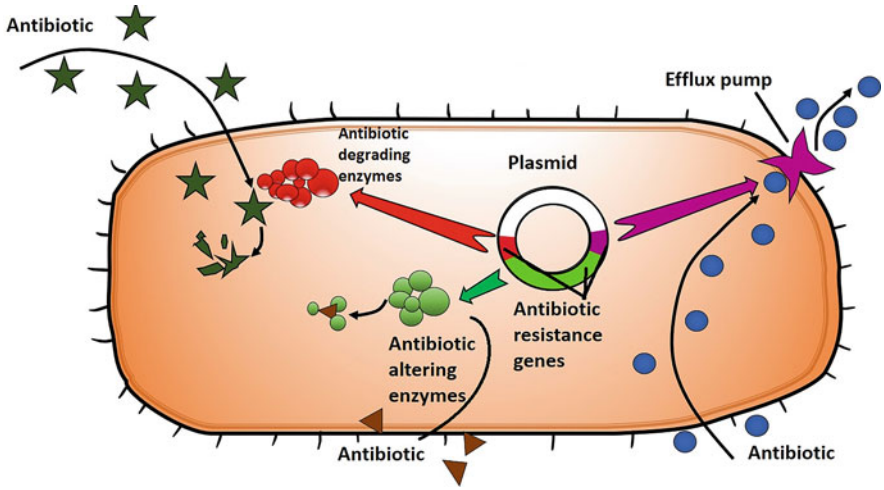


Fig. 1.6 Antibiotic resistance mechanisms in bacteria

1.5.1 Modification of Target Beta-Lactam Antibiotics

The most common and important machinery by which pathogenic bacteria becomes resistant to antibacterial drugs is by acquiring genes coding for enzymes which modify/destroy the target antibiotics. These resistance genes (RGs) are primarily located on mobile genetic elements such as plasmids and transposons; however, they may also be located on bacterial chromosomes. The transposable element RGs can transfer between the chromosomal and other replica, including plasmids. Moreover, RGs can also incorporate into bacterial chromosomes on plasmids. Bacteria become resistance to antibiotics due to the production of β-lactamase, an enzyme that dissects the β-lactam bond in β-lactam antibiotics such as penicillins or cephalosporins (Fig. 1.6). This bond is important for the activity of the antibiotics because it acts as an analogue of the peptide bond which joins the terminal D-alanine to the peptidoglycan monomer. The β-lactamases are comprised of a huge family of enzymes which eventually cleave β-lactam bond (β-lactam ring) to render them inactive (Fig. 1.7). Earlier β-lactamases had a narrow-spectrum activity; however recently, many broad-spectrum β-lactamases have evolved inactivating many of the penicillins and cephalosporins. Many of these families were found to accumulate point mutations in the penicillinase genes. In addition, many new enzymes are currently encoded using auto-transmissible plasmids, which allow these new determinants to spread quickly.

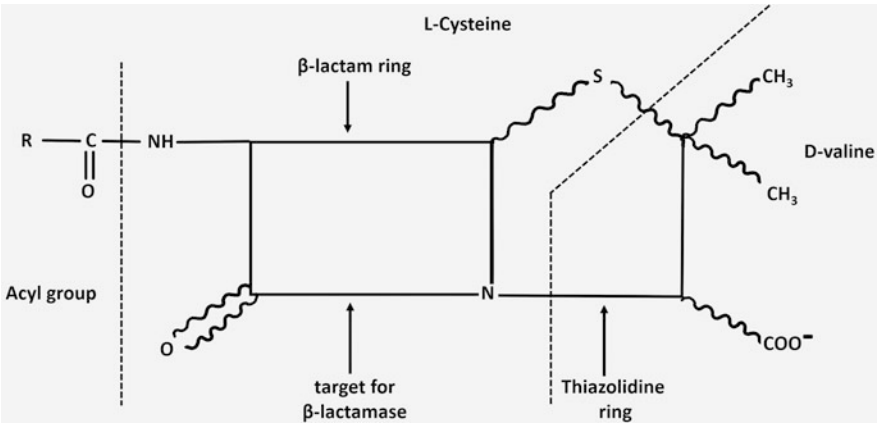


Fig. 1.7 Site of action of β-lactamase on penicillin

1.5.2 Modification in Active Site of Penicillin Binding Proteins

Modifications of the active site of penicillin binding proteins (PBPs) in bacteria can lower cell wall affinity to β-lactam antibiotics, thus rendering bacteria to become resistant to these agents as seen in PBP2x of *Streptococcus pneumoniae* (Laible et al. 1994). Similarly, *Neisseria* spp. and *Streptococcus* spp. have acquired low affinity to PBPs through natural transformation and recombination with DNA from other organisms (Bowler et al. 1994; Page 2007; Zapun et al. 2008). In other organisms such as *Streptococcus mitis*, *Streptococcus sanguis*, and *Streptococcus oralis*, the penicillin resistance had developed due to horizontal transfer of a PBP2b gene from *Streptococcus pneumoniae* (Dowson et al. 1990; Potgieter and Chalkley 1995). Recently, resistance to methicillin in *Staphylococcus* spp. has also appeared as a significant clinical challenge. While there may be many reasons for this resistance, primarily the resistance is conferred by acquisition of the *mecA* gene which is responsible for modification of PBP2a (Chambers 1999).

1.5.3 Permeability-Based Resistance

Another possible mechanism is by lowered expression of outer membrane proteins (OMPs). The β-lactams should spread or cross the porin channels in the bacterial cell walls in order to access PBPs on the internal plasma membranes. Enterobacterales such as *Enterobacter* spp., *Klebsiella pneumoniae*, and *Escherichia coli* exhibit resistance to carbapenems due to loss of OMPs. Loss of OprD has been reported to be associated with imipenem-resistance and reduced susceptibility to meropenem in the non-fermenter *Pseudomonas aeruginosa* (Nikaido 1994; Livermore 2001; Jacoby et al. 2004; Oteo et al. 2008). Imipenem and meropenem resistance has also been related with CarO OMP loss in clinical isolates of multidrug-resistant

Acinetobacter baumannii (Mussi et al. 2005; Poirel and Nordmann 2006). The penetration of β-lactams is usually through porins, OMPF and OMPC, which are proteins inserted into the outer membranes that act as water filled, nonspecific, transmembrane diffusion channels for hydrophilic molecules (Sawai et al. 1982). OMPF is larger than OMPC, which is a major porin responsible for penetration of β-lactam antibiotics. Overall, hydrophobicity, size, and charge of the molecules are responsible to control the rate of penetration, such as cephalosporins are less hydrophobic than penicillins so they have a better penetration rate (Nikaido 1989). Point mutations or insertion sequences in porin genes can produce proteins with a lesser function and result in less permeability to β-lactams (Doumith et al. 2009).

1.5.4 Efflux Pump

The balance in membrane permeability controlling inward and outward traffic of molecules plays a key role in the influx and efflux of antibiotics. The cell wall of Gram-negative bacteria is quite complex containing various protein channels which are involved in inward and outward movement (influx and efflux) of nutrients and also in the movement of noxious compounds like metabolites and drugs. One of these carriers is the efflux pump, which recognizes hazardous substances such as antibiotics and pumping the agent from the periplasm into the cell's environment (Poole 2004), to reduce the intracellular accumulation of the agent. A characteristic of efflux pumps is the range of molecules they can transport, due to poor substrate specificity. This multidrug efflux system plays an important role in providing resistance to a very wide range of compounds in Gram-negative bacteria (Nikaido 1996), particularly in *P. aeruginosa* and *Acinetobacter* spp. Phylogenetically, bacterial antibiotics belong to five families: (1) ABC (ATP-binding cassette); (2) SMR (small multidrug resistance) subfamily of the DMT (drug/metabolite transporters) superfamily; (3) MATE (multi-antimicrobial extrusion) subfamily of the MOP (multidrug/oligosaccharidyl-lipid/polysaccharide flippases) superfamily; (4) MFS (major facilitator superfamily) and (5) RND (resistance/nodulation/division superfamily), which all are secondary ion-driven active transporters (Mahamoud et al. 2007). One of these multidrug efflux pumps, RND pumps are only found in Gram-negative bacteria and demonstrate a wide range of substrates, including antibiotics, antiseptic compounds, dyes or detergents (Levy 2002; Li and Nikaido 2004; Lomovskaya and Totrov 2005; Poole 2005; Piddock 2006). Genome of *E. coli* has the AcrAB-Tol C System; *P. aeruginosa* has the Mex AB-OprM, Mex CD-Opr J, Mex EF-OprN, Mex XY, Mex JK, Mex GHI-Opm D and Mex VW Systems. While other Gram-negative bacteria, such as *B. cepacia* complex with emerging resistance in patients with cystic fibrosis, *S. maltophilia* and *Neisseria gonorrhoeae* may have Ceo AB-Opc M, Amr AB-Opr A, Sme ABC, Sme DEF or Mtr CDE Systems, respectively (Shahid et al. 2009).

1.6 Genetic Mechanisms of Resistance

By acquiring new genetic material from other resistant organisms, bacteria can also gain resistance. This is called horizontal development and may occur between distinct species or genera or between the same species. Bacterial genetic inheritance can occur due to changes in two ways: (1) these modifications could be base changes, deletions of DNA and inversions by mutations that affect the cell's existing DNA (Avison and Bennett 2005), and (2) acquisition of new genetic material like catching new genes in the genome expanding cell. In this phenomenon of acquisition, gene transfer occurs from any outside source, like other bacteria. The mechanisms responsible for genetic exchange between same or different bacterial species include transduction, transformation and conjugation (see Chap. 8).

Acknowledgements Dr. Anuradha Singh is grateful to the Department of Microbiology, JN Medical College and Hospital, AMU, Aligarh, and also to Aligarh Muslim University for providing necessary facilities during her PhD work.

Disclosure Contents of this chapter have been extracted from the PhD thesis of the primary author of this chapter (Dr. Anuradha Singh) under the supervision of Prof. Mohammad Shahid who is also the co-author in this chapter. The thesis was not published in any form, however, the soft copy of the thesis was deposited in the repository of the Maulana Azad Library of the Aligarh Muslim University, the institution from where Dr. Anuradha Singh obtained her PhD. The Library and the Institution have no objection in publishing the part of the thesis as a book chapter. The soft copy of the thesis is available at http://ir.amu.ac.in/9736/1/T%208440.pdf.

References

Abraham EP (1962) The cephalosporins. Pharmacol Rev 14:473–500

Abraham EP, Chain E (1940) An enzyme from bacteria able to destroy penicillin. Nature 146:837–842

Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol 1(134):1–7

Ana MM, Faisca Phillips M (2021) In: Ana MM, Faisca Phillips M (eds) Synthetic approaches to nonaromatic nitrogen heterocycles, 1st edn. Wiley, Hoboken. https://doi.org/10.1002/9781119708841

Avison MB, Bennett PM (2005) Bacterial genetics. In: Borriello SP, Murray PR, Funke G (eds) Topley & Wilson's microbiology & microbial infections, vol 1, 10th edn. Bacteriology Hodder Arnold, London, pp 80–135

Babic M, Hujer AM, Bonomo RA (2006) What's new in antibiotic resistance? Focus on beta-lactamases. Drug Res Update 9:142–156

Barka EA, Vatsa P, Sanchez L et al (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43. https://doi.org/10.1128/MMBR.00019-15

Bowler LD, Zhang QY, Riou JY et al (1994) Interspecies recombination between the *penA* genes of *Neisseria meningitides* and commensal *Neisseria* species during the emergence of penicillin resistance in *N. Meningitides*: natural events and laboratory simulation. J Bacteriol 176:333–337

Bryan LE, Godfrey AJ (1991) β-lactam antibiotics: mode of action and bacterial resistance. In: Lorian V (ed) Antibiotics in laboratory medicine, 3rd edn. Williams and Wilkins, Baltimore, pp 599–664

Chambers HF (1999) Penicillin-binding protein-mediated resistance in pneumococci and staphylococci. J Infect Dis 179(2):S353–S359
Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433
Donowitz GR, Mandell GL (1988) Beta–lactam antibiotics. N Engl J Med 318:419–426
Doumith M, Ellington MJ, Livermore DM et al (2009) Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 63:659–667
Dowson CG, Hutchison A, Woodford N et al (1990) Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of Streptococcus pneumoniae. Proc Natl Acad Sci U S A 87:5858–5862
Fleming A (1929) On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 10:226–236
Flynn EH (1972) Cephalosporins and penicillins: chemistry and biology. Academic Press, Inc, New York
Frere JM, Joris B, Granier B et al (1991) Diversity of the mechanism of resistance to β-lacatam antibiotics. Res Microbial 142:705–710
Georgopapadakou NH, Lin FY (1980) Penicillin-binding proteins in bacteria. Antimicrob Agents Chemother 18:148–157
Ghuysen JM (1988) Bacterial active-site serine penicillin-interactive proteins and domains: mechanism, structure, and evolution. Rev Infect Dis 10:726–732
Hopwood DA (2007) How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? Mol Microbiol 63(4):937–940
Jacoby GA, Archer GL (1991) New mechanism of bacterial resistance to antimicrobial agents. N Engl J Med 324:601–612
Jacoby GA, Mills DM, Chow N (2004) Role of β-lactamases and porins in resistance to ertapenem and other β-lactams in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:3203–3206
Laible G, Spratt BG, Hakenbeck R (1994) Interspecies recombination events during the evolution of altered PBP 2x genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Mol Microbiol 5:1993–2002
Levy SB (2002) Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol 92:65–71
Li XZ, Nikaido H (2004) Efflux-mediated drug resistance in bacteria. Drugs 64:159–204
Livermore DM (2001) Of *Pseudomonas*, porins, pumps and carbapenems. J Antimicrob Chemother 47:247–250
Lomovskaya O, Totrov M (2005) Vacuuming the periplasm. J Bacteriol 187:1879–1883
Mahamoud A, Chevalier J, Alibert-Franco S et al (2007) Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy. J Antimicrob Chemother 59:1223–1229
Mandell GL, Petri WA, Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (1996) Antimicrobial agents: Penicillins, Cephalosporins, and other β–lactam antibiotics. In: The pharmacological basis of therapeutics. McGraw Hill, New York, pp 1073–1101
Mussi MA, Limansky AS, Viale AM (2005) Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of β-barrel outer membrane proteins. Antimicrob Agents Chemother 49:1432–1440
Nikaido H (1989) Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob Agents Chemother 33:1831–1836
Nikaido H (1994) Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264:382–388
Nikaido H (1996) Multidrug efflux pumps of gram-negative bacteria. J Bacteriol 178:5853–5859
Oteo J, Delgado-Iribarren A, Vega D et al (2008) Emergence of imipenem resistance in clinical *Escherichia coli* during therapy. Int J Antimicrob Agents 32:534–537

Page MG (2007) Resistance mediated by penicillin-binding proteins. In: Bonomo RA, Tolmasky ME (eds) Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press, Washington, DC, pp 81–99

Piddock LJ (2006) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 19:382–402

Podolsky, Lawrence M (1998) Cures out of chaos: how unexpected discoveries led to breakthroughs in medicine and health. Harwood Academic Publishers, Amsterdam

Poirel L, Nordmann P (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 12:826–836

Poole K (2004) Efflux-mediated multiresistance in Gram-negative bacteria. Clin Microbiol Infect 10:12–26

Poole K (2005) Efflux-mediated antimicrobial resistance. J Antimicrob Chemother 56:20–51

Potgieter E, Chalkley LJ (1995) Relatedness among penicillin-binding protein 2b genes of *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus pneumoniae*. Microb Drug Resist 1:35–42

Rolinson GN (1998) Forty years of β-lactam research. J Antimicrob Chemother 41:589–603

Sawai T, Hiruma R, Kawana N et al (1982) Outer membrane penetration of β-lactam antibiotics in *Escherichia coli*, *Proteus mirabilis* and *Enterobacter cloaceae*. Antimicrob Agents Chemother 22:585–592

Shahid M, Sobia F, Singh A et al (2009) Beta-lactams and beta-lactamase-inhibitors in current- or potential-clinical practice: a comprehensive update. Crit Rev Microbiol 35(2):81–108

Spencer RC, Wheat PF, Winstanley TG et al (1987) Novel β-lactamase in a clinical isolates of *Klebsiella pneumoniae* conferring unusual resistance to β-lactam antibiotics. J Antimicrob Chemother 20:919–921

Spratt BG (1983) Penicillin-binding proteins and the future of β-lactam antibiotics. J Gen Microbiol 129:1247–1260

Staudinger H (1907) Zur Kenntniss der Ketene. Diphenylketen. Eur J Org Chem 356:51–123. https://doi.org/10.1002/jlac.19073560106

Tidwell, Thomas T (2008) Hugo (Ugo) schiff, schiff bases, and a century of β-lactam synthesis. Angew Chem Int Ed 47(6):1016

Tipper DJ, Strominger JL (1965) Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc Natl Acad Sci U S A 54:1133–1141

Waxman DJ, Strominger JL (1983) Penicillin-binding proteins and the mechanism of action β-lactam antibiotics. Annu Rev Biochem 52:825–869

Zapun A, Contreras-martel C, Vernet T (2008) Penicillin-binding proteins and β-lactam resistance. FEMS Microbiol Rev 32:3847–3852

Trends in Beta-Lactamase Classification

2

Ronni Mol Joji, Ali Al-Mahmeed, Fazal K. Dar, and Mohammad Shahid

Abstract

β-Lactamases are the primary resistance determinants for β-lactam antibiotics in Gram-negative bacteria. These enzymes were first discovered in *Escherichia coli* even before the widespread use of penicillin and named "penicillinase." Several studies and attempts have been made since then to meaningfully group Gram-negative rods using substrate profile, immunological studies, isoelectric point and molecular weight determinations, and nucleotide sequencing. Shortly after a new β-lactam drug is approved for therapeutic use, a new β-lactamase with the ability to destroy this activity is discovered. To date, β-lactamase classification includes 17 functional groups and four designated molecular classes. The β-lactamase classification systems will continue to evolve as and when new enzymes are detected.

Keywords

β-Lactamases · Classification · Ambler's classification · Bush Jacoby classification · Functional group

2.1 Introduction

β-Lactamases are ancient enzymes that have by inference been around for millions of years (Bush 2018). Many antimicrobial therapy failures are due to beta-lactam antibiotic hydrolysis, which is caused by these enzymes (Bush 1989a). In

R. M. Joji (✉) · A. Al-Mahmeed · F. K. Dar · M. Shahid
Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: ronnimj@agu.edu.bh; aliem@agu.edu.bh; fazalkd@agu.edu.bh; mohammeds@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_2

Gram-negative bacteria, β-lactamases are the primary resistance determinants for β-lactam antibiotics. These enzymes, which are around 2771, most likely evolved under selection pressure of natural β-lactams in the environment. Their precursors were most likely penicillin-binding proteins with sequence homology to β-lactamases with an active serine site. There are also metallo-β-lactamases, which contain one or two catalytically active zinc ions (Bush 2018).

Prior to the worldwide clinical use of penicillin for bacterial pathogens, Abraham and Chain observed one of the very first enzymes in *Escherichia coli* in 1940. Because cephalosporins were unknown at the time, they named these enzymes "penicillinase," and the enzymes were thought to be specific for the penicillin beta-lactam bond (Richmond and Sykes 1973). Although the first described β-lactamase was predominantly effective at penicillin hydrolysis, many similar enzymes with varied substrate specificities have since been discovered. Tellingly, immediately as a novel β-lactam antibiotic is approved for use, some previously unknown β-lactamase is discovered to have the ability to destroy this antibacterial activity (Bush 1989a).

2.2 β-Lactamase Classification

Shortly after ampicillin was first used in clinical practice, Ambler (1980) made the first attempt to classify β-lactamases from Gram-negative bacteria. The two enzymes studied in this case were penicillinases, though Fleming and his colleagues had described a β-lactamase that was primarily active against cephalosporins (Fleming et al. 1963). Since then, a wide range of beta-lactamase profiles in enteric bacteria and pseudomonads have been discovered (Richmond and Sykes 1973). Several studies (Sawai et al. 1968; Jaurin and Grundström 1981; Richmond and Sykes 1973) attempted to meaningfully group known β-lactamases based on functionality and biochemical characteristics (Bush 2018).

Sawai et al. (1968) classified β-lactamases into three groups based on substrate profile and response to antisera: (1) Typical cephalosporinase that does not hydrolyze penicillins but hydrolyzes cephaloridine. This enzyme was inducible and was detectable in strains of *E. freundii*, *P. morganii*, *A. aerogenes*. (2) Cephalosporinase, which has the inducible property of penicillinase, with the probability that this type of β-lactamase is a combination of two inducible β-lactamases, penicillinase and cephalosporinase. These enzymes were found in *P. vulgaris* strains as well as *Serratia* group strain GN629. (3) Penicillinase which was found in *K. pneumoniae*, *P. mirabilis*, and *E. coli* strains. This enzyme was discovered to be a constitutive enzyme (Sawai et al. 1968).

In 1970, Jack and Richmond suggested a classification based on substrate profile, cloxacillin inhibition and inhibition by p-chloromercuribenzoate, response to antiserum and electric charge: (1) Class I: cephalosporins; (2) Class II: enzymes primarily active against penicillins; (3) Class III: enzymes with relatively similar activity against penicillins and cephalosporins, but with cloxacillin sensitivity and resistance to p-chloromercuribenzoate; (4) Class IV: enzymes having substrate profile

comparable to Class III but cloxacillin resistant and sensitive to p-chloromercuribenzoate. Cloxacillin is hydrolyzed by some of the enzymes in this class (Richmond and Sykes 1973). Here Richmond and Sykes (1973) suggested the presence of eight types of β-lactamases adding a fifth class and basing their characterization on substrate profile including cloxacillin and p-chloromercuribenzoate inhibition and on electric charge, and molecular weight. Thus, Class V included enzymes with a penicillinase profile that are resistant to sulfhydryl agents (Richmond and Sykes 1973). These classifications have undergone considerable revision over time.

In 1976, Sykes and Matthew also grouped β-lactamases into five classes by taking into account substrate profiles, cloxacillin and p-chloromercuribenzoate inhibition, molecular weight determination, immunological studies, analytical isoelectric focusing with two subgroups of (1) chromosomally mediated: (a) penicillinases, (b) cephalosporinases, and (c) broad spectrum β-lactamases; and (2) R plasmid mediated: (a) enzymes that will not lyse isoxazoyl β-lactam substrates, (b) enzymes that will hydrolyze both isoxazoyl β-lactam substrates and methicillin, and (c) R plasmids specified other β-lactamases. This included at least five other β-lactamases. This categorization was completely different from the earlier classification proposed by Richmond and Sykes in 1973 (Sykes and Matthew 1976).

In light of the molecular structure, four classes of β-lactamases were proposed between 1980 and 1988: (1) Ambler (1980) proposed class A serine penicillinases and class B metalloenzymes based on amino acid sequences of purified proteins (Ambler 1980); (2) Jaurin and Grundström (1981) proposed class C serine cephalosporinases based on sequence of amino acid, from ampC nucleotide sequence translation. According to their study, ampC beta-lactamases with cephalosporins specificity had no remarkable sequence similarity to penicillinase or D-alanine carboxypeptidases (Jaurin and Grundström 1981). Nevertheless, the ampC beta-lactamase region near serine-80 shared wide resemblance with cephalosporinase active-site area, of *Pseudomonas aeruginosa*, prompting Jaurin and Grundstrom to suggest that the ampC and associated cephalosporinases form a separate group of serine beta-lactamases with a specific evolutionary beginning than the serine penicillinases (Jaurin and Grundström 1981); and (3) Huovinen et al. (1988) proposed class D oxacillin hydrolyzing serine-lactamases based on its amino acid sequence. They concluded that there was a homology between PSE-2 and OXA-2, as well as lack of structural similarity with ampC β-lactamases or TEM-1, and thus suggested that these PSE and OXA enzymes have a specific evolutionary beginning and ought to belong to a new class, under class D in Ambler's scheme (Huovinen et al. 1988).

Bush (1988) proposed a functional β-lactamase classification scheme for 28 enzymes. He used substrate profiles and inhibition by clavulanic acid and aztreonam as criteria in a semiempirical classification scheme. (1) Class 1 cephalosporinases: inhibited strongly by aztreonam but only weakly by clavulanate, (2) Class 2 penicillinases and broad-spectrum beta-lactamases: show low affinities for aztreonam but are inhibited by clavulanic acid, (3) Class

Table 2.1 Classification of bacterial β-lactamases (Bush 1989a)

Group	Defining characteristics
1	Hydrolyze cephalosporins and are uninhibited by 10 μM clavulanic acid
2a	Classical penicillinases
2b	Traditional broad-spectrum β-lactamases
2b′	"Extended-broad-spectrum" β-lactam antibiotics hydrolysis, like ceftazidime, cefotaxime, or aztreonam
2c	Penicillinases that hydrolyze carbenicillin
2d	Penicillinases that hydrolyze cloxacillin
2e	Cephalosporinases sensitive to clavulanic acid and have immunological properties similar to penicillinases
3	Enzymatic activity requires a metal ion; all are uninhibited by clavulanic acid
4	Penicillinases resistant to clavulanic acid

3 metalloenzymes (Bush 1988). Bush (1989a, b, c) further proposed a functional classification of four groups as shown in Table 2.1 (Bush 1989b, c).

The major groupings in the 1989 Bush outline were attempted to be preserved. Three changes, however, were noted. Due to the increasing number of β-lactamases such as SHV and TEM, it was agreed to categorize these enzymes into groups with "2b" prefix. Instead of the previous group "2b" designation, the 2be group is assigned to extended-spectrum β-lactamases, implying that these are group 2b enzymes derivatives and have a broader range of activity (Bush et al. 1995). Thus, Bush in alliance with Jacoby and Medeiros in 1995 recommended a functional classification (Table 2.2) for groups 1–4 comprising 217 enzymes, associated with 118 enzymes in molecular classes (Bush 2018).

Rasmussan and Bush in 1997 defined three major subgroups in group 3 MBLs (metallo-β-lactamases) based on substrate profiles: (1) Subgroup 3a: metallo-β-lactamases with a broad range of hydrolytic activity, including hydrolysis of penicillins or cephalosporins at the rate of 60% that of imipenem. They are known to need Zn^{2+} to function optimally. (2) Subgroup 3b: metallo-β-lactamases that hydrolyze carbapenems selectively. (3) Subgroup 3c which includes one enzyme that hydrolyze ampicillin rapidly and, in particular, cephaloridine. This enzyme was notable for its high activity of cephalosporins hydrolysis, as well as of cephamycins and extended-spectrum cephalosporins (Rasmussen and Bush 1997).

In 2005, Hall and Barlow suggested revising Ambler's classification. They proposed that two major groups be designated: S, for serine β-lactamases which includes SA, SC, and SD, which corresponds to the current class A, class C, and class D, respectively, and M, for metallo-β-lactamases including class MB and class ME, which correspond to the current class B subgroups B1 and B2, respectively, and subgroup B3. They came to the conclusion that this classification would enable the inclusion of any newly found groups within the metallo-β-lactamases or serine β-lactamases and ease the identification of any major subdivisions within any class (Hall and Barlow 2005).

Table 2.2 Modifications of bacterial β-lactamase classifications by Bush et al. in 1995 and 2009 (Bush and Jacoby 2010)

Bush et al. (2009)	Bush et al. (1995)	Defining characteristics
1	1	Greater hydrolysis of cephalosporins than benzylpenicillin; cephamycin hydrolysis
1e	Not included	Ceftazidime and other oxyimino-β-lactams hydrolysis at an increased rate
2a	2a	Benzylpenicillin hydrolysis is greater than cephalosporins
2b	2b	Hydrolysis of cephalosporins and benzylpenicillin are similar
2be	2be	Oxyimino-β-lactams hydrolysis at an increased rate (cefotaxime, cefepime, ceftriaxone, ceftazidime, aztreonam)
2br	2br	Tazobactam, sulbactam, and clavulanic acid resistance
2ber	Not included	Oxyimino-β-lactams hydrolysis along with tazobactam, sulbactam, and clavulanic acid resistance
2c	2c	Carbenicillin hydrolysis
2ce	Not included	Carbenicillin, cefpirome and cefepime hydrolysis
2d	2d	Oxacillin or cloxacillin hydrolysis
2de	Not included	Oxacillin or cloxacillin hydrolysis and hydrolysis of oxyimino-β-lactams
2df	Not included	Oxacillin or cloxacillin hydrolysis and hydrolysis of carbapenems
2e	2e	Cephalosporins hydrolysis. Sensitive to clavulanic acid but not aztreonam
2f	2f	Increased hydrolysis of oxyimino-β-lactams, cephamycins, and carbapenems
3a	3	Hydrolysis of broad-spectrum involving carbapenems but no hydrolysis of monobactams
3b	3	Carbapenems preferential hydrolysis
Not included	4	

Due to the complexities of β-lactamase classification, Giske et al. (2009) proposed a more practical scheme that would be useful for health care professionals. They recommended 2be β-lactamases of the functional class be referred to as "class A extended spectrum beta lactamases" (ESBLA), while OXA-ESBLs and plasmid-mediated AmpC be referred to as "miscellaneous ESBLs" (ESBLM). The recommendations for ESBL detection would continue to be applied to the category ESBLA, whereas both genotypic and phenotypic methodologies may be required for the identification and definition of ESBLM enzymes. To enhance semantic accuracy within the novel classification, the ESBLM class may be divided into two groups: ESBLM-C (plasmid-mediated AmpC; class C) and ESBLM-D (OXA-ESBLs; class D) (Giske et al. 2009). Bush (2018) has argued that such classification would be puzzling, especially in circumstances where pathogens producing ESBLs treated with carbapenems, would be rendered ineffective against carbapenemase-producing

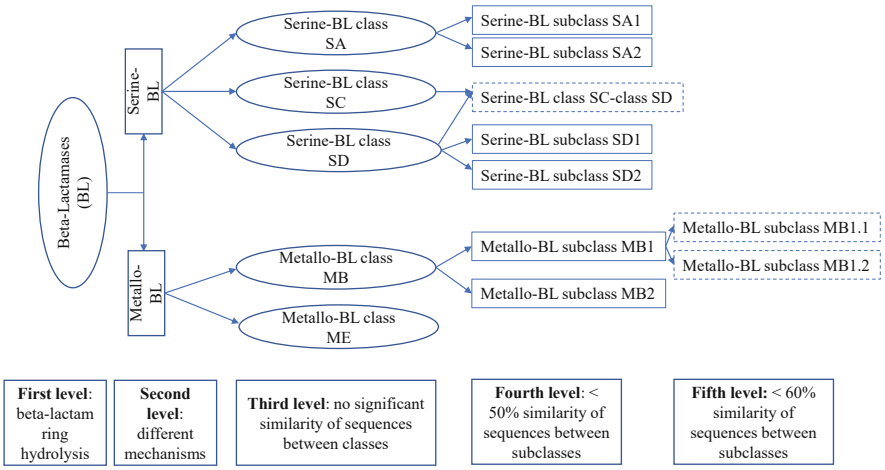


Fig. 2.1 Beta-lactamase hierarchical classification modified from Silveira et al. (2018) (Dotted subclasses are new groups proposed by Silveira et al. (2018))

pathogens if carbapenemases too were referred to as ESBLs (Bush 2018). Bush et al. (2009) in concordance with Bush and Jacoby (2010) agreed that the current classes of carbapenemase, AmpC β-lactamase, and ESBL should be maintained as they are adequately explanatory and comprehensive with well-understood therapeutic effects (Bush et al. 2009).

The Bush and Jacoby (2010) collaboration had expanded and updated the classification of major β-lactamase families, adding new functional subgroups to the scheme (Table 2.2). Group 4 β-lactamases, included in the 1995 functional classification, were thus removed from the new current classification. As more information about these enzymes became available, they may be included in one of the existing enzyme groups. Further categorization would await a further characterization of these enzymes (Bush and Jacoby 2010).

The updated scheme of Bush et al. (2009) has since been broadened, with the inclusion of a differentiating inhibitor called avibactam to separate serine carbapenemases from metallo β-lactamase (Bush 2018). Philippon et al. in 2016 suggested segregation of class A β-lactamases into A1 and A2 subclasses based on the similarities of amino acid sequences of 285 class A β-lactamases (Philippon et al. 2016). More recent classification schemes, particularly for class A/group 2 β-lactamases, are based on the correlation of three dimensional structures and functional information. Structural–functional relationships will become more universal as crystallographic analyses of β-lactamases become available (Bush 2018). Silveira et al. (2018) proposed a five hierarchical grouping levels and a newer β-lactamase class with fused domains, defined by sequence similarity as shown in Fig. 2.1. They suggested that this grouping offers a sound foundation for forthcoming research into the diversity, prevalence, spreading, and advancement of the

various groups and subgroups of this significant enzymatic activity (Silveira et al. 2018).

2.3 Conclusion

In nutshell, since the discovery of β-lactamases, they have been extensively explored as antibiotic resistance determinants. Till date for the β-lactamase classification, 17 functional groups have been identified with four molecular classes. The classification schemes for β-lactamases will change over time, as and when new enzyme variants are described.

References

Ambler RP (1980) The structure of beta-lactamases. Philos Trans R Soc Lond Ser B Biol Sci 289: 321–331

Bush K (1988) Recent developments in beta-lactamase research and their implications for the future. Rev Infect Dis 10:681–690

Bush K (1989a) Characterization of beta-lactamases. Antimicrob Agents Chemother 33:259–263

Bush K (1989b) Classification of beta-lactamases: groups 1, 2a, 2b, and 2b'. Antimicrob Agents Chemother 33:264–270

Bush K (1989c) Classification of beta-lactamases: groups 2c, 2d, 2e, 3, and 4. Antimicrob Agents Chemother 33:271–276

Bush K (2018) Past and present perspectives on beta-lactamases. Antimicrob Agents Chemother 62:e01076

Bush K, Jacoby GA (2010) Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 54:969–976

Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211–1233

Bush K, Jacoby GA, Amicosante G, Bonomo RA, Bradford P, Cornaglia G, Garau J, Giamarellou H, Jarlier V, Martinez-Martinez L, Miriagou V, Palzkill T, Pascual A, Rodriguez-Baño J, Rossolini GM, Sougakoff W, Vatopoulos A (2009) Comment on: redefining extended-spectrum β-lactamases: balancing science and clinical need. J Antimicrob Chemother 64:212–213

Fleming PC, Goldner M, Glass DG (1963) Observations on the nature, distribution, and significance of cephalosporinase. Lancet 1:1399–1401

Giske CG, Sundsfjord AS, Kahlmeter G, Woodford N, Nordmann P, Paterson DL, Cantón R, Walsh TR (2009) Redefining extended-spectrum beta-lactamases: balancing science and clinical need. J Antimicrob Chemother 63:1–4

Hall BG, Barlow M (2005) Revised ambler classification of β-lactamases. J Antimicrob Chemother 55:1050–1051

Huovinen P, Huovinen S, Jacoby GA (1988) Sequence of PSE-2 beta-lactamase. Antimicrob Agents Chemother 32:134–136

Jaurin B, Grundström T (1981) ampC cephalosporinase of Escherichia coli K-12 has a different evolutionary origin from that of beta-lactamases of the penicillinase type. Proc Natl Acad Sci U S A 78:4897–4901

Philippon A, Slama P, Dény P, Labia R (2016) A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. Clin Microbiol Rev 29:29–57

Rasmussen BA, Bush K (1997) Carbapenem-hydrolyzing beta-lactamases. Antimicrob Agents Chemother 41:223–232

Richmond MH, Sykes RB (1973) The beta-lactamases of gram-negative bacteria and their possible physiological role. Adv Microb Physiol 9:31–88

Sawai T, Mitsuhashi S, Yamagishi S (1968) Drug resistance of enteric bacteria. Jpn J Microbiol 12: 423–434

Silveira MC, Azevedo Da Silva R, Faria Da Mota F, Catanho M, Jardim R, Ac RG, De Miranda AB (2018) Systematic identification and classification of β-lactamases based on sequence similarity criteria: β-lactamase annotation. Evol Bioinformatics Online 14:1176934318797351

Sykes RB, Matthew M (1976) The β-lactamases of Gram-negative bacteria and their rôle in resistance to β-lactam antibiotics. J Antimicrob Chemother 2:115–157

Beta-Lactamases and Their Classification: An Overview

3

Anees Akhtar, Nazish Fatima, and Haris M. Khan

Abstract

The discovery of penicillin in 1929 and its introduction in the clinical practice in 1940 were a significant milestone in our ability to treat bacterial infections. While significant studies in the development of newer antimicrobial drugs have been made over time, the beta-lactams remain a mainstay in the antibacterial arsenal. There are primarily four mechanisms by which bacteria can counter and neutralize the effect of beta-lactam antibiotics. The first classification of beta-lactamases was formulated to differentiate beta-lactamases with high hydrolysis rates for cephalosporins from penicillinases which has penicillin-hydrolysing activity. Molecular structure classifications were initially proposed by Ambler in 1980 when only four amino acid sequences of beta-lactamases were known. The conventional system of classification divided beta-lactamases either based on the functional characteristics of the enzymes or based on their primary structure. Another approach towards classifying beta-lactamases is by grouping these enzymes according to their clinical role. This functional classification, albeit a more subjective system, aids the clinician and microbiologist to understand and correlate the properties of a specific enzyme with the observed microbiological resistance profile.

Keywords

Beta-lactamases · Metallo-beta-lactamases · Cephalosporinases

A. Akhtar (✉) · N. Fatima · H. M. Khan
Department of Microbiology, JNMCH, AMU, Aligarh, UP, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_3

3.1 Beta-Lactam Antibiotics: A Short Introduction

The advent of Penicillin in 1921 revolutionized the medical science in relation to antimicrobial treatment. Over the years that followed, vast number of antibiotics were developed but beta-lactams have their own important significance. With continuous research into the field, beta-lactams have been modified in order to improve its clinical usefulness in terms of potency, spectrum, pharmacokinetics and ability to counter antimicrobial resistance (Fleming 1929).

At present, beta-lactams consist of four primary groups based on their chemical structure: The penicillins, the cephalosporins, the carbapenems, and the monobactam. Penicillins have four-membered beta-lactam rings fused to a thiazolidine ring. In cephalosporins, the thiazolidine ring is substituted by dihydrothiazine. Carbapenems on the other hand possess pyrroline which completes the bicyclic conformation. Monobactams, in contrast to the above three bicyclic groups, have a monocyclic structure. The above-mentioned groups were earlier derived naturally but many semi-synthetic derivatives are presently available (Neu 1986).

3.2 How Beta-Lactam Antibiotics Act?

Bacterial cell wall integrity is maintained by the peptidoglycan layer, and beta-lactams suppress the synthesis of this peptidoglycan layer. Penicillin binding proteins (PBPs) are transpeptidases that play important role in the final step of peptidoglycan synthesis by transpeptidation reaction. D-alanyl-D-alanine—the terminal amino acid residues of the nascent peptidoglycan layer is mimicked by beta-lactam antibiotics; because of this molecular mimicry beta-lactam molecule binds to PBP active site in an irreversible way. The irreversible inhibition of the PBPs prevents the final crosslinking (transpeptidation) of the nascent peptidoglycan layer and disrupts cell wall synthesis so the bacterial cell is not able to survive in this environment.

3.3 Resistance Mechanisms Among Beta-Lactam Antibiotics

Antibiotic resistance to beta-lactams has been shown to develop through four primary mechanisms. The first mechanism is induction of changes in the active site of PBPs, thus decreasing the affinity and increasing resistance. Another mechanism is modification of porin proteins leading to production of these proteins lacking full activity. The modifications of these proteins located on the outer membrane of the Gram –ve bacterial cell walls obstruct the transport of beta-lactams to the PBP which are located on the inner membrane. Porins are reduced or lost in some resistant Gram –ve bacteria. For example, loss of OprD in *Pseudomonas aeruginosa* is responsible for its resistance against imipenem. The third mechanism involves development of drug efflux pump systems (mex) which move the antibiotic from the microbial periplasm to the surrounding environment. This efflux pump may be

responsible for multidrug resistance in bacterial pathogens. The fourth mechanism is production of an enzyme called beta-lactamase by the micro organisms. The enzyme, which is a bacterial hydrolase, first binds and acylates the beta-lactams and then hydrolyses it leading to its inactivation (Drawz and Bonomo 2010; Papp-Wallace et al. 2011).

3.4 A Short History of Classification Schemes

Earliest classification systems of beta-lactams were devised to differentiate beta-lactamases having high hydrolysis potential for cephalosporins from penicillinases. This categorization based on function was incorporated in classification by Sawai et al. (1968), where an additional discriminator in terms of response to antisera was also used. Richmond and Sykes also agreed on this functional classification and classified all the known beta-lactamases into five major groups based on their substrate profile. Sykes and Matthew further extended the Richmond and Sykes scheme in 1976 highlighting the use of isoelectric focusing to differentiate plasmid mediated beta-lactamases. Furthermore, Mitsuhashi and Inoue added 'cefuroxime-hydrolyzing beta-lactamase' to the 'penicillinase and cephalosporinase' classification. Another functional classification by Bush in 1989 correlated the substrate and inhibitory properties with molecular structure (Fleming et al. 1963; Sawai et al. 1968; Richmond and Sykes 1973a, b). Ambler first proposed classification based on molecular structure in 1980 at a time when only four amino acid sequences of beta-lactamases were known. This classification scheme included the class A beta-lactamase produced by *Staphylococcus aureus*, PC1 penicillinase; and class B metallo-beta-lactamase from *Bacillus cereus*. Jaurin and Grundstorm added the class C cephalosporinases in 1981, and class D oxacillin-hydrolysing enzymes were segregated from the other serine beta-lactamases (Class A, B and C) in the late 1980s.

In the contemporary scenario, the classification systems incorporate both the functional and molecular characteristics. Until now, 17 functional groups have been identified with four molecular classes. The beta-lactamases are categorized on the basis of their substrate (penicillins, cephalosporins, carbapenems and monobactams). The reactions with clavulanic acid, avibactam and EDTA are utilized for further differentiation (Barthelemy et al. 1988; Baumann et al. 1989).

3.5 Updated Functional Classification of Beta-Lactamases

In the conventional practice, beta-lactamases are classified based on either their primary structure or functional characteristics. On the basis of the protein sequence, beta-lactamases have been divided into four classes from A to D. Classes A, C and D hydrolyse their substrates by forming an acyl enzyme through an active site serine, whereas class B beta-lactamases which are metallo-enzymes utilize at active site zinc ion to facilitate hydrolysis of their substrate.

Classification on the basis of function, based on hydrolytic and inhibitory properties is more practically acceptable as it makes it easier for the clinician to understand and correlate the enzymatic properties with observed resistance profiles of the microorganisms.

The classification suggested by Bush et al. (1995) was further extended by Bush and Jacoby (2010). Newer functional subgroups were later added to the classification scheme, and major families of beta-lactamases were reviewed (Bush and Jacoby 2010). The classification is based on the ability of the enzymes to hydrolyse specific beta-lactam classes and on the inhibition capabilities of beta-lactamase inhibitors clavulanic acid, sulbactam and tazobactam (Bush et al. 1995; Ambler 1980; Richmond and Sykes 1973a, b). Overview of these groups/subgroups is discussed in subsequent sections.

3.6 Group 1 Cephalosporinases

Enterobacteriaceae and a few other microorganisms produce this enzyme which belongs to molecular Class C. They are more active against cephalosporins than on benzylpenicillin, and they demonstrate resistance to inhibition by clavulanic acid. Further, they demonstrate a high affinity for aztreonam. Microorganisms like *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Serratia marcescens*, and *Citrobacter freundii* have low but inducible AmpC on encounter to particular beta-lactams, such as clavulanic acid, ampicillin, imipenem and amoxicillin. On the other hand, *Escherichia coli* and *Acinetobacter baumannii* lack one or more components of the induction system. When produced in large quantity in a host with reduced accumulation of beta-lactams, these enzymes may also generate resistance against carbapenems (Jacoby 2009; Bush 1988; Bush et al. 1985).

Subgroup 1e or extended-spectrum AmpC (ESAC) beta-lactamase has been added in Group 1 as it demonstrates higher activity on ceftazidime and other oxyimino-beta-lactam amino acids. It includes GC1 in *E. cloacae* and plasmid-mediated CMY-10, CMY-19, CMY-37 and others. Recently, an AmpC variant has been discovered in *P. aeruginosa* which can act against imipenem. Clinically significant resistance may arise if there is associated porin mutations within the microorganism (Nordmann and Mammeri 2007; Mammeri et al. 2008).

3.7 Group 2 Serine Beta-lactamases

With continuous identification of ESBLs during the past two decades, this group has become the largest group of beta-lactamases. It includes the molecular classes A and D.

Subgroup 2a penicillinases: A small group with a weak hydrolytic property, are the main beta-lactamases in Gram-positive cocci like staphylococci and enterococci. They are inhibited by clavulanic acid and tazobactam. With the exception of some

staphylococcal penicillinase encoded by plasmid, these enzymes are primarily encoded within the chromosomes (Kernodle et al. 1989).

Subgroup 2b b-lactamases: This group possesses strong hydrolytic action against penicillins and early cephalosporins and demonstrates strong inhibition by clavulanic acid and tazobactam. It consists of the TEM-1, TEM-2 and SHV-1 enzymes. Over the past 25 years, at least 9 TEM and 29 SHV 2b enzymes have been reported often while characterizing other b-lactamases in unusually resistant clinical isolates (Matthew 1979).

Subgroup 2be: This subgroup includes the extended-spectrum beta-lactamases (ESBLs). These enzymes have the additional ability to hydrolyse some oxyimino-beta-lactams, such as cefotaxime, ceftazidime and aztreonam apart from its activity against penicillins and cephalosporins. The largest subset of subgroup 2be has a wider spectrum of substrate activity but a lower capacity to hydrolyse benzylpenicillin and cephaloridine. This arises due to amino acid substitutions in TEM-1, TEM-2 and SHV-1. ESBLs are functionally homologous CTX-M enzymes that are related to chromosomally determined beta-lactamases in species of *Kluyvera*. Most of the CTX-M enzymes exhibit greater activity against cefotaxime than ceftazidime. Many demonstrate activity against cefepime also. CTX-M enzymes are potentially inhibited by tazobactam than clavulanic acid. The subgroup also includes less common ESBLs like BEL-1, BES-1, SFO-1, TLA-1, TLA-2 and members of the PER and VEB enzyme families. Inhibition by clavulanic acid is a characteristic common to this subgroup (Queenan et al. 2004; Bonnet 2004).

Subgroup 2br: This group comprises of broad-spectrum beta-lactamases with subgroup 2b activity and acquired resistance to clavulanic acid and related inhibitors. Thirty-six (including TEM-30 and TEM-31) out of 135 TEM enzymes express this characteristic. Also, five of the corresponding functionally characterized 72 SHV enzymes (e.g. SHV-10) demonstrate this characteristic.

Subgroup 2ber: Consists of TEM enzymes with an extended spectrum and relative resistance to clavulanic acid inhibition. All the enzymes in this subgroup have clavulanic acid IC50s greater than that of TEM-1 with some demonstrating a more modest increase and referred to as CMT (complex mutant TEM) beta-lactamases. This subgroup consists of TEM-50 (CMT-1) (Robin et al. 2005).

Subgroup 2c penicillinases: Characterized by its ability to hydrolyse carbenicillin or ticarcillin by at least 60% faster rate than benzylpenicillin. On the other hand, cloxacillin or oxacillin is hydrolysed at half the rate of that of benzylpenicillin. These are easily inhibited by clavulanic acid or tazobactam. Due to sparse utility of carbenicillin in current clinical practice, newer 2c beta-lactamases have not evolved in the past several year (Bush et al. 1995).

Subgroup 2ce contains the recently described extended spectrum carbenicillinase RTG-4 (CARB-10). It demonstrates expanded activity against cefepime and cefpirome (Potron et al. 2009).

Subgroup 2d: Also referred as OXA enzymes, these constitute the second largest family of beta-lactamases. Characterized by the ability to hydrolyse cloxacillin or oxacillin at a rate of more than 50% that for benzylpenicillin, these enzymes are however primarily identified by their structural amino acid motifs rather than by their

functional attributes. NaCl can inhibit a significant number of enzymes of this category.

Oxyimino-beta-lactams which are cloxacillin- or oxacillin-hydrolysing enzymes with an extended spectrum are included in the new subgroup 2de. Most of the 2de enzymes, for example OXA-11 and OXA-15 are derived by substituting 1–9 amino acid from OXA-10. They have been identified to be produced by *P. aeruginosa* which produces much higher level of resistance than that expressed by *E. coli*. This subgroup demonstrates significantly higher resistance to ceftazidime compared to that of cefotaxime or aztreonam. However, organisms producing a few oxacillinases, such as OXA-1 or OXA-31, may be susceptible to ceftazidime but resistant to cefepime (Aubert et al. 2001).

Acinetobacter baumannii species produce chromosomally encoded OXA enzymes which are included in the subgroup 2df. The plasmid of *Enterobacteriaceae* encodes OXA-23 and OXA-48 enzymes. This subgroup hydrolyses carbapenems. These have been categorized into nine clusters based on the structural configuration of amino acid homologies. The enzymes of this subgroup have not been adequately tested using cloxacillin or oxacillin. Although the organisms producing these enzymes are generally highly resistant to carbapenems, *E. coli* transformants or transconjugants that produce these enzymes are usually susceptible to the carbapenems. These enzymes are not inhibited by clavulanic acid (Walther-Rasmussen and Høiby 2006).

The subgroup 2e cephalosporinases possess the property of hydrolysing extended spectrum cephalosporins while being inhibited by clavulanic acid or tazobactam. These enzymes can be misinterpreted as group 1 AmpC enzymes or ESBLs as they can appear in similar organisms and with comparable resistance profiles. Their poor affinity for aztreonam is the distinguishing feature to differentiate them from Amp C (Bush 1989).

Subgroup 2f: It acts mainly on carbapenems and consists of serine carbapenemases from molecular class A. Tazobactam is more active against these enzymes than clavulanic acid. Extended spectrum cephalosporin may not be effectively hydrolysed by SME and IMI-1 enzymes but most of these enzymes barring GES-3 and GES-4 can hydrolyse aztreonam. The SME family and IMI-1 and NMC-1 beta-lactamases are chromosome encoded. Plasmid encodes KPC and some GES enzymes. KPC carbapenemases has been found to be associated with multidrug-resistant Gram-negative infections outbreaks in hospitals worldwide (Naas et al. 2005).

3.8 Group 3 MBLs

This group consists of metallo-beta-lactamases (MBLs) characterized by their need for zinc ion at the active site. Earlier, the ability of these enzymes to hydrolyse carbapenems was used as a distinguishing trait, but it has been shown that serine beta-lactamases can also possess this characteristic. They have limited hydrolytic activity against monobactams and are not inhibited by clavulanic acid or tazobactam.

This characteristic can be utilized to differentiate it from serine proteases which are inhibited by metal ion chelators such as EDTA, dipicolinic acid, or 1,10-*o*-phenanthroline. The structural classification includes B1, B2 and B3 while functional categorization includes 3a, 3b and 3c. Initially MBLs were determined as chromosomally encoded enzymes expressed in Gram-positive or in a few Gram-negative bacilli, such as *Bacteroides fragilis* or *Stenotrophomonas maltophilia*. Those discovered on transferable elements demonstrated the ability to frequently exchange hosts and underwent genetic transformation leading to the development of enzyme families with several unique variants (Laraki et al. 1999; Queenan and Bush 2007; Garau et al. 2004).

Classification of MBLs into two functional subgroups based on their biochemical characteristics has been proposed.

Subgroup 3a has subclass B1 and has two zinc ions joined with consensus amino acid. This subgroup includes the major plasmid-encoded MBL families, such as the IMP and VIM enzymes that have been reported frequently in non-fermentative bacteria as well as in *Enterobacteriaceae*. L1 MBL from *S. maltophilia* as well as the subclass B3 MBLs, such as CAU-1, GOB-1 and FEZ-1, have also been added to subgroup 3a. The differentiating characteristic of these enzymes from those of subgroup 3a is the difference in the amino acids involved in binding to zinc. However, the need for two bound zinc ions is a prerequisite in both structural subclasses for maximal enzymatic activity. Subgroup 3b is a smaller group of metallo-beta-lactamases that hydrolyse carbapenems more often than penicillins and cephalosporins. These enzymes have been detected with difficultly when chromogenic cephalosporins, such as nitrocefin, are used to monitor the presence of beta-lactamase activity so the chromosomal metallo-beta-lactamases in *Aeromonas* spp. were often missed in carbapenem-resistant isolates because the enzymes did not react with nitrocefin. Carbapenems inhibit their activity when only one zinc binding site is occupied but when the second zinc ion is also present, its inhibitory activity is significantly reduced (Yang and Bush 1996).

Group 4 beta-lactamases have been eliminated from the current classification system as these enzymes have not been completely characterized.

References

Ambler RP (1980) The structure of lactamases. Philos Trans R Soc Lond B 289:321–331

Aubert D, Poirel L, Chevalier J, Leotard S, Pages JM, Nordmann P (2001) Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 45:1615–1620

Barthelemy M, Peduzzi J, Yaghlane HB, Labia R (1988) Single amino acid substitution between SHV-1 b-lactamase and cefotaxime-hydolyzing SHV-2 enzyme. FEBS Lett 231:217–220

Baumann M, Simon H, Schneider KH, Danneel HJ, Kruster U, Giffhorn F (1989) Susceptibility of *Rhodobacter sphaeroides* to b-lactam antibiotics: isolation and characterization of a periplasmic b-lactamase (cephalosporinase). J Bacteriol 171:308–313

Bonnet R (2004) Growing group of extended-spectrum b-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14

Bush K (1988) Lactamase inhibitors from laboratory to clinic. Clin Microbiol Rev 1:109–123

Bush K (1989) Characterization of b-lactamases. Antimicrob Agents Chemother 33:259–263
Bush K, Jacoby GA (2010) Updated functional classification of b-lactamases. Antimicrob Agents Chemother 54:969–976
Bush K, Tanaka SK, Bonner DP, Sykes RB (1985) Resistance caused by decreased penetration of _-lactam antibiotics into *Enterobacter cloacae*. Antimicrob Agents Chemother 27:555–560
Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for b-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211–1233
Drawz SM, Bonomo RA (2010) Three decades of b-lactamase inhibitors. Clin Microbiol Rev 23: 160–201
Fleming A (1929) On the antibacterial action of cultures of a penicillium with special reference to their use in the isolation of B. influenzae. Br J Exp Pathol 10:226–236
Fleming PC, Goldner M, Glass DG (1963) Observations on the nature, distribution, and significance of cephalosporinase. Lancet 1:1399–1401
Garau G, García-Saez I, Bebrone C, Anne C, Mercuri P, Galleni M, Frere JM, Dideberg O (2004) Update of the standard numbering scheme for class B b-lactamases. Antimicrob Agents Chemother 48:2347–2349
Jacoby GA (2009) AmpC b-lactamases. Clin Microbiol Rev 22:161–182
Kernodle DS, Stratton CW, McMurray LW, Chipley JR, McGraw PA (1989) Differentiation of b-lactamase variants of *Staphylococcus aureus* by substrate hydrolysis profiles. J Infect Dis 159: 103–108
Laraki N, Franceschini N, Rossolini GM, Santucci P, Meunier C, de Pauw E, Amicosante G, Frere JM, Galleni M (1999) Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo-b-lactamase IMP-1 produced by *Escherichia coli*. Antimicrob Agents Chemother 43: 902–906
Mammeri H, Nordmann P, Berkani A, Eb F (2008) Contribution of extended-spectrum AmpC (ESAC) b-lactamases to carbapenem resistance in *Escherichia coli*. FEMS Microbiol Lett 282: 238–240
Matthew M (1979) Plasmid mediated b-lactamases of gram-negative bacteria: properties and distribution. J Antimicrob Chemother 5:349–358
Naas T, Nordmann P, Vedel G, Poyart C (2005) Plasmid-mediated carbapenem-hydrolyzing b-lactamase KPC in a *Klebsiella pneumoniae* isolate from France. Antimicrob Agents Chemother 49:4423–4424
Neu HC (1986) Beta-Lactam antibiotics: structural relationships affecting in vitro activity and pharmacologic properties. Rev Infect Dis 8(Suppl. 3):S237–S259
Nordmann P, Mammeri H (2007) Extended spectrum cephalosporinases: structure, detection and epidemiology. Future Microbiol 2:297–307
Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA (2011) Carbapenems: past, present, and future. Antimicrob Agents Chemother 55:4943–4960
Potron A, Poirel L, Croize J, Chanteperdrix V, Nordmann P (2009) Genetic and biochemical characterization of the first extended-spectrum CARB-type b-lactamase, RTG-4, from *Acinetobacter baumannii*. Antimicrob Agents Chemother 53:3010–3016
Queenan AM, Bush K (2007) Carbapenemases: the versatile b-lactamases. Clin Microbiol Rev 20: 440–458
Queenan AM, Foleno B, Gownley C, Wira E, Bush K (2004) Effects of inoculum and b-lactamase activity in AmpC- and extended-spectrum b-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumonia* clinical isolates tested by using NCCLS ESBL methodology. J Clin Microbiol 42:269–275
Richmond MH, Sykes RB (1973a) The lactamases of gram-negative bacteria and their possible physiological roles. Adv Microb Physiol 9:31–88
Richmond MH, Sykes RB (1973b) The b-lactamases of gram-negative bacteria and their possible physiological role. Adv Microb Physiol 9:31–88

Robin F, Delmas J, Chanal C, Sirot D, Sirot J, Bonnet R (2005) TEM-109 (CMT-5), a natural complex mutant of TEM-1 b-lactamase combining the amino acid substitutions of TEM-6 and TEM-33 (IRT-5). Antimicrob Agents Chemother 49:4443–4447

Sawai T, Mitsuhashi S, Yamagishi S (1968) Drug resistance of enteric bacteria. XIV. Comparison of b-lactamases in gram-negative rod bacteria resistant to a-aminobenzylpenicillin. Jpn J Microbiol 12:423–434

Walther-Rasmussen J, Høiby N (2006) OXA-type carbapenemases. J Antimicrob Chemother 57: 373–383

Yang Y, Bush K (1996) Biochemical characterization of the carbapenem-hydrolyzing b-lactamase AsbM1 from *Aeromonas sobria* AER 14M: a member of a novel subgroup of metallo-b-lactamases. FEMS Microbiol Lett 137:193–200

Class A Type B-Lactamases

4

Anuradha Singh, Mohammad Shahid, Hiba Sami, Mohd. Shadab, and Haris M. Khan

Abstract

Extended-spectrum beta-lactamases are able to hydrolyze oxyimino-cephalosporins and monobactams but not carbapenems or cephamycins. Richmond and Sykes (Adv Microb Physiol 9:31–88, 1973) scheme is one out of various functional classification schemes which comprises of all the β-lactamases classified into five major groups based on their substrate profile, some other very commonly used classification systems are Ambler Molecular Classification System and the Bush-Jacoby-Medeiros functional classification system. As per the most commonly used classification scheme, the Ambler classification system, beta-lactamases are classified into Class A, B, C, and D. This chapter will describe class A type beta-lactamases. The most common types of class A ESBLs disseminated worldwide are CTX-M, TEM, and SHV. This chapter will provide significant information regarding nomenclature and variants of class A type beta-lactamases.

Keywords

ESBLs · Class A · SHV · TEM · CTX-M

A. Singh (✉) · H. Sami · H. M. Khan
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid · M. Shadab
Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: mohammeds@agu.edu.bh; shadab@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_4

4.1 Extended-Spectrum Beta-Lactamases

It was in 1983, when first ever plasmid-encoded ß-lactamase with the ability to destroy extended-spectrum ß-lactam antibiotics was described in Germany (Bush and Sykes 1983). ESBLs as mentioned can hydrolyze oxyimino-cephalosporins (e.g., cefotaxime, ceftazidime) and monobactams (e.g., aztreonam), but they cannot hydrolyze the drugs like cephamycins or carbapenems (Bradford 2001). Although ESBLs are frequently identified in *Escherichia coli* and *Klebsiella pneumoniae*, these however have also been described in a range of *Enterobacteriaceae* and *Pseudomonadaceae* across the globe. ESBLs are commonly defined as ß-lactamases, which are able to confer bacterial resistance to the penicillins, aztreonam, and first-, second-, and third-generation cephalosporins but not to carbapenems or cephamycin. The mechanism of action is by hydrolysis of antibiotics, but this action is inhibited by ß-lactamase inhibitors such as clavulanate (Paterson and Bonomo 2005; Bonomo 2014). The scheme of Richmond and Sykes (1973) is one out of various functional classification schemes which includes all the β-lactamases, classified into five major groups based on their substrate profile. Other commonly used classification systems are Bush-Jacoby-Medeiros functional classification system and the ambler molecular classification system (Bush and Jacoby 2010; Ambler 1980), of which Ambler Classification System is the one used widely. According to Ambler's system beta-lactamases are classified into four classes A, B, C, and D. In this chapter, we will discuss about class A-type beta-lactamases.

4.2 Nomenclature of Class A-Type β-Lactamases

β-Lactamases initially were designated as per the names of strains or plasmids which produced these β-lactamases. After that, nomenclature systems have evolved based on biochemical properties, substrates, location of their discovery, gene location on chromosome, peculiarities of sequence, strains of bacteria, and even based on patient's name and to least moderately the name of investigators who illustrate them (Table 4.1). Lately, the use of letters has been started instead of the strain numbers of the families differing by only one through seven amino acids, it is also to be noted that the families of CTX-M and IMP differ from each other by as much as 20% in composition of amino acids. In the early 1960s, TEM-1 was the first plasmid-mediated β-lactamase enzyme originated in Gram-negative bacteria (Datta and Kontomichalou 1965). Originally, it was detected in a strain of *E. coli* cultured from the blood of a patient named Temoniera, in Athens, Greece, so named as TEM (Medeiros 1984). TEM-1 spread to other bacterial species due to plasmids and transposons. TEM-1 became cosmopolitan just after a few years of its isolation, spreading worldwide and currently being found in several different species of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*. SHV-1 (for sulfhydryl) is another common plasmid mediated β-lactamase emerged in *Klebsiella pneumoniae* and *Escherichia coli*. It is a chromosomally mediated enzyme in majority of *K. pneumoniae* isolates, in

Table 4.1 Representative Class A beta-lactamases and their nomenclature

Class A β-lactamase	Origin of name	Country (year)	Organism	UniProt accession No.	Group (s)	Location of gene	Amino acids
ACI-1	*Acidaminococcus*	Spain (2000)	*Acidaminococcus fermentans*	Q9XBM2	2be	Transposon	284
AER-1	*Aeromonas*	UK (1985)	*Aeromonas hydrophila*	Q44056	2c	Transposon	304
AmpC	Ampicillin resistance class	Taiwan (2004)	*Vibrio fischeri*	Q6T3Q5	2b	Chromosome	283
AST-1	*Nocardia asteroides*	France (2001)	*Nocardia asteroides*	Q9EZQ7	2a	Chromosome	310
BCL-1	*Bacillus clausii*	France (2007)	*Bacillus clausii*	A8RR46	2a	Chromosome	307
BEL-1	Belgium extended-spectrum β-lactamase	Belgium (2005)	*Pseudomonas aeruginosa*	Q3SAW3	2be	Plasmid	283
BES-1	Brazil extended-spectrum β-lactamase	Brazil (1996)	*Serratia marcescens*	Q9L6I3	2be	Plasmid	292
BIC-1	Bicêtre carbapenemase	France (2010)	*Pseudomonas fluorescens*	D2WFL1	2f	Chromosome	294
BlaA	β-lactamase	India (2006)	*Yersinia enterocolitica*	Q01166	2e	Chromosome	294
BlaC	β-lactamase	Texas, USA (2006)	*Mycobacterium tuberculosis*	A5U493	2b	Chromosome	307
BlaF	β-lactamase	Belgium (2006)	*Mycobacterium fortuitum*	Q59517	2b	Chromosome	294
BlaL	β-lactamase gene cloned independently in Liège from *Streptomyces cacaoi*	Belgium (1992)	*Streptomyces cacaoi*	Q03680	2d	Chromosome	325
BlaP	β-lactamase	Belgium (2007)	*Bacillus licheniformis*	P00808	2a	Chromosome	307
BlaS	β-lactamase	USA (2005)	*Mycobacterium smegmatis*	Q7WVE1	2be	Chromosome	293

(continued)

Table 4.1 (continued)

Class A β-lactamase	Origin of name	Country (year)	Organism	UniProt accession No.	Group (s)	Location of gene	Amino acids
BlaU	β-lactamase gene cloned independently in Umeå, from *Streptomyces cacaoi*	Belgium (1992)	*Streptomyces cacaoi*	P14560	2a	Chromosome	314
BlaY	β-lactamase	USA (1983)	*Bacillus cereus*	P00809	2a	Chromosome	306
BlaZ	β-lactamase	USA (1986)	*Staphylococcus aureus*	P00807	2a	Plasmid	281
BlaIII	β-lactamase type III	New Jersey (1987)	*Bacillus cereus*	P06548	2a	Chromosome	316
BOR-1	*Bordetella*	France (2005)	*Bordetella bronchiseptica*	Q7WKQ6	2a	Chromosome	305
BPS-1	*Burkholderia pseudomallei*	China (2002)	*Burkholderia pseudomallei*	Q9AGU2	2be	Chromosome	295
BRO	*Branhamella* (*Moraxella*)	Netherlands (1996)	*Moraxella catarrhalis*	Q59514	2c	Plasmid	313
CAD-1	*Carnobacterium divergens*	France (2008)	*Carnobacterium divergens*	Q4QXY0	2a	Chromosome	304
CARB-3	Carbenicillin resistance	Canada (1991)	*Pseudomonas aeruginosa*	P37322	2c	Plasmid	288
CblA	Chromosomal beta-lactamase of class A	Carolina, USA (1994)	*Bacteroides uniformis*	P30898	2e	Chromosome	296
CdiA	*Citrobacter diversus*	UK (1995)	*Citrobacter amalonaticus*	P22390	2e	Chromosome	294
CepA	Cephalosporinase of class A	North Carolina (1993)	*Bacteroides fragilis*	Q57150	2e	Chromosome	300
CfxA	Cefoxitin resistance class A	North Carolina (1993)	*Bacteroides vulgates*	P30899	2e	Chromosome, Transposon	321
CGA-1	*Chryseobacterium gleum* class A	France (2002)	*Chryseobacterium gleum*	Q8VT49	2be	Chromosome	292

CIA-1	*Chryseobacterium indologenes* class A	Japan (2012)	*Chryseobacterium indologenes*	G9M9P7	2be	Chromosome	292
CKO-1	*Citrobacter koseri*	France (2006)	*Citrobacter koseri*	Q8RNV0	2b	Chromosome	300
CME-1	*Chryseobacterium meningosepticum*	Italy (1999)	*Elizabethkingia meningoseptica*	Q9RAZ9	2be	Chromosome	295
CSP-1	*Capnocytophaga sputigena*	France (2010)	*Capnocytophaga sputigena*	D5HKL4	2be?	Chromosome	305
CTX-M-1	Cefotaxime Munich	Germany (1989)	*Escherichia coli*	Q7AVW6	2be	Plasmid	291
CTX-M-2	Cefotaxime Munich	Japan (1986)/ Argentina (1989)	*Salmonella* Typhimurium	P74841	2be	Plasmid	291
CTX-M-8	Cefotaxime Munich	Brazil (1996–1997)	*Citrobacter amalonaticus*	Q9RMT4	2be	Plasmid	291
CumA	Cefuroxime class A	Germany (1994)	*Proteus vulgaris*	P52664	2e	Chromosome	300
DES-1	*Desulfovibrio desulfuricans*	France (2002)	*Desulfovibrio desulfuricans*	Q8KVT3	2be	Chromosome	324
ERP-1	*Erwinia persicina*	France (2002)	*Erwinia persicina*	Q8L1Z4	2be	Chromosome	293
FAR-1	*Nocardia farcinica*	France (1999)	*Nocardia farcinica*	Q5YXD6	2a	Chromosome	313
FEC-1	Fecal *Escherichia coli*	Japan (1988)	*Escherichia coli*	Q8G9E9	2e, 2be	Plasmid	291
FONA-1	*Serratia fonticola* class A	France (1999)	*Serratia fonticola*	Q9RIR3	2be	Chromosome	295
FPH-1	*Francisella philomiragia*	USA (2012)	*Francisella philomiragia*	YP_001676751	2b	Chromosome	294
FRI-1	French imipenemase	France (2015)	*Enterobacter cloacae*	KT192551	2f	Plasmid	294
FTU-1	*Francisella tularensis*	USA (2011)	*Francisella tularensis*	CAJ79318	2a	Chromosome	294

(continued)

Table 4.1 (continued)

Class A β-lactamase	Origin of name	Country (year)	Organism	UniProt accession No.	Group (s)	Location of gene	Amino acids
GES-1	Guiana extended spectrum	France (2000)	*Klebsiella pneumonia*	Q9KJY7	2be	Plasmid	287
GIL-1	*Citrobacter gillenii*	France (2007)	*Citrobacter gillenii*	A4KCT8	2b?	Chromosome	286
GRI-1	*Leminorella grimontii*	France (2007)	*Leminorella grimontii*	A4FRA6	2be	Chromosome	294
HER-1	*Escherichia hermannii*	France (2003)	*Escherichia hermannii*	Q93FN7	2b?	Chromosome	290
HugA	Hôpital Universitaire Genève class A	Switzerland (2002)	*Proteus penneri*	Q8VTN0	2be	Chromosome	298
IBC-1	Integron-borne cephalosporinase	Greece (2000)	*Enterobacter cloacae*	Q83ZP8	2be	Integron	287
IMI-1	Imipenem hydrolyzing	USA (1996)	*Enterobacter cloacae*	Q46991	2f	Chromosome	292
K1	First resistant *Klebsiella* isolate (aztreonam)	UK (1986)	*Klebsiella oxytoca* (*K. aerogenes*)	Q938A8	2be	Chromosome	290
KLUA-1	*Kluyvera ascorbata*	France (2002)	*Kluyvera ascorbata*	Q9RLX4	2be	Chromosome	291
KLUC-1	*Kluyvera cryocrescens*	France (2001)	*Kluyvera cryocrescens*	Q8VVP3	2be	Chromosome	291
KLUG-1	*Kluyvera georgiana*	France (2002)	*Kluyvera georgiana*	Q8GNP9	2be	Chromosome	291
KPC-2	*Klebsiella pneumoniae* carbapenemase	USA (2001)	*Klebsiella pneumonia*	Q93LQ9	2be	Plasmid	293
L2	Second labile enzyme	UK (2000)	*Stenotrophomonas maltophilia*	Q9RBQ1	2e	Chromosome	303
LAP-1	Initials of author's name (Laurent Poirel)	France (2007)	*Enterobacter cloacae*	A0SVI2	2b	Plasmid	285

LEN-1	Name of strain	Japan (1986)	*Klebsiella pneumonia*	P05192	2a	Chromosome	279
LUT-1	*Pseudomonas luteola*	France (2010)	*Pseudomonas luteola*	Q670S6	2e	Chromosome	296
MAL-1	*Levinea malonatica*	(2001)	*Citrobacter koseri*	Q9AL74	2a	Chromosome	300
MEN-1	Named after patient	Germany (1996)	*Escherichia coli*	P28585	2be	Plasmid	291
MIN-1	*Minibacterium massiliensis*	France (2012)	*Minibacterium massiliensis*	A6SVG3	2be	Chromosome	299
MP-1	Name of strain MP-1	Japan (2001)	*Moritella marina*	Q9RA17		Chromosome	287
NMC-A	Not metallocarbapenemase class A	France (1998)	*Enterobacter cloacae*	P52663	2f	Chromosome, Integron	292
OHIO-1	Discovered in the state of Ohio	Ohio (1986)	*Enterobacter cloacae*	P18251	2b	Plasmid	286
OKP-A	Other *Klebsiella pneumoniae*	France (2005)	*Klebsiella pneumonia*	Q2YHZ5	2b	Chromosome	286
ORN-1	*Raoultella ornithinolytica*	France (2004)	*Raoultella ornithinolytica*	Q6W7F0	2b	Chromosome	291
OXY-1	*Klebsiella oxytoca*	Japan (1989)	*Klebsiella oxytoca*	P22391	2be	Chromosome	291
PAU-1	*Pseudomonas aeruginosa*	China (2019)	*Pseudomonas aeruginosa*	MH053445	2be	Transposon	293
PC1	Strain PC1	California (1981)	*Staphylococcus aureus*	M25252	2a	Chromosome	281
PenA	Penicillin resistance class A	Canada (1997)	*Burkholderia cepacia*	O08350	2be	Chromosome	302
PenI	Penicillin resistance	USA (2013)	*Burkholderia pseudomallei*	H7C785	2be	Chromosome	295
PER-1	*Pseudomonas* extended resistant	France (1994)	*Pseudomonas aeruginosa*	P37321	2be	Plasmid, Integron	308

(continued)

Table 4.1 (continued)

Class A β-lactamase	Origin of name	Country (year)	Organism	UniProt accession No.	Group (s)	Location of gene	Amino acids
PLES-1	*Plesiomonas shigelloides*	Spain (2013)	*Plesiomonas shigelloides*	R8AQR8	2b	Chromosome	299
PLA-1	*Raoultella planticola*	France (2004)	*Raoultella planticola*	Q6W7F0	2b	Chromosome	291
PME-1	*Pseudomonas aeruginosa*ESBL-1	Pennsylvania (2008)	*Pseudomonas aeruginosa*	E9N9H5	2be	Plasmid	309
PSE-1	*Pseudomonas*-specific enzyme	Finland (1991)	*Pseudomonas aeruginosa*	Q03170	2c	Plasmid	287
PSE-3	*Pseudomonas*-specific enzyme	London (1984)	*Pseudomonas aeruginosa*	AJ877225	2c	Plasmid	293
R39	Resistant strain no. R39	Belgium (1989)	*Actinomadura* sp.	Q60225	2a, 2d, 2be	Chromosome	304
RAHN-1	*Rahnella aquatilis*	France (2001)	*Rahnella aquatilis*	Q93ET5	2be	Chromosome	295
RIC-1	*Leminorella richardii*	France (2007)	*Leminorella richardii*	A4FRA8	2be	Chromosome	295
ROB-1	Named after patient	USA (1981)	*Haemophilus influenza*	P67918	2b	Plasmid	305
RTG-2	Triad 234ArgThrGly236 (RTG)	France (2000)	*Acinetobacter calcoaceticus*	Q9JP71	2c	Chromosome	298
SCO-1	Author's name (Stephane Corvec)	Argentina (2007)	*Acinetobacter baumannii*	A5Y0S3	2b	Plasmid	288
SED-1	*Citrobacter sedlakii*	France (2001)	*Citrobacter sedlakii*	Q93PQ0	2be	Chromosome	295
SFC-1	*Serratia fonticola*	Portugal (2004)	*Serratia fonticola*	Q6JP75	2be	Chromosome	309
SFO-1	*Serratia fonticola*	Japan (1999)	*Enterobacter cloacae*	Q9XE09	2be	Plasmid	295

SGM-1	*Sphingobium*	USA (2013)	*Sphingobium* sp.	G2IJJ9	2be	Chromosome	316
SHV-1	Sulfhydryl reagent variable	Switzerland (1974)	*Klebsiella pneumonia*	P0AD64	2b	Chromosome, Plasmid	286
SMO-1	Smolensk, Russia		*Ewingella* sp.	R4V074	2be	Chromosome	295
SME-1	*Serratia marcescens* enzyme	UK (1982)	*Serratia marcescens*	P52682	2f	Chromosome	294
SPU-1	*Capnocytophaga sputigena*	France (2010)	*Capnocytophaga sputigena*	E2D9D5	2be	Chromosome	293
STRAL	*Streptomyces albus*	Belgium (1987)	*Streptomyces albus*	P14559	2a	Chromosome	314
STRCE	*Streptomyces cellulosae*	Japan (1994)	*Streptomyces cellulosae*	Q06650	2a	Chromosome	311
STRFR	*Streptomyces fradiae*	Sweden (1990)	*Streptomyces fradiae*	P35392	2a	Chromosome	306
STRLA	*Streptomyces lavendulae*	Sweden (1990)	*Streptomyces lavendulae*	P35393	2b	Chromosome	305
TEM-1	Named after patient Temoniera	France (1985)	*Shigella flexneri*	U48775	2b	Plasmid	286
TER-1	*Raoultella terrigena*	France (2015)	*Raoultella terrigena*	D2D0D6	2b	Chromosome	284
TLA-1	Named after an Inca tribe (Tlahuicas)	Mexico (2000)	*Escherichia coli*	Q9X6W1	2be	Plasmid, Integron	314
TLA-2	Named after an Inca tribe (Tlahuicas)	France (2005)	*Uncultured bacteria*	Q5W3A6-1	2e	Plasmid	304
TOHO-1	Japanese school of medicine (Toho)	Japan (1995)	*Escherichia coli*	Q47066	2be	Plasmid	291
VAK-3	*Vibrio alginolyticus* KV3 isolate	Korea (2012)	*Vibrio alginolyticus*	H9BW95	2b	Chromosome	283
VEB-1	Vietnamese extended-spectrum β-lactamase	France (1999)	*Escherichia coli*	O87489	2be	Plasmid	287

(continued)

Table 4.1 (continued)

Class A β-lactamase	Origin of name	Country (year)	Organism	UniProt accession No.	Group (s)	Location of gene	Amino acids
VHH-1	*Vibrio harveyi* strain HB3	Indonesia (2000)	*Vibrio harveyi*	Q9REJ2	2c	Chromosome	283
VHW-1	*Vibrio harveyi* strain W3B	Indonesia (2000)	*Vibrio harveyi*	Q9REJ3	2c	Chromosome	290
XCC-1	*Xanthomonas campestris* pv. campestris	China (2004)	*Xanthomonas campestris*	O87643	2e	Chromosome	295

Adapted from Philippon et al. (2016)

contrast to *E. coli* where it is usually a plasmid-mediated enzyme. β-Lactamases led to quick development of resistance to expanded-spectrum β-lactam antibiotics. SHV-2, isolated from a strain of *Klebsiella ozaenae* in Germany, was one of the first of these enzymes capable to hydrolyze the newer β-lactams (Kliebe et al. 1985). Their increased activity against oxyimino-cephalosporins led them to be included in extended-spectrum β-lactamases (ESBLs). It was CTX-M (Bauernfeind et al. 1990) which came to designate that family of enzymes. Later, MEN (Bernard et al. 1992) or TLB and Toho-1-like β-lactamase (Yagi et al. 1997) appeared. Recently, Toho enzymes have been assigned CTX-M numbers (http://www.lahey.org/studies). Their designation as CTX is given because of their activity against cefotaxime and because of their first isolation from Munich, they are called as CTX-M (Jacoby 2006). BlaL and blaU were cloned independently in Liège and in Umeå, from *Streptomyces cacaoi*, both beta-lactamases belonged to the class A of beta-lactamases (active-site serine penicillinases) (Magdalena et al. 1992). In May 2004, BEL-1 was found in *P. aeruginosa* which was isolated from a scrotal swab of a 72-year-old patient hospitalized for a dissecting aneurism of the left arteria renalis at the Heilig Hartziekenhuis, Roeselare, Belgium (Poirel et al. 2005). A novel β-lactamase gene of class A, $bla_{\text{PAU-1}}$, associated to mobile genetic elements was detected on a transferable plasmid of *P. aeruginosa* clinical isolate (Wang et al. 2019). Some of these enzymes designated with more than one name for example β-lactamase CTX-1 and CTX-2 were also preferred with one more name TEM-3 and TEM-25, respectively (Bonnet 2004; Shahid et al. 2011). While, CTX-M-1, CTX-M-44, and CTX-M-45 were preferred for MEN-1, Toho-1, and Toho-2, respectively.

4.3 Description of Representative Class A-Type Beta-Lactamases and Their Variants

TEM, SHV, and CTX-M are the most common class A-type beta-lactamases and have higher number of variants. Table 4.2 shows various types of class A beta-lactamases and their existing number of variants. Table 4.3 shows various types of class A beta-lactamases and their KEGG Orthology. Phylogenetic details of various types of Class A beta-lactamases can be checked from webpage https://www.genome.jp/kegg/annotation/br01553.html.

4.4 TEM β-Lactamases

The TEM family is one of the most diverse families of β-lactamases, having about 227 different enzymes (https://www.genome.jp/kegg/annotation/br01553.html) (last accessed April 11, 2020). Table 4.4 shows different variants of TEM searched from the GenBank with details of their GenBank accession number, source organism, and country. Figure 4.1 demonstrates the dendrogram prepared from the available sequences in the GenBank showing genetic relatedness of these TEM enzymes (CLUSTALW was used for the phylogenetic analysis and constructing the dendrogram).

Table 4.2 Representative class A type ESBLs and their variants

β-lactamase name	No. of variants[a,b]	Origin of the name
SHV type	~199	Sulfhydryl variable
TEM type	~227	Patient's name: Temoneira
CTX-M-1 group	~245	Cefotaximase—Munich
CTX-M-2 group		
CTX-M-8 group		
CTX-M-9 group		
CTX-M-25 group		
SFO-1	1	Serratia fonticola
TLA-1	1	Tlahuicas (Inca tribe)
PER	9	Pseudomonas extended resistance
VEB	19	Vietnam extended-spectrum β-lactamase (ESBLs)
BES-1	1	Brazilian ESBLs
GES	33	Guyana ESBLs
BEL-1	9	Belgium ESBLs
TLA-2	1	Amino acid identity with TLA-1
CARB	49	Carbenicillin
LEN	31	Strain name LEN-1
IMI	16	Imipenem
KPC	32	Klebsiella pneumoniae carbapenemase
SME	5	Serratia marcescens ESBL
LAP	2	Author's name (Laurent Poirel)

[a] Details as last accessed on 28 April 2011 at Lahey clinic website (http://www.lahey.org/studies)
[b] Last accessed on 11 April 2020 as updated by KEGG website (https://www.genome.jp/kegg/annotation/br01553.html)

Table 4.3 Beta-lactamase KEGG Orthologys (KOs) for gene variant groups (https://www.genome.jp/kegg/annotation/br01553.html)

Ambler class	Bush-Jacoby group	KO
Class A Serine beta-lactamase	2b	K18698 (TEM)
		K18699 (SHV)
		K18796 (LEN)
		K18767 (CTX-M)
		K18797 (PER)
		K19097 (VEB)
		K19317 (BEL)
	2f	K18768 (KPC)
		K18970 (GES)
		K19316 (IMI)
		K22346 (SME)
	2c	K18795 (CARB-1)
		K19218 (CARB-5)
		K19217 (CARB-17)
	Unclassified	K17836 (PenP)

Adapted from https://www.genome.jp/kegg/annotation/br01553.html

Table 4.4 Different variants of TEM searched from the data available in GenBank with details of their accession number, source organism, and country

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-1	NG_050145	*Escherichia coli*	Plasmid	Canada	2016
TEM-2	NG_050234	*Pseudomonas aeruginosa*	–	–	2016
TEM-3	NG_050259	*Klebsiella pneumoniae*	Plasmid	–	2016
TEM-4	NG_050265	*Klebsiella pneumoniae*	Plasmid	Spain	2016
TEM-6	NG_050277	*Escherichia coli*	–	–	2016
TEM-8	NG_050289	*Klebsiella pneumoniae*	–	–	2016
TEM-9	NG_056168	*Escherichia coli*	–	–	2018
TEM-10	NG_050146	*Morganella morganii*	–	–	2016
TEM-11	NG_050155	*Proteus mirabilis*	-	Hong Kong	2016
TEM-12	NG_050163	*Klebsiella oxytoca*	–	–	2016
TEM-15	NG_050193	*Haemophilus parainfluenzae*	Plasmid	South Africa	2016
TEM-16	NG_050204	*Klebsiella pneumoniae*	–	–	2016
TEM-17	NG_050213	*Capnocytophaga ochracea*	–	–	2016
TEM-19	NG_050227	*Acinetobacter baumannii*	–	–	2016
TEM-20	NG_050235	*Escherichia coli*	–	–	2017
TEM-21	NG_050242	*Pseudomonas aeruginosa*	–	–	2017
TEM-22	NG_050252	*Klebsiella pneumoniae*	–	–	2016
TEM-24	NG_050255	*Klebsiella pneumoniae*	–	–	2016
TEM-26	NG_050256	*Enterobacter kobei*	–	USA	2016
TEM-28	NG_050257	*Escherichia coli*	–	–	2016
TEM-29	NG_050258	*Pseudomonas aeruginosa*	Plasmid	China	2016
TEM-30	NG_050260	*Escherichia coli*	Plasmid	Portugal	2016
TEM-32	NG_050261	*Shigella sonnei*	–	Israel	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-33	NG_050262	*Escherichia coli*	Plasmid	–	2016
TEM-34	NG_050263	*Haemophilus parainfluenzae*	Plasmid	Spain	2016
TEM-35	NG_050264	*Escherichia coli*	–	Netherlands	2016
TEM-36	NG_052650	*Escherichia coli*	–	–	2017
TEM-40	NG_050266	*Escherichia coli*	–	Italy	2016
TEM-43	NG_050267	*Klebsiella pneumoniae*	–	–	2016
TEM-45	NG_050268	*Escherichia coli*	Plasmid	–	2016
TEM-47	NG_050269	*Klebsiella pneumoniae*	–	Poland	2016
TEM-48	NG_050270	*Klebsiella pneumoniae*	–	Poland	2016
TEM-49	NG_050271	*Escherichia coli*	–	Poland	2016
TEM-52	NG_050272	*Klebsiella pneumoniae*	–	–	2016
TEM-53	NG_050273	*Klebsiella pneumoniae*	–	–	2016
TEM-54	NG_050274	*Escherichia coli*	–	–	2016
TEM-55	NG_050275	*Escherichia coli*	–	South Africa	2016
TEM-57	NG_050276	*Escherichia coli*	Plasmid	China	2016
TEM-60	NG_050278	*Providencia stuartii*	Plasmid	–	2016
TEM-63	NG_050279	*Escherichia coli*	–	South Africa	2016
TEM-67	NG_050280	*Proteus mirabilis*	Plasmid	–	2016
TEM-68	NG_050281	*Klebsiella pneumoniae*	Plasmid	Poland	2021
TEM-70	NG_050282	*Escherichia coli*	Plasmid	–	2016
TEM-71	NG_050283	*Klebsiella pneumoniae*	Plasmid	–	2016
TEM-72	NG_050284	*Morganella morganii*	Plasmid	Italy	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-76	NG_050285	*Escherichia coli*	–	France	2016
TEM-77	NG_050286	*Escherichia coli*	–	France	2016
TEM-78	NG_050287	*Escherichia coli*	–	France	2016
TEM-79	NG_050288	*Escherichia coli*	–	France	2016
TEM-80	NG_050290	*Enterobacter cloacae*	Plasmid	–	2016
TEM-81	NG_050291	*Escherichia coli*	–	France	2016
TEM-82	NG_050292	*Escherichia coli*	–	–	2016
TEM-83	NG_050293	*Escherichia coli*	–	France	2016
TEM-84	NG_050294	*Escherichia coli*	–	France	2016
TEM-85	NG_050295	*Klebsiella pneumoniae*	Plasmid	Poland	2016
TEM-86	NG_050296	*Klebsiella pneumoniae*	Plasmid	Poland	2016
TEM-87	NG_050297	*Proteus mirabilis*	Plasmid	Italy	2016
TEM-88	NG_050298	*Klebsiella pneumoniae*	Plasmid	Korea	2016
TEM-90	NG_050299	*Escherichia coli*	Plasmid	–	2016
TEM-91	NG_050300	*Escherichia coli*	–	–	2016
TEM-92	NG_050301	*Proteus mirabilis*	–	–	2016
TEM-93	NG_050302	*Escherichia coli*	Plasmid	Poland	2016
TEM-94	NG_050303	*Escherichia coli*	Plasmid	Poland	2016
TEM-95	NG_050304	*Escherichia coli*	–	Spain	2016
TEM-96	NG_050305	*Escherichia coli*	Plasmid	United Kingdom	2016
TEM-97	NG_050306	*Escherichia coli*	–	Ireland	2016
TEM-98	NG_050307	*Escherichia coli*	–	Ireland	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-99	NG_050308	*Escherichia coli*	–	Ireland	2016
TEM-101	NG_050147	*Escherichia coli*	Plasmid	–	2016
TEM-102	NG_050148	–	Plasmid	–	2016
TEM-104	NG_050149	*Klebsiella pneumoniae*	–	–	2016
TEM-105	NG_050150	*Escherichia coli*	–	–	2016
TEM-106	NG_050151	*Escherichia coli*	–	Korea	2016
TEM-107	NG_050152	*Klebsiella pneumoniae*	–	Korea	2016
TEM-108	NG_050153	*Salmonella enterica* subsp. *enterica serovar Typhimurium*	–	–	2016
TEM-109	NG_050154	*Escherichia coli*	–	–	2016
TEM-110	NG_050156	*Klebsiella pneumoniae*	–	–	2016
TEM-111	NG_050157	*Escherichia coli*	–	–	2016
TEM-112	NG_050158	*Escherichia coli*	–	France	2016
TEM-113	NG_050159	*Proteus mirabilis*	–	France	2016
TEM-114	NG_050160	*Klebsiella aerogenes*	–	France	2016
TEM-115	NG_050161	*Klebsiella pneumoniae*	–	Canada	2016
TEM-116	NG_050162	*Staphylococcus aureus*	Plasmid	–	2016
TEM-120	NG_050164	*Klebsiella oxytoca*	–	Canada	2016
TEM-121	NG_050165	*Escherichia coli*	–	–	2016
TEM-122	NG_050166	*Escherichia coli*	–	United States	2016
TEM-123	NG_050167	*Proteus mirabilis*	–	–	2016
TEM-124	NG_050168	*Morganella morganii*	–	–	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-125	NG_050169	*Escherichia coli*	–	–	2016
TEM-126	NG_050170	*Escherichia coli*	–	France	2016
TEM-127	NG_050171	*Escherichia coli*	Plasmid	Denmark	2016
TEM-128	NG_050172	*Escherichia coli*	Plasmid	Denmark	2016
TEM-129	NG_050173	*Klebsiella oxytoca*	–	France	2016
TEM-130	NG_050174	*Klebsiella pneumoniae*	–	France	2016
TEM-131	NG_050175	*Salmonella enterica* subsp. *enterica serovar Typhimurium*	–	South Africa	2016
TEM-132	NG_050176	*Klebsiella pneumoniae*	Plasmid	Slovakia	2016
TEM-133	NG_050177	*Klebsiella pneumoniae*	–	Spain	2016
TEM-134	NG_050178	*Citrobacter koseri*	–	–	2016
TEM-135	NG_050179	*Salmonella enterica* subsp. *enterica serovar Typhimurium*	Plasmid	Italy	2016
TEM-136	NG_050180	*Klebsiella pneumoniae*	–	–	2016
TEM-137	NG_050181	*Shigella sonnei*	–	Haiti	2016
TEM-138	NG_050182	*Salmonella enterica*	Plasmid	–	2016
TEM-139	NG_050183	*Klebsiella pneumoniae*	Plasmid	Bulgaria	2016
TEM-141	NG_050184	*Enterobacter cloacae*	Plasmid	China	2016
TEM-142	NG_050185	*Escherichia coli*	Plasmid	Germany	2016
TEM-143	NG_050186	*Escherichia coli*	–	–	2016
TEM-144	NG_050187	*Salmonella enterica* subsp. *enterica serovar Derby str. D1*	–	Uruguay	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-145	NG_050188	*Escherichia coli*	–	South Africa	2016
TEM-146	NG_050189	*Escherichia coli*	–	South Africa	2016
TEM-147	NG_050190	*Pseudomonas aeruginosa*	Plasmid	China	2016
TEM-148	NG_050191	*Escherichia coli*	–	Portugal	2016
TEM-149	NG_050192	*Klebsiella aerogenes*	–	–	2016
TEM-150	NG_050194	*Escherichia coli*	–	Portugal	2016
TEM-151	NG_050195	*Escherichia coli*	–	–	2016
TEM-152	NG_050196	*Escherichia coli*	–	–	2016
TEM-153	NG_050197	*Escherichia coli*	–	Portugal	2016
TEM-154	NG_050198	*Escherichia coli*	–	Portugal	2016
TEM-155	NG_050199	*Proteus mirabilis*	–	–	2016
TEM-156	NG_050200	*Proteus mirabilis*	–	Portugal	2016
TEM-157	NG_050201	*Enterobacter cloacae*	–	China	2016
TEM-158	NG_050202	*Escherichia coli*	–	–	2016
TEM-159	NG_050203	*Proteus mirabilis*	–	–	2016
TEM-160	NG_050205	*Proteus mirabilis*	–	–	2016
TEM-162	NG_050206	*Acinetobacter haemolyticus*	Plasmid	India	2016
TEM-163	NG_050207	*Escherichia coli*	–	Buenos Aires	2016
TEM-164	NG_050208	*Klebsiella pneumoniae*	Plasmid	Tunisia	2016
TEM-166	NG_050209	*Escherichia coli*	–	China	2016
TEM-167	NG_050210	*Escherichia coli*	–	Algeria	2016
TEM-168	NG_050211	*Escherichia coli*	–	–	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-169	NG_050212	*Escherichia coli*	Plasmid	–	2016
TEM-171	NG_050214	*Escherichia coli*	Plasmid	–	2016
TEM-176	NG_050215	*Escherichia coli*	–	Peru	2016
TEM-177	NG_050216	*Proteus mirabilis*	–	Italy	2016
TEM-178	NG_050217	*Serratia marcescens*	–	–	2016
TEM-181	NG_050218	*Escherichia* sp. *Sflu5*	Plasmid	–	2016
TEM-182	NG_050219	*Haemophilus parainfluenzae*	–	Spain	2016
TEM-183	NG_050220	*Klebsiella pneumoniae*	–	–	2016
TEM-184	NG_050221	*Escherichia coli*	Plasmid	Italy	2016
TEM-185	NG_050222	*Escherichia coli*	–	–	2016
TEM-186	NG_050223	*Escherichia coli*	Plasmid	Switzerland	2016
TEM-187	NG_050224	*Proteus mirabilis*	Plasmid	France	2016
TEM-188	NG_050225	*Salmonella enterica*	–	Algeria	2016
TEM-189	NG_050226	*Escherichia coli*	–	USA	2016
TEM-190	NG_050228	*Escherichia coli*	–	United Kingdom	2016
TEM-191	NG_052865	*Acinetobacter baumannii*	–	Turkey	2017
TEM-193	NG_050229	*Acinetobacter baumannii*	–	–	2016
TEM-194	NG_050230	*Acinetobacter baumannii*	–	–	2016
TEM-195	NG_050231	*Acinetobacter baumannii*	–	–	2016
TEM-196	NG_055646	*Shigella sonnei*	Plasmid	South Korea	2017
TEM-197	NG_050232	*Klebsiella pneumoniae*	–	Brazil	2016
TEM-198	NG_050233	*Klebsiella pneumoniae*	–	Japan	2016

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-201	NG_050236	*Escherichia coli*	Plasmid	Portugal	2016
TEM-205	NG_050237	*Pseudomonas aeruginosa*		–	2016
TEM-206	NG_050238	*Escherichia coli*	Plasmid	–	2016
TEM-207	NG_050239	*Escherichia coli*	–	–	2016
TEM-208	NG_050240	*Escherichia coli*	–	–	2016
TEM-209	NG_050241	*Klebsiella pneumoniae*	–	Czech Republic	2016
TEM-210	NG_050243	*Escherichia coli*	Plasmid	Switzerland	2016
TEM-211	NG_050244	*Proteus mirabilis*	–	–	2016
TEM-212	NG_050245	*Providencia stuartii*	–	–	2016
TEM-213	NG_051939	*Klebsiella pneumoniae*	–	South Africa	2016
TEM-214	NG_050247	*Escherichia coli*	–	–	2016
TEM-215	NG_050248	*Escherichia coli*	–	–	2016
TEM-216	NG_050249	*Escherichia coli*	–	China	2016
TEM-217	NG_050250	*Enterobacter cloacae*	–	France	2016
TEM-219	NG_050251	*Escherichia coli*	–	India	2016
TEM-220	NG_050253	*Neisseria gonorrhoeae*	–	–	2016
TEM-224	NG_050254	*Klebsiella pneumoniae*	–	Italy	2016
TEM-225	NG_052651	*Escherichia coli*	–	Netherlands	2017
TEM-226	NG_054684	*Escherichia coli*	–	Japan	2017
TEM-227	NG_054696	*Escherichia coli*	–	France	2017
TEM-229	NG_056416	*Acinetobacter haemolyticus*	–	Mexico	2018
TEM-230	NG_056417	*Escherichia coli*	–	–	2018

(continued)

Table 4.4 (continued)

TEM variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission/ publication in GenBank
TEM-231	NG_056418	*Escherichia coli*	–	–	2018
TEM-232	NG_057472	*Klebsiella pneumoniae*	–	USA	2018
TEM-233	NG_057581	*Escherichia coli*	–	–	2018
TEM-234	NG_057609	*Pseudomonas aeruginosa*	–	Kazakhstan	2018
TEM-236	NG_061611	*Escherichia coli*	Plasmid	–	2018
TEM-237	NG_062250	*Escherichia coli*	Plasmid	–	2018

In 1965, TEM-1 is the first member of the TEM family, reported first time from an *Escherichia coli* isolate, with similar substrate and inhibition profiles as of SHV-1 (Datta and Kontomichalou 1965). The production of TEM-1 confers up to 90% of ampicillin resistance to *E. coli* (Livermore 1995). TEM-1 can hydrolyze ampicillin at a rate greater than the likes of carbenicillin, cephalothin, or oxacillin. It shows mild activity against extended-spectrum cephalosporins and is inhibited by clavulanate. Plasmid-mediated TEM-1 is one of the parental enzymes of TEM type ESBLs, and it, in fact, is the most prevalent β-lactam inactivating enzyme found in enteric bacilli (Liu et al. 1992). The second parental enzyme of this family is TEM-2 which differs from TEM-1 at position 39 (Gln39Lys) by an amino acid substitution, while conferring a similar type of resistance (Ambler and Scott 1979; Jacoby and Sutton 1985; Jacoby and Carreras 1990). This substitution also causes a change in the isoelectric point from 5.4 in TEM-1 to 5.6 in TEM-2 (Bradford 2001). Another difference between TEM-1 and TEM-2 is the strength of the promoters present upstream of bla_{TEM} genes, which encode these β-lactamases. $Bla_{\text{TEM-2}}$ gene has a stronger promoter than the promoter for the $bla_{\text{TEM-1}}$. TEM-2 enzyme also shows a higher enzymatic activity when compared to TEM-1 producing strains (Chaibi et al. 1996; Jacoby and Carreras 1990). TEM-3 (originally reported in 1989) was the first enzyme of TEM family to display the ESBL phenotype (Sougakoff et al. 1988). A distinct feature related to genetic environment of the bla_{TEM} genes is seen with the $bla_{\text{TEM-6}}$ gene, which shows a 116 bp IS*1*-like element upstream of coding region (Goussard et al. 1991). This element is responsible for the synthesis of this enzyme at high-level. TEM variants are derived from parental enzymes TEM-1a to TEM-1h and TEM-2, and three transposons, Tn3, Tn2, and Tn1 are encoded from these TEM-1a, TEM-1b, and TEM-2, respectively (Archambault et al. 2006; Partridge and Hall 2005). *K. pneumoniae* isolates showing resistance to multiple antibiotics including oxyimino-cephalosporins were detected in 1987 and these were found to produce CTX-1 β-lactamase with activity against cefotaxime (Sirot et al. 1987).

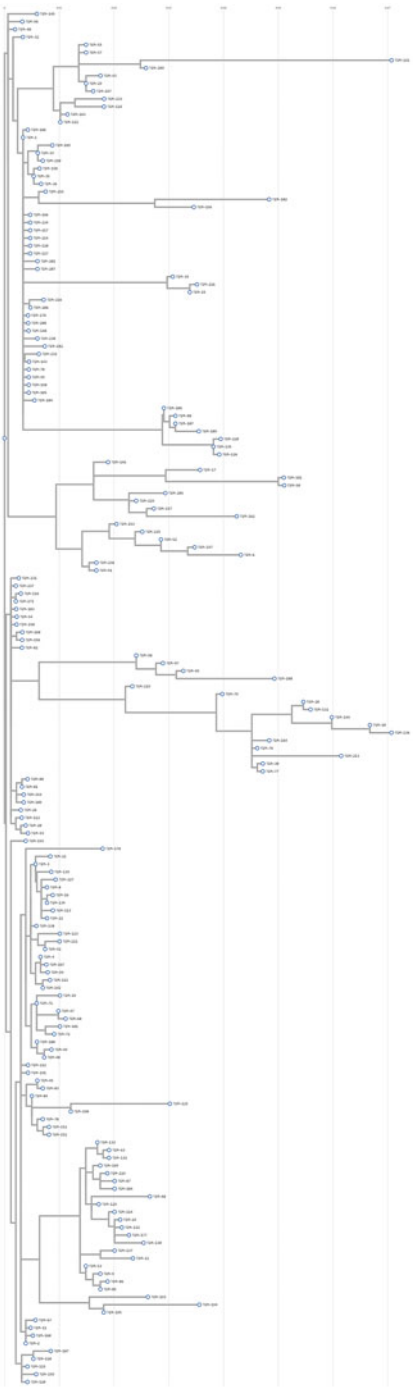


Fig. 4.1 Phylogenetic relation of TEM beta-lactamases

Sequencing of the gene encoding enzyme CTX-1 was related to TEM-2 and was different from its parent enzyme by two amino acids: Glu102Lys and Gly236Ser (Sougakoff et al. 1988). Some variants of TEM family have been characterized by inhibitor resistance β-lactamases; however, most of the enzymes of TEM family show ESBL activity (Canton et al. 2008). Similar to those observed in SHV ESBLs, mutations at several key amino acid residues like Glu104Lys, Arg164Ser, Gly238Ser, and Glu240Lys are important for ESBL activity. Discovery of β-lactamases resistant to clavulanic acid took place in early 1990s. Sequencing revealed that these enzymes were variants of the TEM-1 or TEM-2 β-lactamases. Therefore, initially these enzyme variants were named as IRT (inhibitor-resistant TEM β-lactamase); however, these have later been assigned with numerical TEM designations. These IRT-β-lactamases were found mainly in clinical *E. coli* isolates, but also in some strains of *Klebsiella oxytoca*, *K. pneumonia*, *Citrobacter freundii*, and *P. mirabilis* (Lemozy et al. 1995; Bret et al. 1996). Inhibitor-resistant TEM variants were found resistant to inhibitors clavulanic acid and sulbactam, thus showing clinical resistance to the β-lactam–β-lactamase inhibitor combinations of ticarcillin–clavulanate, amoxicillin–clavulanate, and ampicillin–sulbactam; however, they still remain susceptible to inhibition by tazobactam and thus also to the piperacillin/tazobactam combination (Bonomo et al. 1997; Chaibi et al. 1999). Complex mutant of TEM enzymes (CMT) is the assigned name to a group that comprises of the combination of substitutions associated with ESBL phenotypes and substitutions related with IRT phenotypes. The first appearance of CMT-1, also known as TEM-50 was described in France, in 1997, and represented the ESBL substitutions of TEM-15 and the IRT-substitutions of IRT-4, respectively (Sirot et al. 1997). Other enzymes presenting amino acid substitutions of ESBL and IRT enzymes have also been described in Poland and regions of France (Fiett et al. 2000; Poirel et al. 2004; Robin et al. 2007).

4.5 SHV β-Lactamases

As on April 11, 2020, 199 different enzymes of SHV family have been identified—KEGG database (https://www.genome.jp/kegg/annotation/br01553.html). Table 4.5 shows different variants of SHV searched from the GenBank with details of their GenBank accession number, source organism, and country. Figure 4.2 demonstrates the dendrogram prepared from the available sequences in the GenBank showing genetic relatedness of these SHV enzymes (CLUSTALW was used for the phylogenetic analysis and constructing the dendrogram).

Under Bush-Jacoby-Medeiros classification scheme, SHV enzymes are classified in groups 2b and 2be, and under Ambler classification, these are classified as Class-A ESBLs. These enzymes are found in majority of *K. pneumoniae* strains which possess chromosomal-mediated β-lactamases (Babini and Livermore 2000). SHV-1 (sulfhydryl variable)-type β-lactamase was showing activity against penicillins and against narrow-spectrum cephalosporins, for example, cephalothin and cephaloridine (Livermore 1995). This enzyme was first designated as PIT-2 (from the author's

Table 4.5 Different variants of SHV searched from the data available in GenBank with details of their accession number, source organism, and country

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-1	NG_049989	*Escherichia coli* HB101			2016
SHV-1b-b	NG_050059	*Klebsiella pneumoniae*	Plasmid		2016
SHV-2	NG_050060	*Escherichia coli*			2016
SHV-2a	NG_050067	*Klebsiella pneumoniae*	Plasmid	Switzerland	2016
SHV-3	NG_050068	*Klebsiella pneumoniae*			2016
SHV-5	NG_050087	*Klebsiella pneumoniae*	Plasmid		2016
SHV-7	NG_050104	*Escherichia coli*			2016
SHV-8	NG_050115	*Escherichia coli*			2016
SHV-9	NG_050122	*Klebsiella pneumoniae*	Plasmid		2016
SHV-11	NG_050000	*Klebsiella pneumoniae*	Plasmid	Switzerland	2016
SHV-12	NG_050590	Enterobacter cloacae	Plasmid	China	2016
SHV-13	NG_050008	*Klebsiella pneumoniae*	Plasmid	Netherlands	2016
SHV-14	NG_050014	*Klebsiella pneumoniae*		Netherlands	2016
SHV-15	NG_050024	*Escherichia coli*		India	2016
SHV-16	NG_050035	*Klebsiella pneumoniae*			2016
SHV-18	NG_050047	*Klebsiella pneumoniae*	Plasmid		2016
SHV-24	NG_050061	*Escherichia coli*			2016
SHV-25	NG_050062	*Klebsiella pneumoniae*		Taiwan	2016
SHV-26	NG_050063	*Klebsiella pneumoniae*		Taiwan	2016
SHV-27	NG_050064	*Klebsiella pneumoniae*		Brazil	2016
SHV-28	NG_051877	*Klebsiella pneumoniae*		India	2016
SHV-29	NG_050066	*Klebsiella pneumoniae*			2016
SHV-30	NG_050069	*Enterobacter cloacae*	Plasmid	USA	2016
SHV-31	NG_050070	*Klebsiella pneumoniae*		Netherlands	2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-32	NG_050071	*Klebsiella pneumoniae*		Spain	2016
SHV-33	NG_050072	*Klebsiella pneumoniae*		Spain	2016
SHV-34	NG_050073	*Escherichia coli*	Plasmid	USA	2016
SHV-35	NG_050074	*Klebsiella pneumoniae*	Plasmid		2016
SHV-36	NG_050075	*Klebsiella pneumoniae*	Plasmid	United Kingdom	2016
SHV-37	NG_050076	*Klebsiella pneumoniae*		United Kingdom	2016
SHV-38	NG_050077	*Klebsiella pneumoniae*			2016
SHV-40	NG_050078	*Klebsiella pneumoniae*		Canada	2016
SHV-41	NG_050079	*Klebsiella pneumoniae*		Canada	2016
SHV-42	NG_050080	*Klebsiella pneumoniae*		Canada	2016
SHV-43	NG_050081	*Klebsiella pneumoniae*	Plasmid	China	2016
SHV-44	NG_050082	*Klebsiella pneumoniae*			2016
SHV-45	NG_050083	*Klebsiella pneumoniae*		Brazil	2016
SHV-46	NG_050084	*Klebsiella oxytoca*	Plasmid-encoded		
SHV-48	NG_050085	*Klebsiella pneumoniae*	Plasmid		2016
SHV-49	NG_050086	*Klebsiella pneumoniae*			2016
SHV-50	NG_050088	*Klebsiella pneumoniae*		Canada	2016
SHV-51	NG_050089	*Klebsiella pneumoniae*		Canada	2016
SHV-52	NG_050090	*Klebsiella pneumoniae*		Spain	2016
SHV-55	NG_050091	*Klebsiella pneumoniae*			2016
SHV-56	NG_050092	*Klebsiella pneumoniae*			2016
SHV-57	NG_050093	*Escherichia coli*	Plasmid		2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-59	NG_050094	*Klebsiella pneumoniae*	Plasmid	China	2016
SHV-60	NG_050095	*Klebsiella pneumoniae*		Portugal	2016
SHV-61	NG_050096	*Klebsiella pneumoniae*		Portugal	2016
SHV-62	NG_050097	*Klebsiella pneumoniae*		Portugal	2016
SHV-63	NG_050098	*Klebsiella pneumoniae*		Russia	2016
SHV-64	NG_050099	*Klebsiella pneumoniae*		China	2016
SHV-65	NG_050100	*Klebsiella pneumoniae*		China	2016
SHV-66	NG_050101	*Klebsiella pneumoniae*		China	2016
SHV-67	NG_050102	*Klebsiella pneumoniae*		China	2016
SHV-69	NG_050103	*Klebsiella pneumoniae*		China	2016
SHV-70	NG_050105	*Enterobacter cloacae*	Plasmid	China	2016
SHV-71	NG_050106	*Klebsiella pneumoniae*		Portugal	2016
SHV-72	NG_050107	*Klebsiella pneumoniae*		Portugal	2016
SHV-73	NG_050108	*Klebsiella pneumoniae*		Portugal	2016
SHV-74	NG_050109	*Klebsiella pneumoniae*		Portugal	2016
SHV-75	NG_050110	*Klebsiella pneumoniae*		Portugal	2016
SHV-76	NG_050111	*Klebsiella pneumoniae*		Portugal	2016
SHV-77	NG_050112	*Klebsiella pneumoniae*		Portugal	2016
SHV-78	NG_050113	*Klebsiella pneumoniae*		Portugal	2016
SHV-79	NG_050114	*Klebsiella pneumoniae*		Portugal	2016
SHV-80	NG_050116	*Klebsiella pneumoniae*		Portugal	2016
SHV-81	NG_050117	*Klebsiella pneumoniae*		Portugal	2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-82	NG_050118	*Klebsiella pneumoniae*		Portugal	2016
SHV-85	NG_050119	*Klebsiella pneumoniae*		Brazil	2016
SHV-86	NG_050120	*Klebsiella pneumoniae*		Colombia	2016
SHV-89	NG_050121	*Klebsiella pneumoniae*	Plasmid	China	2016
SHV-92	NG_050123	*Klebsiella pneumoniae*	Plasmid	Spain	2016
SHV-93	NG_050124	*Klebsiella pneumoniae*		China	2016
SHV-94	NG_050125	*Klebsiella pneumoniae*		China	2016
SHV-95	NG_050126	*Citrobacter freundii*		Shanghai	2016
SHV-96	NG_050127	*Acinetobacter baumannii*		Shanghai	2016
SHV-97	NG_050128	*Enterococcus faecalis*		China	2016
SHV-98	NG_050129	*Klebsiella pneumoniae*		Algeria	2016
SHV-99	NG_050130	*Klebsiella pneumoniae*		Algeria	2016
SHV-100	NG_049990	*Klebsiella pneumoniae*		Algeria	2016
SHV-101	NG_049991	*Klebsiella pneumoniae*		France	2016
SHV-102	NG_049992	*Escherichia coli*		Spain	2016
SHV-103	NG_049993	*Klebsiella pneumoniae*		Tunisia	2016
SHV-104	NG_049994	*Klebsiella pneumoniae*	Plasmid		2016
SHV-105	NG_049995	*Klebsiella pneumoniae*			2016
SHV-106	NG_049996	*Klebsiella pneumoniae*		Portugal	2016
SHV-107	NG_049997	*Klebsiella pneumoniae*		Portugal	2016
SHV-108	NG_049998	*Klebsiella pneumoniae*		Portugal	2016
SHV-109	NG_049999	*Klebsiella pneumoniae*		Australia	2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-110	NG_050001	*Klebsiella pneumoniae*		Brazil	2016
SHV-111	NG_050002	*Klebsiella pneumoniae*		Egypt	2016
SHV-119	NG_050003	*Klebsiella pneumoniae*		China	2016
SHV-120	NG_050004	*Escherichia coli*	Plasmid		2016
SHV-121	NG_050005	*Klebsiella pneumoniae*		Germany	2016
SHV-128	NG_050006	*Enterobacter cloacae*	Plasmid		2016
SHV-129	NG_050007	*Escherichia coli*	Plasmid		2016
SHV-132	NG_050009	*Klebsiella pneumoniae*			2016
SHV-133	NG_050010	*Klebsiella pneumoniae*			2016
SHV-134	NG_050011	*Klebsiella pneumoniae*			2016
SHV-135	NG_050012	*Escherichia coli*		China	2016
SHV-137	NG_050013	*Klebsiella pneumoniae*		China	2016
SHV-141	NG_050015	*Klebsiella pneumoniae*		India	2016
SHV-142	NG_050016	*Klebsiella pneumoniae*		China	2016
SHV-143	NG_050017	*Klebsiella pneumoniae*		Taiwan	2016
SHV-144	NG_050018	*Klebsiella pneumoniae*		Malaysia	2016
SHV-145	NG_050019	*Klebsiella pneumoniae*		Portugal	2016
SHV-146	NG_050020	*Klebsiella pneumoniae*		China	2016
SHV-147	NG_050021	*Klebsiella pneumoniae*		USA	2016
SHV-148	NG_050022	*Klebsiella pneumoniae*		USA	2016
SHV-149	NG_050023	*Klebsiella pneumoniae*		USA	2016
SHV-150	NG_050025	*Klebsiella pneumoniae*		USA	2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-151	NG_050026	*Klebsiella pneumoniae*		USA	2016
SHV-152	NG_050027	*Klebsiella pneumoniae*		USA	2016
SHV-153	NG_050028	*Klebsiella pneumoniae*		USA	2016
SHV-154	NG_050029	*Klebsiella pneumoniae*		USA	2016
SHV-155	NG_050030	*Klebsiella pneumoniae*		USA	2016
SHV-156	NG_050031	*Klebsiella pneumoniae*		USA	2016
SHV-157	NG_050032	*Klebsiella pneumoniae*		USA	2016
SHV-158	NG_050033	*Klebsiella pneumoniae*		USA	2016
SHV-159	NG_050034	*Klebsiella pneumoniae*		USA	2016
SHV-160	NG_050036	*Klebsiella pneumoniae*		USA	2016
SHV-161	NG_050037	*Klebsiella pneumoniae*		USA	2016
SHV-162	NG_050038	*Klebsiella pneumoniae*		USA	2016
SHV-163	NG_050039	*Klebsiella pneumoniae*		USA	2016
SHV-164	NG_050040	*Klebsiella pneumoniae*			2016
SHV-165	NG_050041	*Klebsiella pneumoniae*		USA	2016
SHV-168	NG_050042	*Klebsiella pneumoniae*		Canada	2016
SHV-172	NG_050043	*Klebsiella pneumoniae*			2016
SHV-173	NG_050044	*Klebsiella pneumoniae*			2016
SHV-178	NG_050045	*Klebsiella pneumoniae*			2016
SHV-179	NG_050046	*Klebsiella pneumoniae*			2016
SHV-180	NG_050048	*Klebsiella pneumoniae*			2016
SHV-182	NG_050049	*Klebsiella pneumoniae*			2016

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-183	NG_050050	*Enterobacter cloacae*		Slovenia	2016
SHV-185	NG_050051	*Klebsiella pneumoniae*		India	2016
SHV-186	NG_050052	*Klebsiella pneumoniae*			2016
SHV-187	NG_050053	*Klebsiella pneumoniae*			2016
SHV-188	NG_050054	*Klebsiella pneumoniae*			2016
SHV-189	NG_050055	*Klebsiella pneumoniae*			2016
SHV-190	NG_050056	*Klebsiella pneumoniae*		China	2016
SHV-191	NG_050057	*Klebsiella pneumoniae*		China	2016
SHV-193	NG_050058	*Klebsiella pneumoniae*		China	2016
SHV-194	NG_051169	*Klebsiella pneumoniae*		Portugal	2016
SHV-195	NG_051484	*Klebsiella pneumoniae*			2016
SHV-196	NG_051521	*Klebsiella pneumoniae*			2016
SHV-197	NG_052582	*Escherichia coli*		China	2017
SHV-198	NG_055284	*Klebsiella pneumoniae*		Turkey	2017
SHV-199	NG_055503	*Escherichia coli*		DR of Congo	2017
SHV-200	NG_055588	*Klebsiella pneumoniae*	Plasmid		2017
SHV-201	NG_062244	*Klebsiella pneumoniae*		Bangladesh	2018
SHV-202	NG_062245	*Klebsiella pneumoniae*		Bangladesh	2018
SHV-203	NG_055668	*Klebsiella pneumoniae*		Canada	2017
SHV-204	NG_057611	*Klebsiella pneumoniae*		Canada	2018
SHV-205	NG_062276	*Klebsiella pneumoniae*		Mexico	2018
SHV-206	NG_062278	*Klebsiella pneumoniae*	Plasmid	Indonesia	2018

(continued)

Table 4.5 (continued)

SHV variants	Accession number	Organisms	Plasmid origin	Country of origin (based on GenBank data)	Year of submission in GenBank
SHV-207	NG_062279	*Klebsiella pneumoniae*	Plasmid	France	2018
SHV-208	NG_062280	*Klebsiella pneumoniae*	Plasmid	Taiwan	2018
SHV-209	NG_062281	*Klebsiella pneumoniae*	Plasmid	France	2018
SHV-210	NG_062282	*Klebsiella pneumoniae*	Plasmid		2018
SHV-211	NG_062283	*Klebsiella pneumoniae*	Plasmid	France	2018
SHV-212	NG_062284	*Klebsiella pneumoniae*	Plasmid	Madagascar	2018
SHV-213	NG_062285	*Klebsiella pneumoniae*	Plasmid	Madagascar	2018
SHV-214	NG_062286	*Klebsiella pneumoniae*	Plasmid	Madagascar	2018
SHV-215	NG_062287	*Klebsiella pneumoniae*	Plasmid	Madagascar	2018
SHV-216	NG_062288	*Klebsiella pneumoniae*	Plasmid	Cambodia	2018
SHV-217	NG_062289	*Klebsiella pneumoniae*	Plasmid	Cambodia	2018
SHV-218	NG_062290	*Klebsiella pneumoniae*	Plasmid	Cambodia	2018
SHV-219	NG_062291	*Klebsiella pneumoniae*	Plasmid	Cambodia	2018
SHV-220	NG_062292	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-221	NG_062293	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-222	NG_062294	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-223	NG_062295	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-224	NG_062296	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-225	NG_062297	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-226	NG_062298	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-227	NG_062299	*Klebsiella pneumoniae*	Plasmid	Senegal	2018
SHV-228	NG_062300	*Klebsiella pneumoniae*	Plasmid	Senegal	2018

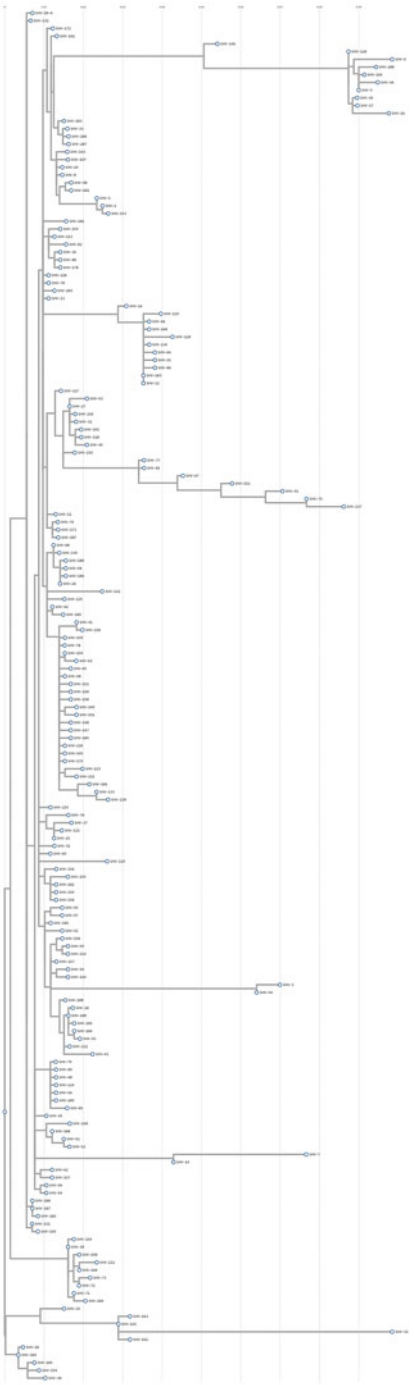


Fig. 4.2 Phylogenetic relation of SHV beta-lactamases

name Pitton) and was also detected in other *Enterobacteriaceae* as a plasmid-mediated β-lactamases (Matthew 1979; Pitton 1972). In 1983, the first plasmid-mediated resistance mechanism against oxyimino-cephalosporins appeared in clinical isolates of *S. marcescens, Klebsiella ozaenae,* and *K. pneumoniae* (Knothe et al. 1983). Many of *K. pneumoniae* strains are equipped with a chromosomal copy of either $bla_{\text{SHV-1}}$ or $bla_{\text{SHV-11}}$ or close relatives, which encode for non-extended-spectrum enzymes (Chaves et al. 2001; Lee et al. 2006). It has been suggested that bla_{SHV} genes originated from the chromosome of *K. pneumoniae* and descended to plasmid by IS*26*-mediated mobilization events (Ford and Avison 2004). Some studies have reported the presence of $bla_{\text{SHV-5}}$ between two IS26 elements together with the sequences identical to part of the *K. pneumoniae* chromosome (Preston et al. 2004; Miriagou et al. 2005). Plasmid-mediated SHV-2a, SHV-11, and SHV-12 bear evidence to IS*26* insertion into the bla_{SHV} promoter in the plasmid (Podbielski et al. 1991; Nuesch-Inderbinen et al. 1997). Some studies have shown that promoter strength is increased by this IS*26* insertion through the introduction of a different 35 regions (Podbielski et al. 1991). SHV type enzymes probably owe its origin to the antibiotic era from a common ancestor, and these descendants include both penicillinases and extended-spectrum β-lactamases (Hall and Barlow 2004). Sequence identity of SHV shared with TEM enzymes is about 67%. In fact, substrate binding cavity of SHV-1 is just 0.7–1.2 Å larger than in TEM-1 (Kuzin et al. 1999; Reynolds et al. 2006). SHV-type β-lactamases can have amino acid substitutions which can render an increased resistance level to both narrow and extended-spectrum cephalosporins and to monobactams and β-lactamase inhibitors too. Amino acid substitutions in residues Asp179, Gly238, and Glu240 have been identified as responsible for third generation cephalosporins resistance in clinical strains. Residue Asp179, associated to the ESBL phenotype, is found to be located in the Ω loop of the SHV active site. Probably, the movement of the Ω loop is increased by amino acid substitution of Asp179 by Ala (SHV-6), Asn (SHV-8), and Gly (SHV-24) (Knothe et al. 1983), which confer high resistance to ceftazidime but not to cefotaxime in the enzymes isolated in Japan (SHV-24), France (SHV-6), and the United States (SHV-8) (Arlet et al. 1997; Rasheed et al. 1997; Kurowaka et al. 2000). SHV β-lactamase substitutions in the residue Gly238 play a significant part in conferring high-level resistance against extended-spectrum cephalosporins. It is by the substitution Gly238Ser that pushes the β-strand out and away from the reactive Ser70, which thus expands the active site to permit greater substrate versatility against penicillins and cephalosporins (Hujer et al. 2001). Higher resistance to cefotaxime than to ceftazidime is conferred by enzymes like SHV-2, SHV-2A, and SHV-3 (Barthelemy et al. 1988; Podbielski et al. 1991). It is by the substitution of residue Glu240 that increases the hydrolyzing activity against ceftazidime. In fact, enzymes such as SHV-5 and SHV-12 show increased resistance to ceftazidime by holding at least both Gly238Ser and Glu240Lys substitutions (Gutmann et al. 1995; Nuesch-Inderbinen et al. 1997). In SHV-26, an amino acid substitution, Ala187Thr, has also been shown to have low level resistance to β-lactamase inhibitors (Chang et al. 2001). Enzymes like SHV-2, SHV-2A, SHV-5, and SHV-12 were detected in strains of *Pseudomonadaceae*,

Moraxelleceae, and *Enterobacteriaceae* isolated in hospitals, in the community settings, animals, and food samples from Africa, Asia, Europe, and Oceania (Paterson and Bonomo 2005; Damjanoba et al. 2007; Jouini et al. 2007; Chiaretto et al. 2008).

4.6 CTX-M-Type β-Lactamases: Brief Description

Recent years have seen the emergence of a novel family of plasmid-mediated ESBLs, CTX-M-type ESBLs. CTX-M-type ESBLs form a distinct lineage of molecular class A β-lactamases and are a quickly expanding group. A clinical isolate of *E. coli* was found in 1989, to produce a non-TEM, non-SHV ESBL and was designated as CTX-M-1 due to its hydrolytic activity against cefotaxime (Bauernfeind et al. 1990). Quintessential characteristic of all CTX-M producing isolates is the higher resistance to cefotaxime in comparison to ceftazidime (Bonnet 2004; Chen et al. 2005). Chromosomal genes present in the members of the genus *Kluyvera* is the source of CTX-M determinants. The genus *Kluyvera* includes several environmental species with few to non-pathogenic activity against humans. CTX-M-type enzymes were found in *Salmonella enterica* serovar, *S. typhimurium*, and *E. coli* strains mainly although this group was also found even in other *Enterobacteriaceae* species (Bradford 2001). Recently, this group of ESBLs has emerged as the predominant type in many parts of the world, including Canada, Europe, South America, India, and parts of the United States (Lewis et al. 2007; Livermore et al. 2007; Pitout et al. 2007; Rossolini et al. 2008; Shahid et al. 2011).

4.7 Classification and Origins of CTX-M ß-Lactamases

The CTX-M ß-lactamases are now exceedingly over 245 different variants (https://www.genome.jp/kegg/annotation/br01553.html). Table 4.6 shows the variants of CTX-M which were updated from the NCBI based on the data available till March 2021. On the basis of their amino acid identities, they can be classified into five genogroups (Bonnet 2004): the CTX-M-genogroup-1 includes CTX-M-1, -3, -10, -11, -12, -15 (UOE-1), -22, -23, -28, -30, -28, -29, -30, -32, -33, -36, -54; the CTX-M-genogroup-2 includes CTX-M-2, -4, -5, -6, -7, -20, -31, -44 (previously Toho-1), and FEC-1; the CTX-M-genogroup-8 includes CTX-M-8, -40; the CTX-M-genogroup-9 includes CTX-M-9, -13, -14 (UOE-2 and Toho-3), -16, -17,-18, -19, -21, -24, -27, -45 (formerly Toho-2), -46, -47, -48, -49, -50; and the CTX-M-genogroup-25 with CTX-M-25, -26, -25, -39, -41. CTX-M-14 was later found identical to CTX-M-18 (Shahid et al. 2011). But lately CTX-M-45 is being considered as a distinct group of CTX-M type β-lactamases (Rossolini et al. 2008). Studies suggest that β-lactamases of CTX-M genogroups are in fact structurally related to the naturally produced β-lactamases present in various *Kluyvera* species (Decousser et al. 2001; Humeniuk et al. 2002; Poirel et al. 2002; Olson et al. 2005;

Table 4.6 Different variants of CTX searched from the data available in GenBank with details of their accession number, source organism, and country

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-1	X92506	*Escherichia coli*	Muenchen FRG	18-Apr-2005
CTX-M-2	X92507	*Salmonella typhimurium*	Muenchen FRG	18-Apr-2005
CTX-M-3	Y10278	*Citrobacter freundii*	Poland	18-Apr-2005
CTX-M-4	Y14156	*Salmonella typhimurium*	Greece	18-Apr-2005
CTX-M-5	U95364	*Salmonella typhimurium*	Latvia, USA	26-Aug-1998
CTX-M-6	AJ005044	*Salmonella typhimurium*	Greece	15-Apr-2005
CTX-M-7	AJ005045	*Salmonella typhimurium*	Greece	15-Apr-2005
CTX-M-8	AF189721	*Citrobacter amalonaticus*	Brazil	7-Jul-2000
CTX-M-9	AF174129	*Escherichia coli*	Spain	1-Aug-2002
CTX-M-10	AF255298	*Escherichia coli*	Spain	1-Mar-2001
CTX-M-11	AY005110	*Klebsiella pneumoniae*	China	1-Aug-2000
CTX-M-12	AF305837	*Klebsiella pneumoniae*	Kenya	19-June-2001
CTX-M-13	AF252623	*Klebsiella pneumoniae*	China	2-May-2003
CTX-M-14	AF252622	*Escherichia coli*	China	22-Dec-2006
CTX-M-15	AY044436	*Escherichia coli*	India	11-Jan-2007
CTX-M-16	AY029068	*Escherichia coli*	France	3-Oct-2001
CTX-M-17	AY033516	*Klebsiella pneumoniae*	France	30-Sep-2003
CTX-M-18	AF325133	*Klebsiella pneumoniae*	France	21-Nov-2001
CTX-M-19	AF325134	*Klebsiella pneumoniae*	France	21-Nov-2001
CTX-M-20	AJ416344	*Proteus mirabilis*	France	15-Apr-2005
CTX-M-21	AJ416346	*Escherichia coli*	France	15-Apr-2005
CTX-M-22	AY080894	*Klebsiella pneumoniae*	China	4-Jan-2007
CTX-M-23	AF488377	*Escherichia coli*	Germany	9-Aug-2004
CTX-M-24	AY143430	*Klebsiella pneumoniae*	China	4-Jan-2007
CTX-M-25	AF518567	*Escherichia coli*	Canada	29-Nov-2004
CTX-M-26	AY157676	*Klebsiella pneumoniae*	UK	7-Mar-2003
CTX-M-27	AY156923	*Escherichia coli*	France	1-Jul-2003
CTX-M-28	AJ549244	*Escherichia coli*	France	15-Apr-2005
CTX-M-29	AY267213	*Escherichia coli*	China	4-Jan-2007
CTX-M-30	AY292654	*Citrobacter freundii*	Canada	27-Oct-2004
CTX-M-31	AJ567481	*Providencia* sp.	Argentina	15-Apr-2005
CTX-M-32	AJ557142	*Escherichia coli*	Spain	15-Apr-2005
CTX-M-33	AY238472	*Escherichia coli*	Greece	14-May-2007
CTX-M-34	AY515297	*Escherichia coli*	China	12-Dec-2005
CTX-M-35	AB176534	*Klebsiella oxytoca*	Canada	6-Jan-2005

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-36	AB177384	*Escherichia coli*	Argentina	17-Apr-2004
CTX-M-37	AY649755	*Enterobacter cloacae*	Mongolia	26-Jul-2004
CTX-M-38	AY822595	*Klebsiella pneumoniae*	China	29-Nov-2004
CTX-M-39	AY954516	*Escherichia coli*	Israel	27-Oct-2005
CTX-M-40	AY750914	*Escherichia coli*	UK	13-Jun-2006
CTX-M-41	DQ023162	*Proteus mirabilis*	Israel	22-May-2005
CTX-M-42	DQ061159	*Escherichia coli*	Russia	25-Jun-2005
CTX-M-43	DQ102702	*Acinetobacter baumannii*	Bolivia	16-May-2006
CTX-M-44	D37830	*Escherichia coli*	Japan	18-Jun-1999
CTX-M-45	D89862	*Escherichia coli*	Japan	20-May-1998
CTX-M-46	AY847147	*Klebsiella pneumoniae*	China	18-Dec-2004
CTX-M-47	AY847143	*Escherichia coli*	China	18-Dec-2004
CTX-M-48	AY847144	*Klebsiella pneumoniae*	China	18-Dec-2004
CTX-M-49	AY847145	*Klebsiella pneumoniae*	China	18-Dec-2004
CTX-M-50	AY847146	*Klebsiella pneumoniae*	China	18-Dec-2004
CTX-M-51	DQ211987	*Escherichia coli*	Spain	11-Oct-2005
CTX-M-52	DQ223685	*Klebsiella pneumoniae*	Beijing PRC	29-Oct-2005
CTX-M-53	DQ268764	*Salmonella enterica*	France	19-Nov-2005
CTX-M-54	DQ303459	*Klebsiella pneumoniae*	Korea	26-July-2006
CTX-M-55	DQ885477	*Escherichia coli*	Thailand	30-Oct-2007
CTX-M-56	EF374097	*Escherichia coli*	Latin America	7-Aug-2007
CTX-M-57	DQ810789	*Salmonella enterica*	UK	4-Jan-2008
CTX-M-58	EF210159	*Escherichia coli*	Germany	3-Feb-2007
CTX-M-59	DQ408762	*Klebsiella pneumoniae*	Brazil	30-Apr-2008
CTX-M-60	AM411407	*Klebsiella pneumoniae*	Colombia	8-Jan-2007
CTX-M-61	EF219142	*Salmonella typhimurium*	France	6-Feb-2007
CTX-M-62	EF219134	*Klebsiella pneumoniae*	Australia	24-Jan-2008
CTX-M-63	AB205197	*Klebsiella pneumoniae*	Japan	2-Nov-2006
CTX-M-64	AB284167	*Shigella sonnei*	Japan	20-Jan-2009
CTX-M-65	EU213262	*Escherichia coli*	USA	3-Mar-2008
CTX-M-66	EF576988	*Proteus mirabilis*	Taiwan	10-Apr-2008
CTX-M-67	EF581888	*Escherichia coli*	Spain	2-Dec-2008
CTX-M-68	EU177100	*Klebsiella* sp. ARS06-441	New Zealand	7-Oct-2007
CTX-M-69	EU402393	*Escherichia coli*	China	9-Feb-2008
CTX-M-70	Not assigned			
CTX-M-71	FJ815436	*Klebsiella pneumoniae*	Bulgaria	24-Sep-2009
CTX-M-72	AY847148	*Klebsiella pneumoniae*	China	10-Jul-2009

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-73	Not assigned			
CTX-M-74	GQ149243	*Enterobacter cloacae*	Brazil	29-Oct-2009
CTX-M-75	GQ149244	*Providencia stuartii*	Brazil	29-Oct-2009
CTX-M-76	AM982520	*Kluyvera ascorbata*	Argentina	29-Jun-2008
CTX-M-77	AM982521	*Kluyvera ascorbata*	Argentina	29-Jun-2008
CTX-M-78	AM982522	*Kluyvera ascorbata*	Argentina	7-Oct-2008
CTX-M-79	EF426798	*Escherichia coli*	China	7-Oct-2008
CTX-M-80	EU202673	*Klebsiella pneumoniae*	China	11-Aug-2008
CTX-M-81	EU136061	*Klebsiella pneumoniae*	Southern China	3-Nov-2008
CTX-M-82	DQ256091	*Escherichia coli*	China	17-Apr-2009
CTX-M-83	FJ214366	*Salmonella enterica*	China	15-Dec-2008
CTX-M-84	FJ214367	*Salmonella enterica*	China	15-Dec-2008
CTX-M-85	FJ214368	*Salmonella enterica*	China	15-Dec-2008
CTX-M-86	FJ214369	*Salmonella enterica*	China	15-Dec-2008
CTX-M-87	EU545409	*Escherichia coli*	China	11-May-2009
CTX-M-88	FJ873739	*Salmonella enteric*	Iran	25-Apr-2009
CTX-M-89	FJ971899	*Proteus mirabilis*	Pennsylvania, USA	1-Sep-2009
CTX-M-90	FJ907381	*Salmonella sp.*	China	22-Jul-2009
CTX-M-91	GQ870432	*Proteus mirabilis*	(NCBI) USA[a]	6-Oct-2009
CTX-M-92	GU127598	*Escherichia coli*	Lithuania	15-Nov-2009
CTX-M-93	Not assigned			
CTX-M-94	HM167760	*Escherichia coli*	Belgium	14-Jul-2010
CTX-M-95	FN813245	*Kluyvera ascorbata*	Argentina	23-Oct-2010
CTX-M-96	AJ704396	*Klebsiella pneumoniae*	Argentina	15-Apr-2005
CTX-M-97	HM776707	*Escherichia coli*	Israel	21-Aug-2010
CTX-M-98	HM755448	*Escherichia coli*	China	11-Oct-2010
CTX-M-99	HM803271	*Klebsiella pneumoniae*	France	11-Aug-2010
CTX-M-100	Not assigned			
CTX-M-101	HQ398214	*Escherichia coli*	China	27-Feb-2011
CTX-M-102	HQ398215	*Escherichia coli*	China	27-Feb-2011
CTX-M-103	Not assigned			
CTX-M-104	HQ833652	*Escherichia coli*	South China	27-Feb-2011
CTX-M-105	HQ833651	*Escherichia coli*	South China	27-Feb-2011
CTX-M-106	HQ\\913565	*Escherichia coli*	China	14-Mar-2011
CTX-M-107	JF274244	*Shigella* sp.	China	27-Oct-2011
CTX-M-108	JF274245	*Shigella* sp.	China	27-Oct-2011
CTX-M-109	JF274248	*Shigella* sp.	China	27-Oct-2011

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-110	JF274242	*Shigella* sp.	China	27-Oct-2011
CTX-M-111	JF274243	*Shigella* sp.	China	27-Oct-2011
CTX-M-112	JF274246	*Shigella* sp.	China	27-Oct-2011
CTX-M-113	JF274247	*Shigella* sp.	China	27-Oct-2011
CTX-M-114	GQ351346	*Providencia rettgeri*	Korea	6-May-2011
CTX-M-115	KJ911020	*Acinetobacter baumannii*	Russia	26-Jul-2016
CTX-M-116	JF966749	*Proteus mirabilis*	Russia	25-Jul-2016
CTX-M-117	JN227085	*Escherichia coli*	Switzerland	25-Jul-2016
CTX-M-118	Not assigned			
CTX-M-119	Not assigned			
CTX-M-120	Not assigned			
CTX-M-121	JN790862	*Escherichia coli*	China	26-Jul-2016
CTX-M-122	JN790863	*Escherichia coli*	China	26-Jul-2016
CTX-M-123	JN790864	*Escherichia coli*	China	26-Jul-2016
CTX-M-124	JQ429324	*Escherichia coli*	France	22-Apr-2012
CTX-M-125	JQ724542	*Enterobacter cloacae*	China	26-Jul-2016
CTX-M-126	AB703103	*Escherichia coli*	Japan	25-Apr-2014
CTX-M-127	MF196229	*Escherichia coli*	Denmark	22-Jun-2017
CTX-M-128	Not assigned			
CTX-M-129	JX017364	*Escherichia coli*	China	26-Jul-2016
CTX-M-130	JX017365	*Escherichia coli*	China	26-Jul-2016
CTX-M-131	JN969893	*Providencia rettgeri*	Brazil	22-May-2015
CTX-M-132	JX313020	*Escherichia coli*	China	16-Jul-2014
CTX-M-133	Not assigned			
CTX-M-134	JX896165	*Escherichia coli*	IHMA, USA[a]	20-Nov-2012
CTX-M-135	Not assigned			
CTX-M-136	KC351754	*Proteus mirabilis*	Russia	26-Jul-2016
CTX-M-137	KF790923	*Escherichia coli*	China	12-Aug-2014
CTX-M-138	KF526119	*Escherichia coli*	China	16-Jul-2014
CTX-M-139	NG_048928	*Escherichia coli*	France	8-Jun-2016
CTX-M-140	NG_068166	*Proteus mirabilis*	China	21-Apr-2020
CTX-M-141	KC964871	*Klebsiella pneumonia*	Brazil	5-Jun-2013
CTX-M-142	KF240809	*Escherichia coli*	India	25-Sep-2013
CTX-M-143	MN715319	*Escherichia coli*	Jordan	27-Nov-2019
CTX-M-144	KJ020573	*Escherichia coli*	China	26-Jul-2016

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-145	Not assigned			
CTX-M-146	KY938173	*Escherichia coli*	Germany	30-Dec-2017
CTX-M-147	KF513180	*Klebsiella pneumonia*	IHMA, USA[a]	24-Nov-2013
CTX-M-148	KJ020574	*Escherichia coli*	South China	26-Jul-2016
CTX-M-149	Not assigned			
CTX-M-150	NG_048936	*Escherichia coli*	France	8-Jun-2016
CTX-M-151	AB916359	*Salmonella enterica*	Japan	23-Aug-2014
CTX-M-152	KJ461948	*Kluyvera* sp.	India	4-May-2014
CTX-M-153	MT156338	*Escherichia coli*	China	21-Mar-2020
CTX-M-154	MN752689	*Klebsiella pneumoniae*	Denmark	10-Dec-2019
CTX-M-155	KM211508	*Klebsiella pneumoniae*	India	12-Nov-2014
CTX-M-156	KM211509	*Klebsiella pneumoniae*	India	12-Nov-2014
CTX-M-157	KM211510	*Klebsiella pneumonia*	India	12-Nov-2014
CTX-M-158	KM211691	*Escherichia coli*	Germany	10-Feb-2015
CTX-M-159	NG_048943	*Klebsiella pneumonia*	Japan	8-Jun-2016
CTX-M-160	KP050493	*Proteus mirabilis*	IHMA, USA[a]	14-Dec-2015
CTX-M-161	NG_048946	*Escherichia coli*	NCBI, USA[a]	8-Jun-2016
CTX-M-162	NG_048947	*Klebsiella oxytoca*	Russia	8-Jun-2016
CTX-M-163	KP681698	*Escherichia coli*	Russia	23-Nov-2016
CTX-M-164	KP727571	*Proteus mirabilis*	Russia	23-Nov-2016
CTX-M-165	KP727572	*Klebsiella pneumonia*	Chile	23-Nov-2016
CTX-M-166	LN830266	*Escherichia coli*	Portugal	12-May-2015
CTX-M-167	KR537428	*Proteus mirabilis*	Lithuania	23-Nov-2016
CTX-M-168	KR537429	*Escherichia coli*	Viet Nam	23-Nov-2016
CTX-M-170	NG_048956	*Escherichia coli*	Russia	8-Jun-2016
CTX-M-171	NG_051164	*Proteus mirabilis*	Chile	4-Aug-2016
CTX-M-172	NG_048957	*Escherichia coli*	NCBI, USA[a]	8-Jun-2016
CTX-M-173	NG_048958	*Klebsiella pneumoniae*	Kuwait	8-Jun-2016
CTX-M-174	NG_048959	*Escherichia coli*	Viet Nam	8-Jun-2016
CTX-M-175	NG_048960	*Escherichia coli*	Poland	8-Jun-2016
CTX-M-176	NG_048961	*Klebsiella pneumoniae*	Italy	8-Jun-2016
CTX-M-177	NG_048962	*Enterobacter cloacae*	Chile	8-Jun-2016
CTX-M-178	NG_056408	*Escherichia coli*	China	26-Jun-2018
CTX-M-179	NG_048963	*Escherichia coli*	South Korea	8-Jun-2016
CTX-M-180	NG_048964	*Escherichia coli*	Guatemala	8-Jun-2016
CTX-M-181	KX056900	*Escherichia coli*	USA	8-Jun-2016
CTX-M-182	NG_048966	*Escherichia coli*	Thailand	8-Jun-2016
CTX-M-183	NG_050812	*Klebsiella pneumoniae*	Lithuania	9-Nov-2018
CTX-M-184	NG_050813	*Escherichia coli*	NCBI, USA[a]	9-Nov-2018

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-185	NG_050944	*Kluyvera ascorbata*	NCBI, USA[a]	4-Aug-2016
CTX-M-186	NG_051165	*Escherichia coli*	Pakistan	9-Nov-2018
CTX-M-187	NG_056409	*Enterobacter cloacae*	China	26-Jun-2018
CTX-M-188	NG_051467	*Escherichia coli*	Canada	19-Sep-2016
CTX-M-189	NG_051468	*Escherichia coli*	NCBI, USA[a]	19-Sep-2016
CTX-M-190	NG_051509	*Escherichia coli*	China	19-Sep-2016
CTX-M-191	NG_056165	*Escherichia coli*	NCBI, USA[a]	25-Jan-2018
CTX-M-192	NG_056166	*Escherichia coli*	NCBI, USA[a]	25-Jan-2018
CTX-M-193	NG_052899	*Escherichia coli*	USA	23-Feb-2017
CTX-M-194	NG_052900	*Escherichia coli*	Thailand	23-Feb-2017
CTX-M-195	NG_052901	*Escherichia coli*	France	23-Feb-2017
CTX-M-196	NG_052902	*Escherichia coli*	Austria	23-Feb-2017
CTX-M-197	NG_054686	*Klebsiella pneumoniae*	Czech Republic	13-Jun-2017
CTX-M-198	NG_054687	*Escherichia coli*	Russia	13-Jun-2017
CTX-M-199	NG_054961	*Escherichia coli*	China	13-Jun-2017
CTX-M-200	NG_054961	*Escherichia coli*	Ecuador	10-Aug-2017
CTX-M-201	NG_055501	*Klebsiella pneumoniae*	Taiwan	10-Aug-2017
CTX-M-202	NG_055502	*Klebsiella pneumoniae*	Canada	10-Aug-2017
CTX-M-203	NG_055272	*Escherichia coli*	Denmark	10-Aug-2017
CTX-M-204	NG_055283	*Klebsiella pneumoniae*	Germany	10-Aug-2017
CTX-M-205	NG_055667	*Kluyvera georgiana*	Canada	21-Aug-2017
CTX-M-206	NG_056171	*Pseudomonas aeruginosa*	France	25-Jan-2018
CTX-M-207	NG_056173	*Escherichia coli*	Japan	25-Jan-2018
CTX-M-208	NG_057474	*Klebsiella pneumoniae*	France	26-Jun-2018
CTX-M-209	NG_057475	*Klebsiella pneumoniae*	Kuwait	26-Jun-2018
CTX-M-210	NG_057476	*Klebsiella pneumoniae*	Poland	26-Jun-2018
CTX-M-211	NG_057477	*Escherichia coli*	Poland	26-Jun-2018
CTX-M-212	NG_057478	*Proteus mirabilis*	Chile	26-Jun-2018
CTX-M-213	NG_057473	*Kluyvera ascorbata*	Spain	26-Jun-2018
CTX-M-214	NG_057483	*Escherichia coli*	UK	26-Jun-2018
CTX-M-215	NG_063838	*Escherichia coli*	China	17-Jan-2019
CTX-M-216	NG_057608	*Escherichia coli*	Thailand	26-Jun-2018
CTX-M-217	NG_057610	*Providencia stuartii*	Brazil	26-Jun-2018
CTX-M-218	NG_057613	*Escherichia coli*	Mexico	26-Jun-2018
CTX-M-219	NG_059336	*Klebsiella pneumoniae*	Germany	26-Jun-2018
CTX-M-220	NG_060560	*Klebsiella pneumoniae*	Slovenia	26-Jun-2018
CTX-M-221	NG_061413	*Serratia marcescens*	Poland	1-Aug-2018
CTX-M-222	NG_061609	*Escherichia coli*	Netherlands	23-Aug-2018
CTX-M-223	NG_062275	*Escherichia coli*	Canada	7-Nov-2018

(continued)

Table 4.6 (continued)

CTX-M type	Accession no.	Source organism	Country of publication/ origin	Publication date
CTX-M-224	NG_062241	*Enterobacter kobei*	Brazil	7-Nov-2018
CTX-M-225	NG_064720	*Escherichia coli*	India	1-May-2019
CTX-M-226	NG_064721	*Escherichia coli*	China	1-May-2019
CTX-M-227	NG_065865	*Escherichia coli*	Mexico	26-Aug-2019
CTX-M-228	NG_065866	*Enterobacter cloacae*	NCBI, USA[a]	26-Aug-2019
CTX-M-229	NG_065867	*Pseudomonas aeruginosa*	Brazil	26-Aug-2019
CTX-M-230	NG_067139	*Escherichia coli*	Mexico	12-Dec-2019
CTX-M-231	NG_067140	*Klebsiella pneumoniae*	Netherlands	12-Dec-2019
CTX-M-232	NG_067141	*Escherichia coli*	Canada	12-Dec-2019
CTX-M-233	NG_067142	*Escherichia coli*	South Korea	12-Dec-2019
CTX-M-234	NG_068168	*Escherichia coli*	China	21-Apr-2020
CTX-M-235	NG_068169	*Escherichia coli*	Netherlands	21-Apr-2020
CTX-M-236	NG_068506	*Klebsiella pneumoniae*	Taiwan	21-Apr-2020
CTX-M-237	NG_070730	*Klebsiella pneumoniae*	Italy	21-Sep-2020
CTX-M-238	NG_070731	*Escherichia coli*	Japan	21-Sep-2020
CTX-M-239	NG_070732	*Escherichia coli*	Portugal	21-Sep-2020
CTX-M-240	NG_070733	*Escherichia coli*	Russia	21-Sep-2020
CTX-M-241	NG_070734	*Escherichia coli*	USA	21-Sep-2020
CTX-M-242	NG_070784	*Escherichia coli*	Denmark	9-Nov-2020
CTX-M-243	Not assigned			
CTX-M-244	NG_073460	*Escherichia coli*	Denmark	24-Feb-2021
CTX-M-245	MN928785	*Serratia marcescens*	Iraq	9-Mar-2021

[a] Submitted to: International Health Management Associates (IHMA); National Centre for Biotechnology Information (NCBI)

Pitout et al. 2005). CTX-M-genogroup-1 ß-lactamases are closely related to the ß-lactamases of *Kluyvera cryocrescens* (Decousser et al. 2001) while CTX-M-genogroup-2 enzymes show structural relationship with the naturally produced ß-lactamase of *Kluyvera ascorbata* (Humeniuk et al. 2002). An enzyme identical to CTX-M-3 was also isolated from a *K. ascorbata* strain (Rodriguez et al. 2004). The precursors of CTX-M-8 and CTX-M-9 genogroups are related to ß-lactamase of *Kluyvera Georgiana* (Poirel et al. 2002; Olson et al. 2005). The CTX-M-genogroup-9 is related to enzymes from *Kluyvera* spp. isolated in Guyana, which is identical with CTX-M-14 (Boyd et al. 2004).

Figure 4.3 demonstrates the dendrogram prepared from the available sequences in the GenBank showing genetic relatedness of these CTX enzymes (CLUSTALW was used for the phylogenetic analysis and constructing the dendrogram).

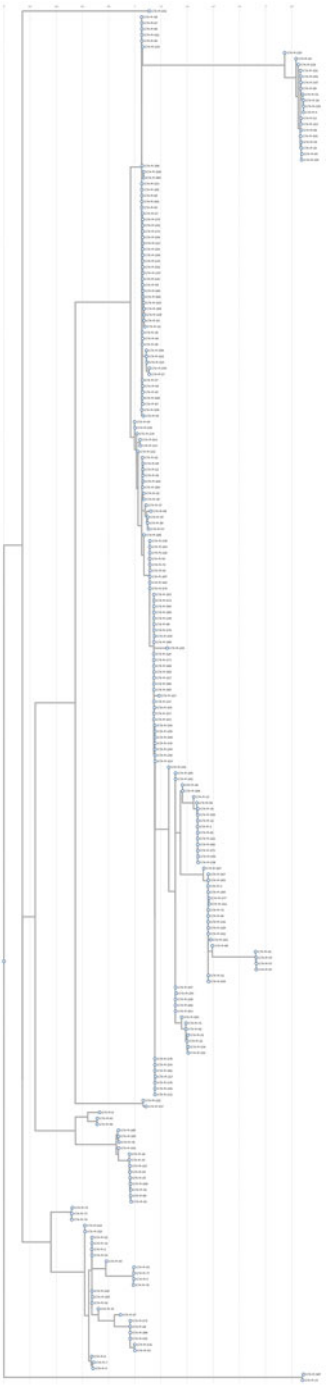


Fig. 4.3 Phylogenetic relation of CTX-M beta-lactamases

Acknowledgments Dr. Anuradha Singh is grateful to the Department of Microbiology, JN Medical College and Hospital, AMU, Aligarh, and also to Aligarh Muslim University for providing necessary facilities during her PhD work.

Disclosure Contents of this chapter have been extracted from the PhD thesis of the primary author of this chapter (Dr. Anuradha Singh) under the supervision of Prof. Mohammad Shahid who is also the co-author in this chapter. The thesis was not published in any form; however, the soft copy of the thesis was deposited in the repository of the Maulana Azad Library of the Aligarh Muslim University, the institution from where Dr. Anuradha Singh obtained her PhD. The Library and the Institution have no objection in publishing the part of the thesis as a book chapter. The soft copy of the thesis is available at http://ir.amu.ac.in/9736/1/T%208440.pdf

References

Ambler RP (1980) The structure of beta-lactamases. Philos Trans R Soc Lond Ser B Biol Sci 289: 321–331

Ambler RP, Scott GK (1979) Partial amino acid sequence of penicillinase coded by *Escherichia coli* plasmid R6K. Proc Natl Acad Sci USA 75:3732–3736

Arlet G, Rouveau M, Philippon A (1997) Substitution of alanine for aspartate at position 179 in the SHV-6 extended-spectrum β-lactamase. FEMS Microbiol Lett 152:163–167

Archambault M, Petrov P, Hendriksen RS et al (2006) Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among *Salmonella enterica* serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb Drug Resist 12:192–198

Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14:933–951

Bonnet R (2004) Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14

Bonomo RA (2014) β-Lactamases: a focus on current challenges. Cold Spring Harb Perspect Med 7:a025239

Bush K, Sykes RB (1983) β-lactamase inhibitors in perspective. J Antimicrob Chemother 11:97–107

Babini GS, Livermore DM (2000) Are SHV β-lactamases universal in *Klebsiella pneumoniae*? Antimicrob Agents Chemother 44:2230

Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54(3):969–976

Bauernfeind A, Grimm H, Schweighart S (1990) A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. Infection 18:294–298

Bonomo RA, Rudin SA, Shlaes DM (1997) Tazobactam is a potent inactivator of selected inhibitor-resistance class A β-lactamases. FEMS Microbiol Lett 148:59–62

Barthelemy M, Peduzzi J, Yaghlane HB et al (1988) Single aminoacid substitution between SHV-1 β-lactamase and cefotaxime-hydrolysing SHV-2 enzyme. FEBS Lett 231:217–220

Bernard H, Tancrede C, Livrelli V et al (1992) A novel plasmid-mediated extended-spectrum beta-lactamase not derived from TEM- or SHV-type enzymes. J Antimicrob Chemother 29:590–592

Boyd DA, Tyler S, Christianson S et al (2004) Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum β-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 48:3758–3764

Bret L, Chanal C, Sirot D, Labia R, Sirot J (1996) Characterization of an inhibitor-resistant enzyme IRT-2 derived from TEM-2 β-lactamase produced by Proteus mirabilis strains. J Antimicrob Chemother 38(2):183–191

Chaibi EB, Farzaneh S, Peduzzi J (1996) An additional ionic bond suggested by molecular modelling of TEM-2 might induce a slight discrepancy between catalytic properties of TEM-1 and TEM-2 beta-lactamases. FEMS Microbiol Lett 143:121–125

Chaibi EB, Sirot D, Paul G et al (1999) Inhibitor-resistant TEM-β-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother 43:447–458

Chang FY, Siu LK, Fung CP (2001) Diversity of SHV and TEM beta-lactamases in *Klebsiella pneumoniae*: gene evolution in Northern Taiwan and two novel beta-lactamases, SHV-25 and SHV-26. Antimicrob Agents Chemother 45:2407–2413

Chaves J, Ladona MG, Segura C et al (2001) SHV-1 β-lactamasen is mainly a chromosomally encoded species-specific enzyme in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 45: 2856–2861

Chen Y, Delmas J, Sirot J et al (2005) Atomic resolution structures of CTX-M β-lactamases extended spectrum activities from increased mobility and decreased stability. J Mol Biol 348: 349–362

Canton R, Morosini MI, Martin O et al (2008) IRT and CMT β-lactamases and inhibitor resistance. Clin Microbiol Infect 14:53–62

Chiaretto G, Zavagnin P, Bettini F et al (2008) Extended-spectrum β-lactamase SHV-12-producing *Salmonella* from poultry. Vet Microbiol 128:406–413

Datta N, Kontomichalou P (1965) Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. Nature 208:239–241

Decousser JW, Poirel L, Nordmann P (2001) Characterization of a chromosomally encoded extended-spectrum class A beta-lactamase from *Kluyvera cryocrescens*. Antimicrob Agents Chemother 45:3595–3598

Damjanoba I, Toth A, Paszti J et al (2007) Epidemiology of SHV-type beta-lactamase-producing *Klebsiella* spp. from outbreaks in five geographically distant Hungarian neonatal intensive care units: widespread dissemination of epidemic R-plasmids. Int J Antimicrob Agents 29:665–671

Ford PJ, Avison MB (2004) Evolutionary mapping of the SHV β-lactamase and evidenced for two separate IS*26*-dependent bla_{SHV} mobilization events from *Klebsiella pneumoniae* chromosome. J Antimicrob Chemother 54:69–75

Fiett J, Palucha A, Miaczynska B et al (2000) A novel complex mutant β-lactamase, TEM-68 identified in a *Klebsiella pneumoniae* isolate from an outbreak of extended-spectrum β-lactamase-producing Klebsiellae. Antimicrob Agents Chemother 44:1499–1505

Goussard S, Sougakoff W, Mabilat C et al (1991) An IS*1*-like element is responsible for high-level synthesis of extended-spectrum β-lactamase TEM-6 in *Enterobacteriaceae*. J Gen Microbiol 137:2681–2687

Gutmann L, Ferre B, Goldstein FW et al (1995) SHV-5, a novel SHV-type beta-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. Antimicrob Agents Chemother 33:951–956

Hall BG, Barlow M (2004) Evolution of the serine β-lactamases: past, present and future. Drug Resist Updat 7:111–123

Hujer AM, Hujer KM, Bonomo RA (2001) Mutagenesis of amino acid residues in the SHV-1 β-lactamase: the premier role of Gly238Ser in penicillin and cephalosporins resistance. Biochem Biophys Acta 1547:37–50

Humeniuk C, Arlet G, Gautier V et al (2002) Beta-lactamases of *Kluyvera ascorbata*, probable progenitors ofsome plasmid-encoded CTX-M types. Antimicrob Agents Chemother 46:3045–3049

Jacoby GA (2006) β-lactamase nomenclature. Antimicrob Agents Chemother 50:1123–1129

Jacoby GA, Sutton L (1985) β-lactamases and β-lactam resistance in *Escherichia coli*. Antimicrob Agents Chemother 28:703–706

Jacoby GA, Carreras I (1990) Activities of β-lactam antibiotic against *Escherichia coli* strains reducing extended-spectrum β-lactamases. Antimicrob Agents Chemother 34:858–862

Jouini A, Vinue L, Slana KB et al (2007) Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J Antimicrob Chemother 60:1137–1141

Knothe H, Shah P, Krcmery V et al (1983) Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection 11:315–317

Kliebe C, Nies BA, Meyer SF et al (1985) Evolution of plasmid-coded resistance to broad-spectrum cephalosporin. Antimicrob Agents Chemother 28:302–307

Kuzin AP, Nukaga M, Nukaga Y et al (1999) Structure of SHV-1 β-lactamase. Biochemistry 38: 5720–5727

Kurowaka H, Yagi T, Shibata N et al (2000) A new SHV-derived extended-spectrum β-lactamase (SHV-24) that hydrolyzes ceftazidime through a single-amino-acid substitution (D179G) in the Ω-loop. Antimicrob Agents Chemother 44:1725–1727

Livermore DM (1995) β-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 8: 557–584

Lemozy J, Sirot D, Chanal C (1995) First characterization of inhibitor resistant TEM (IRT) β-lactamases in *Klebsiella pneumoniae* strains. Antimicrob Agents Chemother 33:2580–2582

Liu PYF, Gur D, Hall LMC et al (1992) Survey of the prevalence of β-lactamases amongst 1000 gram-negative bacilli isolated consecutively at the Royal London Hospital. J Antimicrob Chemother 30:429–447

Lee YH, Cho B, Bae K et al (2006) *Klebsiella pneumoniae* strains carrying the chromosomal SHV-11 β-lactamase gene produce the plasmid-mediated SHV-12 extended-spectrum β-lactamase more frequently than those carrying the chromosomal SHV-1 β-lactamase gene. J Antimicrob Chemother 57:1259–1261

Lewis JS, Herrera M, Wickes B et al (2007) First report of the emergence of CTX-M-type extended-spectrum β-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother 51:4015–4021

Livermore DM, Canton R, Gniadkowski M et al (2007) CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 59:165–174

Matthew M (1979) Plasmid mediated β-lactamases of gram-negative bacteria: distributions and properties. J Antimicrob Chemother 5:349–358

Medeiros AA (1984) β-lactamases. Br Med Bull 40:18–27

Magdalena J, Forsman M, Lenzini MV (1992) Two different beta-lactamase genes are present in *Streptomyces cacaoi*. FEMS Microbiol Lett 99:101–106

Miriagou V, Carattoli A, Tzelepi E et al (2005) IS*26*-associated In4-type integrons forming multiresistance loci in Enterobacterial plasmids. Antimicrob Agents Chemother 49(8): 3541–3543

Nuesch-Inderbinen MT, Kayser FH, Hachler H (1997) Survey and molecular genetics of SHV β-lactamases in *Enterobacteriaceae* in Switzerland: two novel enzymes, SHV-11 and SHV-12. Antimicrob Agents Chemother 41:943–949

Olson AB, Silverman M, Boyd DA et al (2005) Identification of a progenitor of the CTX-M-9 group of extended-spectrum β-lactamases from *Kluyvera georgiana* isolated in Guyana. Antimicrob Agents Chemother 49:2112–2115

Pitton JS (1972) Mechanism of bacterial resistance to antibiotics. Rev Physiol 65:15–93

Partridge SR, Hall RM (2005) Evolution of transposons containing bla_{TEM} genes. Antimicrob Agents Chemother 49:1267–1268

Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamase: a clinical update. Clin Microbiol Rev 18:657–686

Poirel L, Kampfer P, Nordmann P (2002) Chromosome-encoded Ambler class A beta-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum beta-lactamases. Antimicrob Agents Chemother 46:4038–4040

Poirel L, Mammeri H, Nordmann P (2004) TEM-121, a novel complex mutant of TEM-type β-lactamase from *Enterobacter aerogenes*. Antimicrob Agents Chemother 48:4528–4531

Preston KE, Venezia RA, Stellrecht KA (2004) The SHV-5 extended-spectrum β-lactamase gene of pACM1 is located on the remnant of a compound transposon. Plasmid 51:48–53

Podbielski A, Schonling J, Melzer B et al (1991) Different promoters of SHV-2 and SHV-2a β-lactamase lead to diverse levels of cefotaxime resistance in their bacterial producers. J Gen Microbiol 137:1667–1675

Poirel L, Brinas L, Verlinde A et al (2005) BEL-1, a novel clavulanic acid-inhibited extended-spectrum β-lactamase, and the class 1 integron In120 in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 49(9):3743–3748

Pitout JD, Gregson DB, Church DL et al (2005) Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. J Clin Microbiol 43:2844–2849

Pitout JD, Hamilton N, Church DL et al (2007) Development and clinical validation of a molecular diagnostic assay to detect CTX-M-type β-lactamases in Enterobacteriaceae. Clin Microbiol Infect 13:291–297

Philippon A, Slama P, Dény P et al (2016) A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. Clin Microbiol Rev 29(1):29–57

Richmond MH, Sykes RB (1973) The β-lactamases of gram negative bacteria and their possible physiological role. Adv Microb Physiol 9:31–88

Rossolini GM, D'Andrea MM, Mugnaioli C (2008) The spread of CTX-M-type extended-spectrum β-lactamases. Clin Microbiol Infect 14(Suppl. 1):33–41

Rasheed JK, Jay C, Metchock B et al (1997) Evolution of extended-spectrum β-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob Agents Chemother 41:647–653

Rodriguez MM, Power P, Radice M et al (2004) Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. Antimicrob Agents Chemother 48:4895–4897

Reynolds KA, Thomson JM, Corbett KD et al (2006) Structural and computational characterization of the SHV-1 β-lactamase- β-lactamase inhibitor protein interface. J Biol Chem 281:26745–26753

Robin F, Delmas J, Brebion A et al (2007) TEM-158 (CMT-9), a new member of the CMT-type extended-spectrum β-lactamases. Antimicrob Agents Chemother 51:4181–4183

Sougakoff W, Goussard S, Courvalin P (1988) The TEM-3 β-lactamase, which hydrolyzes broad-spectrum cephalosporins, is derived from the TEM-2 penicillinase by two amino acid substitutions. FEMS Microbiol Lett 56:343–348

Sirot D, Sirot J, Labia R et al (1987) Transferable resistance to third generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel beta-lactamase. J Antimicrob Chemother 20:323–334

Sirot D, Recule C, Chaibi EB et al (1997) A complex mutant of TEM β-lactamase with mutation encountered in both IRT-14 and extended-spectrum TEM-15, produced by an *Escherichia coli* clinical isolate. Antimicrob Agents Chemother 41:1322–1325

Shahid M, Singh A, Sobia F et al (2011) An overview of CTX-M β-lactamases. Rev Med Microbiol 22:28–40

Wang J, Xu T, Ying J et al (2019) PAU-1, a novel plasmid-encoded ambler class A β-lactamase identified in a clinical *Pseudomonas aeruginosa* isolate. Infect Drug Resist 12:3827–3834

Yagi T, Kurokawa H, Senda K et al (1997) Nosocomial spread of cephem-resistant *Escherichia coli* strains carrying multiple Toho-1-like beta-lactamase genes. Antimicrob Agents Chemother 41: 2606–2611

Class B-Type Beta-Lactamases: Treatment Strategies

5

Farrukh Sobia, Shahazad Niwazi, Amani Awwadh Alotaibi, and Hanan Abdullah Almaimani

Abstract

Improper usage of antibiotics in treatment of patients as well as in farming serves as major impetus responsible for increase in antibiotic resistance. Moreover, programs and interventions implemented locally and internationally aiming at improving the usage of antimicrobials are collectively termed as "antimicrobial stewardship."

Class A carbapenemases (KPC and GES) showed an inhibitory effect on clavulanic acid and tazobactam. The class C cephalosporinases are encoded by chromosomal ampC gene of Enterobacterial isolates. Furthermore, β-lactamase class D are oxacillinases that are located both on chromosomes (carbapenem-resistant *A. baumannii*) and intestinal bacterial plasmids. The class B beta-lactamase possess a double zinc moiety as a characteristic feature, and hence, the term metallo-beta-lactamase (MBL) was assigned. They show strong inhibitory action against carbapenems and cephalosporins but are unable to hydrolyze monobactams (e.g., Aztreonam).

MBLs are grouped in three different subclasses (namely B1–B3) according to the sequence of their amino acids. The enzymes NDM, VIM, and IMP were included in Subclass B1. In addition, in the active center, subclass B2 has a zinc2+ moiety and hence demonstrates a narrow range of activities. The third subclass, i.e., B3 displays more extensive degradation of substrates as it possess two zinc molecules (Zn1, Zn2).

F. Sobia (✉) · S. Niwazi · A. A. Alotaibi · H. A. Almaimani
Department of Health Education and Promotion, Faculty of Public Health and Tropical Medicine, Jazan University, Jizan, Saudi Arabia
e-mail: fsobia@jazanu.edu.sa; squrashi@jazanu.edu.sa; alotaibi@jazanu.edu.sa; aalmaimani@jazanu.edu.sa

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_5

Keywords

Antimicrobial drug resistance · Class beta lactamase · Novel carbapenemase inhibitor · Beta-lactams · *Enterobacteriaceae*

5.1 History of Carbapenems

Carbapenems are the β-lactams which possess a β-lactam ring along with a five-membered unsaturated ring having a carbon in place of sulfur; hence, they are different from penicillin (Brunton et al. 2018; Walsh 2003). This unique structure also provides them remarkable stability against ESBLs (El-Gamal et al. 2017; Meletis 2016). The first carbapenem was Thienamycin that was discovered in 1976; this was a compound naturally derived from *Streptomyces cattleya* (Birnbaum et al. 1985; Kahan et al. 1979). Thienamycin was shown to be unstable in water, and this feature limits its clinical use (Lee and Bradley 2019). Later on, a semisynthetic compound, N-formimidoyl derivative was produced to overcome this instability, and this compound was named as imipenem (Foye et al. 2013; Grayson 2012). Moreover, dehydropeptidase (a renal tubular dipeptidase enzyme) degrades imipenem, and hence, cilastatin (a competitive antagonist) is co-administered with imipenem as it prevents the degradation of imipenem by dehydropeptidase and the patients' kidney also get protected from toxicity due to higher doses of imipenem (Grayson 2012; Buckley et al. 1992; Fischer and Ganellin 2006). Thienamycin displayed an unusually broad-spectrum activity ranging against majority of Gram-positive bacteria (GPB) and Gram-negative bacteria (GNB), most strikingly against *Pseudomonas aeruginosa* (WHO 2018), and this unique property differentiates it from another natural antibiotic. Interestingly, beta-lactamase that is effectively inhibiting penicillin and cephalosporin does not affect its activity, and thus, it began a potentially promising era of chemotherapy by beta-lactams (Holmes et al. 2016). However, chemical instability and restricted solubility of thienamycin prevented their medicinal development. On the other hand, it displays unique affinity to PBP and inhibit beta-lactamase effectively, and these features fostered concern in the derivatives of thienamycin (Tenover 2006).

Imipenem became the first commercially available carbapenem in 1985 (Walsh 2003), but in the same year, there was an initial emergence of beta-lactamases, SHV and TEM families (class A ESBLs) among Enterobacterial isolates. However, Imipenem (the first market-driven carbapenem) results in the treatment of ESBL-producing Enterobacterales infections (El-Gamal et al. 2017). During the last three decades, meropenem, ertapenem, and doripenem (and other carbapenems) have played a vibrant role in tackling of serious infections in critically ill patients caused by drug-resistant microorganisms. Unfortunately, the usefulness of carbapenems is now severely compromised due to the emergence of carbapenem-resistant bacteria, more specifically in Enterobacterales (CRE).

CRE infections are mostly associated with poor clinical outcome. More often, carbapenem-resistant bacterial isolates affect chronically and critically ill patients as

well as elderly and immunosuppressed. Most of the CRE infections are linked to significant healthcare-related exposures (Birnbaum et al. 1985; Lee and Bradley 2019) and CRE-transmissions in the post-acute care services, which contributes significantly to the CRE epidemiology (Foye et al. 2013).

Changes in carbapenem permeability in bacterial cell is blamed for resistance in CREs, which is caused due to alterations in porins [outer membrane proteins (OMPs)] such as OmpK35 and OmpK36. Furthermore, in some situations, increasing control of efflux pumps (like AcrABTolC), particularly when paired with cephalosporinases, is increasingly described as contributing factor to carbapenem resistance (e.g., CTX-M-15, SHV-5, ACT, DHA, and CMY). However, intrinsic carbapenemase activity has not been observed in these enzymes (Yang et al. 2009; Tzouvelekis et al. 2012; Blair et al. 2015; Durante-Mangoni et al. 2019).

5.2 Antimicrobial Drug Resistance

Antimicrobial resistance or AMR can be defined as the potential of human pathogenic organism to resist the effect of antibiotics and is considered as one of the biggest crises encountered by humanity in the twenty first century. This situation occurs due to various reasons. One aspect could be overpopulation that results in quick spread of bacterial diseases among humans, and between animals and humans, which could get worsened by the ease of crossing international boundaries.

The most often used antibacterial agents are β-lactam antibiotics belonging to penicillin-, cephalosporin-, and carbapenem-groups; β-lactam and carbapenem were developed as specific agents to treat penicillin-resistant bacteria. Pressed with continuously increasing usage of carbapenems in healthcare settings, resistance to carbapenems appeared too. The resistance to carbapenem consequently represents a significant hazard to immunocompromised patients who are susceptible to multi-drug-resistant bacteria infection worldwide.

5.3 Beta-Lactamases

Beta-lactamases are classified into four classes (Class A–D) depending on the protein motifs in Ambler classification. The β-lactamase enzymes belonging to classes A, C, and D utilizes “serine” as an enzyme-active center, on the other hand those of class B utilizes “zinc ion.” The functional classification of β-lactamases was given by Bush-Jacobi-Medeiros; the β-lactamases were categorized into groups 1–3. Group 1 consists of cephalosporinases (grouped under class C in molecular/structural classification). Group 2 comprises non-Group 1 β-lactamases (possess serine and included classes A and D of molecular/structural classification). Moreover, Group 3 represents metallo-beta-lactamases (commonly called as MBLs) which corresponds to class B of Ambler classification. This functional classification of β-lactamases depends on β-lactam substrate degradation and effect of inhibitors.

Class B type of β-lactamase is an MBL which possesses the Zn^{2+} at an active site (Palzkill 2013). *P. Aeruginosa* harboring the MBL destroys all β-lactam drugs except monobactams. The activity of class B β-lactams can be suppressed by the chelating substance (ethylene-diamine-tetraacetic acid or EDTA) as it links with metal ion situated at the enzyme active site. Interestingly, an MBL gene can be located on various genetic structures like integron, transposon, plasmid or chromosome. In 1989, the components of an integron carrying antibiotic-resistant genes belonging to class B MBLs and extended-spectrum beta-lactamases (ESBLs) belonging to classes A and D were reported (Stokes and Hall 1989; Gillings 2014; Deng et al. 2015). Gene cassettes have usually been integrated into the genome by interacting attI with attC, and the genetic recombination has been mediated by the integrase enzyme. Usually, the promoter is present upstream of insertion site (in the integrase gene) and thereby controls the expression of inserted antibiotic resistance genes embedded in the gene cassette. IMP, VIM, SPM, GIM, NDM, and FIM are the carbapenem-resistant MBLs reported to date. The earliest MBL, found in the 1990s, were the IMP and VIM-type, and their new variants are constantly being identified. Variant mutations influence the spectrum of activity of carbapenem (e.g., activity against meropenem, imipenem, and doripenem). For instance, in IMP-6, there is only one amino acid substitution of IMP-1 (serine to glycine at 214), but it enhances meropenem resistance. Similarly, VIM-4 only has an insertion of an amino acid "arginine" at position 44 and a substitution of amino acid "serine" with "arginine" at position 265, from VIM-1.

NDM-1 was isolated in *E. coli* and *Klebsiella* obtained from patient who came back to Sweden in 2008 after vacation to India. In them, $bla_{\text{NDM-1}}$ gene was found to be located on the plasmid. Interestingly, NDM was not noticed in the integron structure, whereas VIM and IMP were usually located there.

MBLs are grouped into three subclasses, namely B1–B3 depending on amino acid sequences (Rasmussen and Bush 1997; Palzkill 2013). NDM, IMP, VIM, and SPM were categorized into subclass B1, and subclass B3 includes GOB-1, CAU-1, and FEZ-1, both possess double Zn^{2+} molecules in the enzyme-active center (i.e., Zn1 and Zn2), and were classified as subgroup 3a in Bush-Jacoby functional classification system (Palzkill 2013). Moreover, the binding site for Zn1 in the B1-enzyme involves three histidine proteins (namely His116, His118, and His196) (Cadag et al. 2012). Aspartate, cystine, and histidine are the binding sites for Zn2 of B1 (DCH, Asp-120, Cys-221, His-263) (Cadag et al. 2012). CphA, Sfh-I, and ImiS are subclass B2 mbls with one Zn^{2+} at the active center and display range of target degradation substrate (Garau et al. 2005; Fonseca et al. 2011).

5.4 Treatment Options for Class B Beta-Lactamase-Producing Organisms

The outbreaks of carbapenemase-producers and bacteria resistant to extended-spectrum β-lactams are becoming a major concern that not only makes the patient's harder to treat but also worsen prognosis of sick people. The hydrolytic activity of

ESBLs and carbapenemases is responsible for beta-lactam resistance in Gram-negative bacteria. Hence, the combination of beta-lactam/beta lactamase inhibitor proved successful, but later on they lost their effectiveness in the bacterial isolates that possess multiple beta-lactamase in one organism.

5.5 Currently Available Beta-Lactams and Their Spectrum of Activity

The latest developed β-lactams are carbapenems (imipenem/cilastatin, meropenem, doripenem, and ertapenem) which are frequently considered as last resort available for use. They showed a vast range of activity, and hence, they are generally kept reserved for the patients having infections caused by MDR pathogens.

Imipenem/cilastatin is currently considered in the treatment of a wide range of bacterial infections, such as infections of the urinary tract and lower airways, particularly those caused by bacteria which are resistant to cephalosporins. There is no need to prescribe meropenem with cilastatin because it is not hydrolyzed by dehydropeptidase I enzyme. Moreover, meropenem displayed better activity than imipenem against Gram-negative bacteria (GNB) but is less active against Gram-positive bacteria (more specifically *Enterococcus*). The pyrrolidinyl substituent which is at the second position of the side chain of meropenem was proved to be responsible for better activity against GNB as well as improved stability toward dehydropeptidase enzyme.

Doripenem's range of activity is found to be somewhat comparable to meropenem, but it shows improved action against some resistant *Pseudomonas* strains. Ertapenem nevertheless has less activity than imipenem and meropenem against *Pseudomonas aeruginosa, Enterococcus,* and *Acinetobacter* spp., but has a longer half-life, allowing treatment once every day. In addition, ertapenem has fair anti-anaerobic bacteria activity and also active against Enterobacterial isolates. Additionally, the Infectious Disease Society of America (VA, USA) recommends it as a first-line drug for community-acquired intra-abdominal infections. Whereas, high-risk community-acquired as well as nosocomial and abdominal infections can be treated by doripenem, imipenem, and meropenem.

The sub-inhibitory concentrations of plazomicin along with colistin, meropenem, and fosfomycin have been reported displaying synergistic bactericidal activity against carbapenemase-producing *K. pneumoniae*. Interestingly, the action of KPC and ESBLs has been counteracted by the vaborbactam, the avibactam, and the relebactam (novel inhibitors of β-lactamase). USFDA (MD, USA) approved another recently discovered β-lactam/β-lactamase inhibitor combination (ceftazidime/avibactam, meropenem/vaborbactam, and imipenem/cilastatin/relebactam) for the treatment of infections caused by CREs. Cefiderocol (S-649266) was recently developed to treat antibiotic-resistant organisms as an additional cephalosporin. By active transportation, the siderophores reach the periplasmic region, connect additionally to PBP3 from Gram negative bacteria and hinder the production of the cell wall. It was reported as stable against carbapenemases and other ESBLs, whereas

eravacycline is a novel tetracycline having broader spectrum of activity, active against CRE too.

5.6 New Carbapenemase Inhibitors

The major new groups of recently discovered inhibitors are diazobicyclooctanes (DBOs) and boronic acid derivatives (Tehrani and Martin 2018; González-Bello et al. 2020). Avibactam and relebactam are the only approved diazobicyclooctanes, whereas vaborbactam is the boronic acid derivative that has been approved. However, these new inhibitors have been developed and commercialized, but still there is a need of newer compounds. The extremely limited treatment options and more often the unavailability of options for carbapenemase carrying microorganism maintain beta-lactam resistance (more specifically carbapenems) as one of the major issues globally in the healthcare sector (Somboro et al. 2018; Arca-Suárez et al. 2019; Papp-Wallace 2019).

5.7 Avibactam (Aztreonam/Avibactam)

Avibactam exhibits inhibitory effect against AmpC, ESBLs, and carbapenemase-producing Enterobacterales but was found unable to inhibit MBLs produced by Enterobacterales (Ehmann et al. 2013; Livermore et al. 2018; Tsivkovski et al. 2020). Recently, a combination was developed targeting MBLs. Aztreonam, the only approved monobactam to date was selected as it evades the MBL's action by showing low affinity (Drawz et al. 2014; Shields and Doi 2020). Moreover, ESBLs and AmpC enzymes can hydrolyze aztreonam, so the combination of aztreonam/avibactam displays benefit over the combination of ceftazidime/avibactam, as it acts against class-B-carbapenemase-producing strains also. It is considered as a very noteworthy option as it is a combination of double drugs which has been accepted already for clinical use. But further research still is need to assess the activity of avibactam against *P. aeruginosa* strains producing MBL, as it can turn out as a potential combination against MBL-producing enterobacteria (Wenzler et al. 2017; Lee et al. 2020). Moreover, an alternative combination (ceftazidime/avibactam) along with aztreonam showed promising results for the treatment of infections caused by strains carrying various classes of β-lactamases, including MBLs (Shaw et al. 2018; Benchetrit et al. 2020; Sieswerda et al. 2020). The well-established efficacy and safety of both compounds in this combination is another favorable aspect for this combination.

5.8 Taniborbactam (Cefepime/Taniborbactam)

A cyclic boronate compound (Taniborbactam, formerly VNRX-5133) displayed broad-spectrum activity against OXA-48, KPC, and MBLs like NDM and VIM, but not against IMP (Krajnc et al. 2019; Wang et al. 2020). It is probably the first inhibitor to show direct inhibition of all four types of Ambler Classes (Class A, B, C, and D). Taniborbactam used two distinctive mechanisms for inhibition of serine-beta-lactamases (SBL) and metallo-beta-lactamases (MBL). The SBLs are slowly dissociated and are used as a competitive inhibitor for reversible MBLs with quick dissociation and low inhibitor constant (ki) for MBLs (Hamrick et al. 2020). Taniborbactam was developed for treating complicated infections by MDR pathogens along with cefepime and meropenem.

5.9 QPX7728 (Meropenem/QPX7728)

QPX7728 is an ultra-wide-spectrum cyclic boronic acid β-lactamase inhibitor, which is active against both MBLs and SBLs (Tsivkovski et al. 2020). Meropenem/QPX7728 was reported as an effective β-lactam–β-lactamase inhibitor combination that was tested against majority of carbapenem-resistant groups in Enterobacterales having multiple resistance mechanisms (like KPC and MBL carbapenemases) (Nelson et al. 2020). This combination of Meropenem/QPX7728 showed antimicrobial activity against KPC-producing *P. aeruginosa* strains and on collection of carbapenem-resistant *A. baumannii* isolates which were producing NDM, CHDLs, and KPC carbapenemases also (Nelson et al. 2020; Lomovskaya et al. 2020). This inhibitor displays exceptional affinity for significant carbapenemases (NDM-1, KPC-2, OXA-23, IMP-1, VIM-1, and OXA-48), with low Ki values. The combinations of QPX7728 with ceftibuten and tebipenem have also been tested against carbapenem-resistant Enterobacterial isolates (Rubio-Aparicio et al. 2019).

5.10 Other Promising MBL Inhibitors

Any clinically useful inhibitors of the MBL enzymes have not been approved yet. In recent years, new structures focusing on the inhibition of MBLs have been developed. Probably ANT2681 (Antabio) represents one of the most promising compounds. It displays the inhibitory activity of MBLs by interacting with Zn^{2+} cluster located at the active site of these enzymes (Everett et al. 2018). Interestingly, it shows the highest affinity for NDM-1, lower affinity for VIM-1, but very poor affinity for IMP-1. Although meropenem was found to be ineffective in dropping tissue burden but its co-administration with Antabio yielded a statistically significant decline in colony-forming units. Hence, ANT2681 is undergoing preclinical evolution in combination with meropenem and could be used for targeting serious infections caused by MBL-producing CRE. Interestingly, the combination of several bicyclic and tricyclic heterocycles with 6-methylidine penem results in improved

action against class B carbapenemases (Venkatesan et al. 2004, 2006; Weiss et al. 2004). Furthermore, CcrA and IMP-1 were found to be inhibited by J-110,441, which is a 1β-methylcarbapenem with a benzothenyl moiety at the C-2 position (Nagano et al. 1999). IMP-1 gets inhibited by J-111,225 also which is a new 1β-methylcarbapenem possessing a trans-3,5 disubstituted pyrrolidinylthio moiety in C-2 (Nagano et al. 2000a, b). There are cephalosporin-derived molecules named as "Reverse hydroxamates" that showed activity against the MBL, GIM-1 (Ganta et al. 2009). A new class of dipicolinic acid derivatives inhibitors against MBLs is also being used for fragment-based drug development (Chen et al. 2017). Triazole inhibitors are other most recently developed inhibitors against MBLs, whose efficacy remains yet to be established in preclinical trials (Muhammad et al. 2020).

5.11 Major Pitfalls in the Development of New Carbapenemase Inhibitors

The most difficult challenge is probably to develop inhibitors of MBL type and *A. baumannii* CHDL enzymes. The vast range of genetic diversity in the enzymes could be considered as one of the major difficulties in designing beta-lactamase inhibitors for class B enzymes, for example, NDM and VIM can be inhibited by taniborbactam, but it cannot inhibit IMP enzymes. In contrast, tiny compounds capable of binding and chelating zinc inhibiting MBLs have been described; however, they also block human metalloenzymes and could therefore likewise be hazardous to live tissues (Somboro et al. 2019; Boyd et al. 2020). Moreover, there is a great variance between the in vitro conditions used for determining antibiotic susceptibility, and the actual situation at the site of infection (Asempa et al. 2020). Hence, it seems quite challenging to design and then evaluate these inhibitors against MBLs, thus suggesting a need for further research in this area. A CHDL-producing *A. baumannii*, was found to be resistant to the action of most classical inhibitors (Evans and Amyes 2014). Durlobactam (Durand-Réville et al. 2017) and LN-1-255 (Vázquez-Ucha et al. 2017) are the only two compounds that showed useful activity against these enzymes. Besides, co-occurrence of various β-lactamases in the same pathogen is another important challenge. Future inhibitors must be highly effective and must be able to simultaneously inhibit different kinds of lactamases, which need significant structural and biochemical development (Spyrakis et al. 2020).

5.12 Conclusion

Carbapenems are considered as the most effective β-lactam antibiotics which showed a broad-spectrum of antibacterial activity, and they do not cause any adverse reaction also. Their molecular structure is responsible for providing great stability against hydrolysis. Hence, these compounds serve as the most suitable last-resort treatment for severe infections. For such reasons, carbapenem resistance mediated by carbapenemases is seen as a major public health problem of global concern. It

compromises treatment options for infections caused by carbapenem-resistant bacteria. Unfortunately, co-occurrence of genetic determinants for resistance to another antibiotic (e.g., aminoglycoside and quinolone) and to carbapenem antibiotics have also been noticed quite often. The bacteria are therefore often found susceptible only to fosfomycin and colistin, but these antibiotics have issues related to toxicity and effectiveness. The only rescue medication available could be tigecycline; however, resistance to this medicine has also developed. In reducing resistance, a major role will be played by sensible use of carbapenems and proper control and preventative measures of infection.

References

Arca-Suárez J, Fraile-Ribot P, Vázquez-Ucha JC, Cabot G, Martínez-Guitián M, Lence E, González-Bello C, Beceiro A, Rodríguez-Iglesias M, Galán-Sánchez F et al (2019) Challenging antimicrobial susceptibility and evolution of resistance (OXA-681) during treatment of a long-term nosocomial infection caused by a *Pseudomonas aeruginosa* ST175 clone. Antimicrob Agents Chemother 63:e01110

Asempa TE, Abdelraouf K, Nicolau DP (2020) Metallo-β-lactamase resistance in Enterobacteriaceae is an artefact of currently utilized antimicrobial susceptibility testing methods. J Antimicrob Chemother 75:997–1005

Benchetrit L, Mathy V, Armand-Lefevre L, Bouadma L, Timsit JF (2020) Successful treatment of septic shock due to NDM-1-producing Klebsiella pneumoniae using ceftazidime/avibactam combined with aztreonam in solid organ transplant recipients: report of two cases. Int J Antimicrob Agents 55:105–842

Birnbaum J, Kahan FM, Kropp H, Macdonald JS (1985) Carbapenems, a new class of beta-lactam antibiotics: discovery and development of imipenem/cilastatin. Am J Med 78(6):3–21

Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13(1):42–51

Boyd SE, Livermore DM, Hooper DC, Hope WW (2020) Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrob Agents Chemother 64:e00397

Brunton LL, Knollmann BC, Hilal-Dandan R (2018) In: Shanahan JF, Lebowitz H (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 13th edn. McGraw Hill Medical, New York

Buckley MM, Brogden RN, Barradell LB, Goa KL (1992) Imipenem/cilastatin. Drugs 44(3): 408–444

Cadag E, Vitalis E, Lennox KP, Zhou CL, Zemla AT (2012) Computational analysis of pathogen-borne metallo beta-lactamases reveals discriminating structural features between B1 types. BMC Res Notes 5:96

Chen AY, Thomas PW, Stewart AC, Bergstrom A, Cheng Z, Miller C, Bethel CR, Marshall SH, Credille CV, Riley CL et al (2017) Dipicolinic acid derivatives as inhibitors of New Delhi metallo-β-lactamase-1. J Med Chem 60:7267–7283

Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, Chen D, Bian H, Li Y, Yu G (2015) Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob 14:45

Drawz SM, Papp-Wallace KM, Bonomo RA (2014) New β-lactamase inhibitors: a therapeutic renaissance in an MDR world. Antimicrob Agents Chemother 58:1835–1846

Durand-Réville TF, Guler S, Comita-Prevoir J, Chen B, Bifulco N, Huynh H, Lahiri S, Shapiro AB, McLeod SM, Carter NM et al (2017) ETX2514 is a broad-spectrum β-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. Nat Microbiol 2:17104

Durante-Mangoni E, Andini R, Zampino R (2019) Management of carbapenem-resistant Enterobacteriaceae infections. Clin Microbiol Infect 25(8):943–950

Ehmann DE, Jahic H, Ross PL, Gu RF, Hu J, Durand-Réville TF, Lahiri S, Thresher J, Livchak S, Gao N et al (2013) Kinetics of avibactam inhibition against Class A, C, and D β-lactamases. J Biol Chem 288:27960–27971

El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A (2017) Recent updates of carbapenem antibiotics. Eur J Med Chem 131:185–195

Evans BA, Amyes SGB (2014) OXA β-lactamases. Clin Microbiol Rev 27:241–263

Everett M, Sprynski N, Coelho A, Castandet J, Bayet M, Bougnon J, Lozano C, Davies DT, Leiris S, Zalacain M et al (2018) Discovery of a novel metallo-β-lactamase inhibitor that potentiates meropenem activity against carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 62:e00074

Fischer J, Ganellin CR (2006) Analogue-based drug discovery, 6th edn. Wiley, Weinheim

Fonseca F, Bromley EH, Saavedra MJ, Correia A, Spencer J (2011) Crystal structure of Serratia fonticola Sfh-I: activation of the nucleophile in mono-zinc metallo-beta-lactamases. J Mol Biol 411:951–959

Foye WO, Lemke TL, Williams DA (2013) Foye's principles of medicinal chemistry, 7th edn. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia

Ganta SR, Perumal S, Pagadala SRR, Samuelsen O, Spencer J, Pratt RF, Buynak JD (2009) Approaches to the simultaneous inactivation of metallo- and serine-beta-lactamases. Bioorg Med Chem Lett 19:1618–1622

Garau G, Bebrone C, Anne C, Galleni M, Frere JM, Dideberg O (2005) A metallo-beta- lactamase enzyme in action: crystal structures of the mono-zinc carbapenemase CphA and its complex with biapenem. J Mol Biol 345:785–795

Gillings MR (2014) Integrons: past, present, and future. Microbiol Mol Biol Rev 78:257–277

González-Bello C, Rodríguez D, Pernas M, Rodríguez Á, Colchón E (2020) β-Lactamase inhibitors to restore the efficacy of antibiotics against superbugs. J Med Chem 63:1859–1881

Grayson ML (2012) Kucers' the use of antibiotics: a clinical review of antibacterial, antifungal, antiparasitic and antiviral drugs, 6th edn. CRC Press, Boca Raton

Hamrick JC, Docquier JD, Uehara T, Myers CL, Six DA, Chatwin CL, John KJ, Vernacchio SF, Cusick SM, Trout REL et al (2020) VNRX-5133 (Taniborbactam), a broad-spectrum inhibitor of serineand metallo-β-lactamases, restores activity of cefepime in Enterobacterales and Pseudomonas aeruginosa. Antimicrob Agents Chemother 64:e01963

Holmes AH, Moore LSP, Sundsfjord A et al (2016) Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 387(10014):176–187

Kahan JS, Kahan FM, Goegelman R et al (1979) Thienamycin, a new beta-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J Antibiot 32(1):1–12

Krajnc A, Brem J, Hinchliffe P, Calvopiña K, Panduwawala TD, Lang PA, Kamps JJAG, Tyrrell JM, Widlake E, Saward BG et al (2019) Bicyclic boronate VNRX-5133 inhibits metallo- and serine-β-lactamases. J Med Chem 62:8544–8556

Lee Y, Bradley N (2019) Overview and insights into carbapenem allergy. Pharmacy 7(3):110–116

Lee M, Abbey T, Biagi M, Wenzler E (2020) Activity of aztreonam in combination with ceftazidime-avibactam against serine- and metallo-β-lactamase-producing Pseudomonas aeruginosa. Diagn Microbiol Infect Dis 99:115–227

Livermore DM, Meunier D, Hopkins KL, Doumith M, Hill R, Pike R, Staves P, Woodford N (2018) Activity of ceftazidime/avibactam against problem Enterobacteriaceae and Pseudomonas aeruginosa in the UK, 2015–2016. J Antimicrob Chemother 73:648–657

Lomovskaya O, Nelson K, Rubio-Aparicio D, Tsivkovski R, Sun D, Dudley MN (2020) Impact of intrinsic resistance mechanisms on potency of QPX7728, a new ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-beta-lactamases in Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii. Antimicrob Agents Chemother 64:e00552

Meletis G (2016) Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis 3(1):15–21

Muhammad Z, Skagseth S, Boomgaren M, Akhter S, Fröhlich C, Ismael A, Christopeit T, Bayer A, Leiros HKS (2020) Structural studies of triazole inhibitors with promising inhibitor effects against antibiotic resistance metallo-β-lactamases. Bioorg Med Chem 28:115–598

Nagano R, Adachi Y, Imamura H, Yamada K, Hashizume T, Morishima H (1999) Carbapenem derivatives as potential inhibitors of various beta-lactamases, including class B metallo-beta-lactamases. Antimicrob Agents Chemother 43:2497–2503

Nagano R, Adachi Y, Hashizume T, Morishima H (2000a) In vitro antibacterial activity and mechanism of action of J-111,225, a novel 1beta-methylcarbapenem, against transferable IMP-1 metallo-beta-lactamase producers. J Antimicrob Chemother 45:271–276

Nagano R, Shibata K, Adachi Y, Imamura H, Hashizume T, Morishima H (2000b) In vitro activities of novel trans-3,5-disubstituted pyrrolidinylthio-1beta-methylcarbapenems with potent activities against methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa. Antimicrob Agents Chemother 44:489–495

Nelson K, Rubio-Aparicio D, Sun D, Dudley M, Lomovskaya O (2020) In vitro activity of the ultrabroad-spectrum-beta-lactamase inhibitor QPX7728 against carbapenem-resistant Enterobacterales with varying intrinsic and acquired resistance mechanisms. Antimicrob Agents Chemother 64:e00757–e00720

Palzkill T (2013) Metallo-beta-lactamase structure and function. Ann N Y Acad Sci 1277:91–104

Papp-Wallace KM (2019) The latest advances in β-lactam/β-lactamase inhibitor combinations for the treatment of Gram-negative bacterial infections. Expert Opin Pharmacother 20:2169–2184

Rasmussen BA, Bush K (1997) Carbapenem-hydrolyzing beta-lactamases. Antimicrob Agents Chemother 41:223–232

Rubio-Aparicio D, Nelson K, Griffith DC, Dudley MN, Lomovskaya O (2019) QPX7728: in vitro activity in combination with oral beta-lactam antibiotics against Enterobacteriaceae. In Proceedings of the ASM 2019, San Francisco, CA, USA, 20–24 June 2019

Shaw E, Rombauts A, Tubau F, Padullés A, Càmara J, Lozano T, Cobo-Sacristán S, Sabe N, Grau I, Rigo-Bonnin R et al (2018) Clinical outcomes after combination treatment with ceftazidime/avibactam and aztreonam for NDM-1/OXA-48/CTX-M-15-producing Klebsiella pneumoniae infection. J Antimicrob Chemother 73:1104–1106

Shields RK, Doi Y (2020) Aztreonam combination therapy: an answer to metallo-β-lactamase-producing gram-negative bacteria? Clin Infect Dis 71:1099–1101

Sieswerda E, van den Brand M, van den Berg RB, Sträter J, Schouls L, van Dijk K, Budding AE (2020) Successful rescue treatment of sepsis due to a pandrug-resistant, NDM-producing Klebsiella pneumoniae using aztreonam powder for nebulizer solution as intravenous therapy in combination with ceftazidime/avibactam. J Antimicrob Chemother 75:773–775

Somboro AM, Osei Sekyere J, Amoako DG, Essack SY, Bester LA (2018) Diversity and proliferation of metallo-β-lactamases: a clarion call for clinically effective metallo-β-lactamase inhibitors. Appl Environ Microbiol 84:e00698–e00618

Somboro AM, Osei Sekyere J, Amoako DG, Kumalo HM, Khan R, Bester LA, Essack SY (2019) In vitro potentiation of carbapenems with tannic acid against carbapenemase-producing Enterobacteriaceae: Exploring natural products as potential carbapenemase inhibitors. J Appl Microbiol 126:452–467

Spyrakis F, Santucci M, Maso L, Cross S, Gianquinto E, Sannio F, Verdirosa F, De Luca F, Docquier JD, Cendron L et al (2020) Virtual screening identifies broad-spectrum β-lactamase inhibitors with activity on clinically relevant serine- and metallo-carbapenemases. Sci Rep 10:12763

Stokes HW, Hall RM (1989) A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1669–1683

Tehrani KHME, Martin NI (2018) β-lactam/β-lactamase inhibitor combinations: an update. Med Chem Commun 9:1439–1456

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control 34(5):S3–S10

Tsivkovski R, Totrov M, Lomovskaya O (2020) Biochemical characterization of QPX7728, a new ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-beta-lactamases. Antimicrob Agents Chemother 64:e00130–e00120

Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 25(4):682–707

Vázquez-Ucha JC, Maneiro M, Martínez-Guitián M, Buynak J, Bethel CR, Bonomo RA, Bou G, Poza M, González-Bello C, Beceiro A (2017) Activity of the β-lactamase inhibitor LN-1-255 against carbapenem-hydrolyzing class D β-lactamases from *Acinetobacter baumannii*. Antimicrob Agents Chemother 61:e01172

Venkatesan AM, Gu Y, Dos Santos O, Abe T, Agarwal A, Yang Y, Petersen PJ, Weiss WJ, Mansour TS, Nukaga M et al (2004) Structure-activity relationship of 6-methylidene penems bearing tricyclic heterocycles as broad-spectrum beta-lactamase inhibitors: crystallographic structures show unexpected binding of 1,4-thiazepine intermediates. J Med Chem 47:6556–6568

Venkatesan AM, Agarwal A, Abe T, Ushirogochi H, Yamamura I, Ado M, Tsuyoshi T, Dos Santos O, Gu Y, Sum FW et al (2006) Structure-activity relationship of 6-methylidene penems bearing 6,5 bicyclic heterocycles as broad-spectrum beta-lactamase inhibitors: evidence for 1,4-thiazepine intermediates with C7 R stereochemistry by computational methods. J Med Chem 49:4623–4637

Walsh C (2003) Antibiotics: actions, origins, resistance. ASM Press, Washington, DC

Wang X, Zhao C, Wang Q, Wang Z, Liang X, Zhang F, Zhang Y, Meng H, Chen H, Li S et al (2020) In Vitro activity of the novel β-lactamase inhibitor taniborbactam (VNRX-5133), in combination with cefepime or meropenem, against MDR Gram-negative bacterial isolates from China. J Antimicrob Chemother 75:1850–1858

Weiss WJ, Petersen PJ, Murphy TM, Tardio L, Yang Y, Bradford PA, Venkatesan AM, Abe T, Isoda T, Mihira A et al (2004) In vitro and in vivo activities of novel 6-methylidene penems as beta-lactamase inhibitors. Antimicrob Agents Chemother 48:4589–4596

Wenzler E, Deraedt MF, Harrington AT, Danizger LH (2017) Synergistic activity of ceftazidime-avibactam and aztreonam against serine and metallo-β-lactamase-producing gram-negative pathogens. Diagn Microbiol Infect Dis 88:352–354

WHO (2018) Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017–2018. www.apps.who.int/iris/bitstream/handle/10665/279656/9789241515061-eng.pdf?ua=1

Yang D, Guo Y, Zhang Z (2009) Combined porin loss and extended spectrum β-lactamase production is associated with an increasing imipenem minimal inhibitory concentration in clinical Klebsiella pneumoniae strains. Curr Microbiol 58(4):366–370

Class C type β-lactamases (AmpC β-lactamases)

6

P. Ronni Mol, Ganesan Shanthi, Ali Al-Mahmeed, Khalid M. Bindayna, and Mohammad Shahid

Abstract

β-lactams are the drug of choice for Gram-negative bacterial infections. The most common primary resistance determinants to β-lactams are the presence of extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes. AmpC resistance can be grouped into three categories including inducible resistance by the encoding of chromosomal AmpC genes, chromosomal resistance that is not inducible due to the mutation of the promoter and/or attenuator, and plasmid-mediated resistance. Plasmid-mediated AmpC β-lactamases (pAmpC) include MIR/ACT, ACC, DHA, FOX, CIT, and MOX. AmpC isolates have been identified in cultures from rehabilitation facilities and health care centers. There are no Clinical Laboratory Standards Institute (CLSI) recommendations or other accepted standards for AmpC detection. Various phenotypic tests are used for AmpC detection. The gold standard for the identification of AmpC producers is the molecular method (PCR). A significant alarm has been raised in several studies for the management of diseases caused by AmpC strains. Therefore, lab

P. Ronni Mol (✉)
Division of Medical Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India

Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: ronnimj@agu.edu.bh

G. Shanthi
Division of Medical Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India

A. Al-Mahmeed · K. M. Bindayna · M. Shahid
Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: aliem@agu.edu.bh; bindayna@agu.edu.bh; mohammeds@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_6

diagnosis and treatment should be performed with caution. For AmpC beta-lactamases, carbapenems are the drug of choice including alternatives like the cefepime and beta-lactam/beta-lactamase inhibitors.

Keywords

AmpC resistance · Carbapenems · Cefoxitin resistance · Plasmid-mediated AmpC β-lactamases

6.1 Introduction

The antibiotic discovery was a significant moment in human history that modernized medicine and saved countless lives. Unfortunately, these "magic bullets" were followed by the budding of resistant pathogenic strains. Currently antimicrobial resistance is a major public health issue worldwide (Aslam et al. 2018). In this age of growing antibiotic resistance, monitoring and surveillance activity is yet necessary because failure to identify antibiotic resistant determinants can lead to the global spread of resistant microbes and can indirectly complicate patient therapy (Mohd Khari et al. 2016).

β-lactams are the drug of choice for Gram-negative infections due to their limited side effects and good bactericidal properties. In Gram-negative bacteria, β-lactamases are the primary resistance determinants to β-lactams. The development of extended-spectrum β-lactamases (ESBLs) and AmpC enzymes are the most common β-lactamases (Mohamudha Parveen et al. 2010). Even in initially sensitive strains, β-lactam drug exposure can result in significant resistance to β-lactam and AmpC development. The chance of AmpC induction varies according to the species and β-lactam drugs and makes treatment decisions more challenging (Tamma et al. 2019).

AmpC β-lactamase of *Escherichia coli* (*E. coli*) was the first of its kind that destroyed penicillin (Jacoby 2009). In 1965, a research conducted on the genetic makeup of *E. coli* for penicillin resistance reported resistance which was termed amp A and amp B (Eriksson-Grennberg et al. 1965). An amp A mutation which showed reduced resistance was termed AmpC (Jacoby 2009). AmpC enzymes are of Class C β-lactamases in the Ambler structural classification while they are of category 1 in the Bush et al. functional classification system (Jacoby 2009; Bush et al. 1995). These enzymes contain serine deposits at their active catalytic site under the Ambler classification scheme. AmpC resistance can be grouped into three classes: (1) Inducible resistance by the encoding of chromosomal AmpC genes (e.g. *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter cloacae*, etc.), (2) Chromosomal resistance that is not inducible due to the mutation of the promoter and/or attenuator (e.g. *Shigella* species, *Escherichia coli*, *Acinetobacter baumannii*), (3) Plasmid-mediated resistance (e.g. *Klebsiella pneumoniae*, *E. coli*, *Salmonella* species, etc.) (Tamma et al. 2019).

Most of the chromosomal mediated AmpC enzymes can be found in *Enterobacteriaceae* of group II like *Enterobacter* spp., *Citrobacter freundii*, *Providencia* spp., *Hafnia alvei*, *Serratia* spp., *Morganella morganii*, and in other Gram-negative bacteria like *Acinetobacter* spp. and *Pseudomonas* spp. (Rensing et al. 2019). In the beginning it was considered that AmpC genes are transmitted from chromosomal source of *Enterobacteriaceae* to mobile elements enabling the dissemination of enzymes. Accordingly, nowadays they are found in bacteria that have no or low expressions of chromosomal AmpC gene like *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., and *Shigella* spp., which belongs to *Enterobacteriaceae* group I (Halat et al. 2016; Rensing et al. 2019). *Acinetobacter* spp. has various acquired β-lactamases, but their AmpC enzyme is mostly due to the oxyimino-β-lactam resistance. Usually, the enzyme expression is low and is uninducible, but excessive expression occurs due to upstream addition of ISAba1, an insertion element (Jacoby 2009). AmpC enzyme overexpression also plays a key role in the rising resistance of *P. aeruginosa*, besides porins, pumps, and acquired β-lactamases, which are also significant. Since *P. aeruginosa* possesses minimum of three *ampD* genes, increased development of AmpC occurs stepwise, generating resistance to oxyimino-cephalosporins, antipseudomonal penicillins, and, with complete depression, cefepime (Juan et al. 2006).

pAmpC enzymes have been discovered all over the world, including DHA, CIT, ACC, MOX, and FOX as well as MIR/ACT, which are derivatives of *Enterobacteriaceae* chromosomal AmpC genes. Plasmid-mediated class C enzymes are named based on the type of β-lactamase, such as Ambler class C (ACC) or AmpC type (ACT), and to the resistance produced to moxalactam (MOX) or latamoxef (LAT), cefoxitin (FOX), cephamycins (CMY), to the location of discovery, such as the Dhahran hospital (DHA) in Saudi Arabia or the Miriam Hospital in Providence, R.I. (MIR-1) and even named after a patient (Bilal) like BIL-1 (Philippon et al. 2002). Plasmid-mediated AmpCs are derived from chromosomal enzymes and may coexist in positive chromosomal species, like *E. Coli*, to increase their expression. The first pAmpC was discovered in South Korea (CMY-1) (1989). Subsequently, many more of pAmpCs (FOX, CIT, MOX, DHA, EBC, and ACC) have been characterized, with the CMY-2 enzyme (CIT-type) being the most frequent subtype (Oliveira et al. 2019). Table 6.1 provides information on some of the AmpC types (chromosomal/plasmid mediated) detected from several Gram-negative bacteria, as well as their accession numbers, year, and native country. Figure 6.1 exhibits the phylogenetic relation of these AmpC types (CLUSTALW was used for the phylogenetic analysis and constructing the dendrogram).

Three proteins, namely ampG, ampD, and ampR, control the expression of AmpC. Firstly, AmpG is a permease which is membrane-bound that permits the access of 1,6-anhydromuropeptides. Secondly, N-acetyl-muramyl-l-alanine amidase (AmpD) is a protein in the cytoplasm that forms a peptide from 1,6-anhydromuropeptides which is transformed to UDP-N-acetyl-muramic acid (UDP-NAM) a cell wall precursor. Lastly, AmpR is an AmpC expression transcriptional regulator (Mizrahi et al. 2020). In a wild-type isolate, AmpC transcription is suppressed due to the bonding of ampR to UDP-NAM (Jacobs et al. 1995). In the

Table 6.1 Some AmpC β-lactamases (chromosomal and plasmid mediated) reported in Gram-negative bacterial species, their accession numbers, year, and country of origin in chronological order

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
MHN1	X08082	1988	–	Chromosomal	*Enterobacter cloacae*
MOX-1	D13304	1992	Japan	Plasmid	*K. pneumoniae*
MIR-1	M37839	1993	USA	Plasmid	*K. pneumoniae*
BIL-1	X74512	1993	UK	Plasmid	*E. coli*
FOX-1	X77455	1994	Spain	Plasmid	*K. pneumoniae*
LAT-1	X78117	1994	Greece	Plasmid	*K. pneumoniae*
GC1	D44479	1994	Japan	Chromosomal	*Enterobacter cloacae*
CMY-1	X92508	1995	Germany	Plasmid	*K. pneumoniae*
ACT-1	U58495	1996	USA	Plasmid	*K. pneumoniae*
LAT-2	S83226	1996	Greece	Plasmid	*K. pneumoniae*
FOX-2	Y10282	1996	Germany	Plasmid	*E. coli*
FOX-3	Y11068	1997	France	Plasmid	*K. oxytoca*
LAT-3	Y15411	1997	Greece	Plasmid	*E. coli*
SRT-1	AB005484	1997	Japan	Chromosomal	*Serratia marcescens*
SST-1	AB008455	1997	Japan	Chromosomal	*Serratia marcescens*
LAT-4	Y15412	1997	Greece	Plasmid	*E. coli*
CMY-12	Y16785	1998	France	Plasmid	*Proteus mirabilis*
CMY-5	Y17716	1998	Sweden	Plasmid	*K. oxytoca*
CMY-4	AJ007826	1998	UK	Plasmid	*E. coli*
CMY-6	AJ011293	1998	India	Plasmid	*E. coli*
CMY-7	AJ011291	1998	India	Plasmid	*E. coli*
CMY-8	AF167990	1999	Taiwan	Plasmid	*K. pneumoniae*
ACC-2	AF180952	1999	France	Plasmid	*Hafnia alvei*
MOX-2	AJ276453	2000	France	Plasmid	*K. pneumoniae*

DHA-2	AF259520	2000	France	Plasmid	*K. pneumoniae*
FOX-5	AY007369	2000	USA	Plasmid	*K. pneumoniae*
FOX-4	AJ277535	2000	Spain	Plasmid	*E. coli*
FOX-6	AY034848	2001	USA	Plasmid	*K. pneumoniae*
CMY-10	AF381618	2001	South Korea	Plasmid	*K. pneumoniae*
CMY-11	AF381626	2001	Korea	Plasmid	*E. coli*
CMY-9	AB061794	2001	Japan	Plasmid	*E. coli*
K992004.1	AF411144	2001	South Korea	Chromosomal	*Enterobacter cloacae*
K995120.1	AF411145	2001	South Korea	Chromosomal	*Enterobacter cloacae*
K99230	AF411146	2001	South Korea	Chromosomal	*Enterobacter cloacae*
K9911729	AF411147	2001	South Korea	Chromosomal	*Enterobacter cloacae*
K9973	AF411148	2001	South Korea	Chromosomal	*Enterobacter cloacae*
K9914325	AF411149	2001	South Korea	Chromosomal	*Enterobacter cloacae*
DHA-3	AY494945	2003	Taiwan	Plasmid	*K. pneumoniae*
MIR-2	AY227752	2003	Portugal	Chromosomal	*Enterobacter cloacae*
CMY-14	AJ555825	2003	Poland	Plasmid	*Proteus mirabilis*
FOX-7	AJ703795	2004	Italy	Plasmid	*K. pneumoniae*
CMY-19	AB194410	2004	Japan	Plasmid	*K. pneumoniae*
MIR-3	AY743435	2004	Korea	Chromosomal	*Enterobacter cloacae*
CMY-16	AJ781421	2004	Italy	Plasmid	*Proteus mirabilis*
CMY-18	AY743434	2004	Korea	Plasmid	*E. coli*
CMY-21	DQ139328	2005	UK	Plasmid	*E. coli*
CMY-2	DQ173299	2005	UK	Plasmid	*E. coli*
ACT-2	AM076977	2005	Italy	–	*Enterobacter asburiae*
CMY-22	DQ256079	2005	China	Plasmid	*E. coli*
CMY-23	DQ438952	2006	UK	Plasmid	*E. coli*
CMY-37	AB280919	2006	Israel: Palestine	Chromosomal	*Citrobacter freundii*

(continued)

Table 6.1 (continued)

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
CMY-31	EF622224	2007	Switzerland	Plasmid	*K. pneumoniae*
MIR-4	EF417572	2007	China	Plasmid	*E. coli*
ACC-1	EF554600	2007	Ireland	Plasmid	*K. pneumoniae*
ACC-4	EF504260	2007	Greece	Plasmid	*E. coli*
CMY-26	AB300358	2007	Japan	Plasmid	*K. oxytoca*
CMY-24	EF415650	2007	Singapore	Plasmid	*E. coli*
CMY-28	EF561644	2007	Ireland	Plasmid	*E. coli*
CMY-34	EF394370	2007	China	–	*Citrobacter freundii*
CMY-35	EF394371	2007	China	–	*Citrobacter freundii*
CMY-38	AM931008	2007	Poland	–	*Proteus mirabilis*
CMY-25	EU515249	2008	Spain	–	*K. pneumoniae*
CMY-27	EU515250	2008	Spain	–	*E. coli*
CMY-40	EU515251	2008	Spain	–	*E. coli*
MIR-8	FJ237367	2008	China	Plasmid	*K. pneumoniae*
MOX-3	EU515248	2008	Spain	–	*Aeromonas sp*
MOX-4	FJ262599	2008	China	–	*Aeromonas caviae*
ACT-5	FJ237369	2008	China	–	*E. coli*
CMY-32	EU496815	2008	USA	–	*E. coli*
CMY-33	EU496816	2008	USA	–	*E. coli*
CMY-43	FJ360626	2008	South Korea	–	*E. coli*
CMY-44	FJ437066	2008	USA	–	*E. coli*
CMY-45	FN546177	2008	Poland	–	*Proteus mirabilis*
MOX-5	GQ152600	2009	Spain	–	*Aeromonas caviae*
MOX-6	GQ152601	2009	Spain	–	*Aeromonas caviae*
MOX-7	GQ152602	2009	Spain	–	*Aeromonas caviae*

ACT-8	FN645445	2009	Portugal	–	*E. coli*
CMY-46	FN556186	2009	Portugal	–	*E. coli*
CMY-49	GQ402541	2009	China	–	*Citrobacter freundii*
CMY-50	FN645444	2009	Portugal	–	*E. coli*
CMY-15	AJ555823	2009	Poland	–	*Proteus mirabilis*
DHA-1	HM568877	2010	China	Plasmid	*K. pneumoniae*
FOX-8	HM565917	2010	Spain	Plasmid	*E. coli*
DHA-6	HQ322612	2010	Spain	Plasmid	*E. coli*
DHA-7	HQ456945	2010	Spain	Plasmid	*Enterobacter cloacae*
ACT-9	HQ693810	2010	Taiwan	–	*Pantoea agglomerans*
CMY-42	HM146927	2010	Germany	Plasmid	*E. coli*
CMY-47	HM046998	2010	Spain	–	*Citrobacter freundii*
CMY-53	HQ336940	2010	Denmark	Plasmid	*E. coli*
CMY-54	HM544039	2010	Spain	Plasmid	*E. coli*
CMY-55	HM544040	2010	Spain	Plasmid	*E. coli*
CMY-56	HQ322613	2010	Spain	Plasmid	*K. pneumoniae*
CMY-57	HQ285243	2010	Spain	–	*E. coli*
CMY-58	HQ185697	2010	China	–	*E. coli*
CMY-63	HQ650104	2010	China	–	*Citrobacter freundii*
CMY-64	HQ832678	2010	China	–	*E. coli*
FOX-9	JF896803	2011	Spain	Plasmid	*K. pneumoniae*
DHA-5	JF273491	2011	Spain	–	*Morganella morganii*
CMY-48	HM569226	2011	Spain	–	*Citrobacter freundii*
CMY-60	JF460794	2011	China	Plasmid	*E. coli*
CMY-61	JF460795	2011	China	Plasmid	*E. coli*
CMY-62	JF460796	2011	China	Plasmid	*E. coli*
CMY-65	JF780936	2011	China	Plasmid	*Citrobacter sp. 913*

(continued)

Table 6.1 (continued)

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
CMY-66	JN714478	2011	Spain	–	*Citrobacter freundii*
FOX-10	JX049131	2012	USA	Plasmid	*K. pneumoniae*
CMH-1	JQ673557	2012	Taiwan	Plasmid	*Enterobacter cloacae*
ACC-5	HE819401	2012	Portugal	–	*Hafnia alvei*
MIR-6	JQ664733	2012	Taiwan	Plasmid	*Enterobacter cloacae*
MOX-8	JX173956	2012	Thailand	–	*Aeromonas caviae*
ACT-12	JX440355	2012	Spain	–	*Enterobacter cloacae*
ACT-13	HE819402	2012	Portugal	–	*Enterobacter asburiae*
ACT-14	JX440354	2012	Spain	–	*Enterobacter cloacae*
ACT-15	JX440356	2012	Spain	–	*Enterobacter cloacae*
CMY-51	JQ733571	2012	Spain	–	*Citrobacter freundii*
CMY-67	JQ711185	2012	Spain	–	*Citrobacter freundii*
CMY-69	JX049132	2012	USA	Plasmid	*E. coli*
CMY-71	JQ711184	2012	Spain	–	*Citrobacter freundii*
CMY-72	JX440352	2012	Spain	–	*Citrobacter freundii*
CMY-75	JQ733572	2012	Spain	–	*Citrobacter freundii*
CMY-76	JQ733573	2012	Spain	–	*Citrobacter freundii*
CMY-78	JQ733575	2012	Spain	–	*Citrobacter freundii*
CMY-79	JQ733576	2012	Spain	–	*Citrobacter freundii*
CMY-80	JQ733577	2012	Spain	–	*Citrobacter freundii*
CMY-81	JQ733578	2012	Spain	–	*Citrobacter freundii*
CMY-84	JQ733579	2012	Spain	–	*Citrobacter freundii*
DHA-12	HG798963	2013	Argentina	–	*P. mirabilis*
ACT-17	KF992026	2013	Spain	–	*Enterobacter cloacae*
ACT-18	KF992028	2013	Spain	–	*Enterobacter cloacae*

ACT-19	KF992029	2013	Spain	–	*Enterobacter cloacae*
ACT-22	KF992027	2013	Spain	–	*Enterobacter cloacae*
ACT-23	KF515536	2013	Czech Republic	–	*Enterobacter cloacae*
CMY-108	KF564648	2013	USA	–	*E. coli*
CMY-110	AB872957	2013	Japan	–	*E. coli*
CMY-93	KF992025	2013	Spain	–	*Citrobacter freundii*
CMY-99	KF305673	2013	Bulgaria	Plasmid	*Proteus mirabilis*
FOX-12	HG975301	2014	Portugal	Plasmid	*Aeromonas caviae*
MIR-10	KM087858	2014	USA	–	*Enterobacter asburiae*
MIR-11	KM087859	2014	USA	–	*Enterobacter cloacae*
MIR-12	KM087863	2014	USA	–	*Enterobacter cloacae*
MIR-13	KM087862	2014	USA	–	*Enterobacter asburiae*
MIR-15	KM087851	2014	USA	–	*Enterobacter kobei*
MIR-16	KM087861	2014	USA	–	*Enterobacter asburiae*
MIR-17	LN515535	2014	Netherlands	–	*Enterobacter cloacae*
MIR-18	LN609373	2014	Portugal	–	*Enterobacter sp. ET82*
MIR-23	KJ949106	2014	China	–	*Enterobacter cloacae*
ACC-6	KM087831	2014	USA	–	*P. mirabilis*
DHA-10	KP050490	2014	USA	–	*Morganella morganii*
DHA-13	KM087855	2014	–	–	*Morganella morganii*
DHA-14	KM087854	2014	–	–	*Morganella morganii*
DHA-15	KM087853	2014	–	–	*K. pneumoniae*
DHA-16	KM087852	2014	–	–	*Morganella morganii*
DHA-17	KM087850	2014	–	–	*Morganella morganii*
DHA-18	KM087841	2014	–	–	*Morganella morganii*
DHA-19	KM087849	2014	–	–	*Morganella morganii*
DHA-20	KM087848	2014	–	–	*Citrobacter koseri*

(continued)

Table 6.1 (continued)

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
DHA-21	KM087847	2014	–	–	*Morganella morganii*
DHA-22	KM087856	2014	–	–	*E. coli*
DHA-9	KJ207201	2014	Spain	Plasmid	*Morganella morganii*
MIR-7	KJ207200	2014	Spain	–	*Enterobacter cloacae*
MIR-9	KM087860	2014	–	–	*Enterobacter asburiae*
MOX-10	LN609374	2014	–	–	*Aeromonas caviae*
MOX-9	KJ746495	2014	Italy	–	*Citrobacter freundii*
ACT-24	KJ207207	2014	Spain	–	*Enterobacter cloacae*
ACT-25	KJ207208	2014	Spain	–	*Enterobacter cloacae*
ACT-27	KJ207209	2014	Spain	–	*Enterobacter cloacae*
ACT-28	KJ207206	2014	Spain	–	*Enterobacter cloacae*
ACT-29	KM087832	2014	USA	–	*Enterobacter asburiae*
ACT-30	KM087833	2014	–	–	*Enterobacter cloacae*
ACT-31	KM087843	2014	–	–	*Enterobacter cloacae*
ACT-32	KM087835	2014	–	–	*Enterobacter cloacae*
ACT-33	KM087834	2014	–	–	*Enterobacter cloacae*
ACT-34	HG975300	2014	Portugal	–	*Enterobacter cloacae*
ACT-35	LC004922	2014	Japan	–	*Enterobacter cloacae*
ACT-36	KM926621	2014	Spain	–	*Enterobacter cloacae*
ACT-37	KM926622	2014	Spain	–	*Enterobacter cloacae*
CMY-105	KJ207205	2014	Spain	–	*Citrobacter freundii*
CMY-106	KM983294	2014	Spain	–	*Citrobacter freundii*
CMY-112	KM087837	2014	USA	–	*Citrobacter freundii*
CMY-113	KM087836	2014	USA	–	*Citrobacter freundii*
CMY-114	KM087846	2014	USA	–	*Citrobacter freundii*

CMY-115	KM087839	2014	USA	–	*Citrobacter freundii*
CMY-116	KM087840	2014	USA	–	*Citrobacter freundii*
CMY-117	KM087844	2014	USA	–	*Citrobacter freundii*
CMY-118	KM087838	2014	USA	–	*Citrobacter freundii*
CMY-119	KM087845	2014	USA	–	*Citrobacter freundii*
CMY-121	KM507172	2014	France	Plasmid	*E. coli*
CMY-124	KM985462	2014	China	–	*Citrobacter freundii*
CMY-82	KJ207203	2014	Spain	–	*Citrobacter freundii*
CMY-85	KJ207202	2014	Spain	–	*Citrobacter freundii*
CMY-86	KJ207204	2014	Spain	–	*Citrobacter freundii*
CMY-127	KM985465	2014	China	–	*Citrobacter freundii*
CMY-128	KM985466	2014	China	–	*Citrobacter freundii*
CMY-129	KM985467	2014	China	–	*Citrobacter freundii*
CMH-2	KP823454	2015	India	–	*K. pneumoniae*
ACT-38	KP836350	2015	South Africa	–	*Citrobacter freundii*
CMY-132	KP862820	2015	Argentina	–	*E. coli*
CMY-133	KP862819	2015	USA	–	*E. coli*
CMY-138	KT997883	2015	USA	–	*Proteus mirabilis*
FOX-13	KU641008	2016	Spain	Plasmid	*Providencia rettgeri*
FOX-14	KX263248	2016	Switzerland	Plasmid	*Pseudomonas aeruginosa*
MIR-19	KX192155	2016	USA	–	*Enterobacter asburiae*
MIR-20	KX192156	2016	USA	–	*Enterobacter asburiae*
MIR-21	KX192157	2016	USA	–	*Enterobacter asburiae*
CMH-3	KX192165	2016	USA	–	*Enterobacter cloacae*
DHA-23	KX068223	2016	Taiwan	–	*K. pneumoniae*
DHA-24	KU759569	2016	France	Plasmid	*K. oxytoca*

(continued)

Table 6.1 (continued)

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
ACT-39	KU884289	2016	Algeria	–	*Enterobacter cloacae*
ACT-40	KX192159	2016	–	–	*Enterobacter cloacae*
ACT-41	KX192160	2016	–	–	*Enterobacter cloacae*
ACT-42	KX192161	2016	–	–	*Enterobacter cloacae*
ACT-43	KX192162	2016	–	–	*Enterobacter cloacae*
ACT-44	KX192163	2016	–	–	*Enterobacter cloacae*
ACT-45	KX192164	2016	–	–	*Enterobacter cloacae*
ACT-46	KX192166	2016	–	–	*Enterobacter cloacae*
CMY-139	KU641016	2016	France	Plasmid	*E. coli*
CMY-140	KX354367	2016	Taiwan	–	*E. coli*
CMY-141	KX537750	2016	Turkey	–	*E. coli*
CMY-142	KX881969	2016	China	–	*E. coli*
CMY-143	KY009534	2016	Australia	Plasmid	*E. coli*
CMY-145	KX470426	2016	France	–	*E. coli*
CMY-146	KX034085	2016	France	–	*E. coli*
FOX-15	MF795087	2017	Poland	Plasmid	*Kluyvera sp.*
MIR-22	MG028659	2017	Canada	–	*Enterobacter cloacae*
ACC-7	MG028657	2017	Canada	–	*Hafnia alvei*
DHA-25	KY563770	2017	Israel	Plasmid	*K. pneumoniae*
MOX-13	MF795086	2017	Poland	Plasmid	*Citrobacter sp.*
ACT-54	MG028658	2017	Canada	–	*Enterobacter cloacae*
CMY-147	KY563765	2017	Kuwait	–	*E. coli*
CMY-148	KY624573	2017	Jordan	–	*E. coli*
CMY-149	KY624574	2017	Greece	–	*Proteus mirabilis*
CMY-151	KY780116	2017	China	–	*Citrobacter freundii*

CMY-152	KY978224	2017	China	–	*Citrobacter freundii*
CMY-153	MF042206	2017	USA	–	*E. coli*
CMY-154	MF196231	2017	Denmark	–	*E. coli*
CMY-156	MF770636	2017	Japan	–	*E. coli*
CMY-158	MF804901	2017	Myanmar	–	*E. coli*
CMY-159	MF804902	2017	Myanmar	–	*E. coli*
FOX-16	MH067964	2018	USA	Plasmid	*K. pneumoniae*
CMH-4	MH469276	2018	France	–	*Enterobacter cloacae*
CMH-5	MH469277	2018	France	–	*Enterobacter cloacae*
DHA-26	MH067966	2018	Australia	Plasmid	*K. pneumoniae*
DHA-27	MH067965	2018	Taiwan	Plasmid	*E. coli*
DHA-28	MK088090	2018	China	Plasmid	*Morganella morganii*
ACT-58	MH469279	2018	France	–	*Enterobacter cloacae complex sp*
ACT-62	MH469270	2018	France	–	*Enterobacter cloacae complex sp.*
CMY-160	MH243359	2018	UAE	–	*E. coli*
CMY-161	MH067963	2018	Taiwan	–	*E. coli*
CMY-162	MH378228	2018	Denmark	–	*E. coli*
CMY-163	MH450208	2018	UAE	–	*E. coli*
CMH-6	MN692202	2019	France	–	*Enterobacter cloacae*
DHA-29	MN219696	2019	–	Plasmid	*Citrobacter freundii*
MOX-14	MN550985	2019	South Korea	Plasmid	*Proteus mirabilis*
ACT-72	MN082690	2019	Australia	–	*Enterobacter cloacae*
ACT-77	MN692201	2019	France	–	*Enterobacter cloacae*
ACT-78	MN862370	2019	France	–	*Enterobacter cloacae*
ACT-79	MN862371	2019	France	–	*Enterobacter cloacae*

(continued)

Table 6.1 (continued)

Organisms/ AmpC variants	Accession numbers	Year reported in chronological order	Country of origin/submission (based on Genbank data)	Chromosomal/ plasmid mediated	Organisms
ACT-80	MN862372	2019	France	–	*Enterobacter cloacae*
ACT-81	MN862373	2019	France	–	*Enterobacter cloacae*
ACT-82	MN862374	2019	France	–	*Enterobacter cloacae*
CMY-164	MK965968	2019	Chile	–	*E. coli*
CMY-165	MN104598	2019	Taiwan	–	*E. coli*
CMY-167	MN219694	2019	Switzerland	–	*Proteus mirabilis*
CMY-169	MN550987	2019	Colombia	–	*Providencia rettgeri*
CMY-170	MN550988	2019	Poland	–	*K. pneumoniae*
CMY-171	MN164523	2019	UK	–	*E. coli*
FOX-17	MT135152	2020	Switzerland	Plasmid	*Providencia stuartii*
ACC-8	MT872413	2020	Ireland	–	*K. pneumoniae*
CMY-173	MT994367	2020	UK	–	*E. coli*

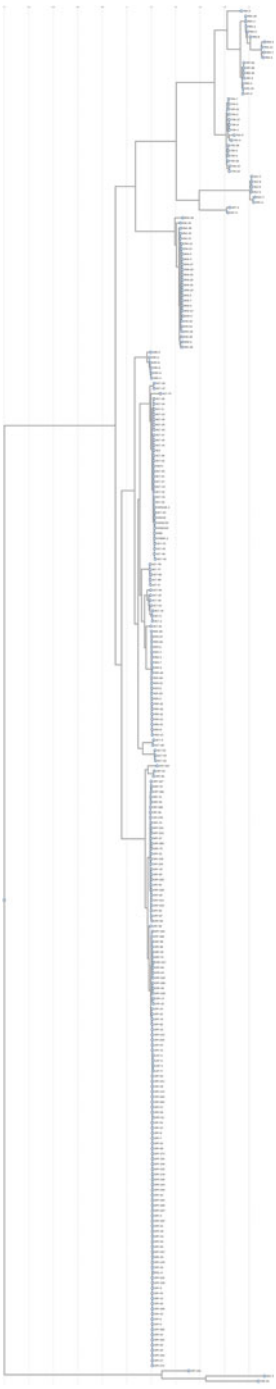


Fig. 6.1 Phylogenetic relation of AmpC type beta-lactamases

basal state, degradation products of the cell wall enter via ampG and are transformed by ampD into UDP-NAM. The interaction between the degradation product (1,6-anhydromuropeptides) and ampR is then exhibited at a low level (Mizrahi et al. 2020). Several opportunistic Gram-negative pathogens carry chromosomal Amp C genes that are not expressed under normal conditions. However, derepression of these, due to genetic mutations that mark the AmpC regulation and transcription or through specific β-lactams induction, can result in high-level expression of AmpC with a subsequent rise in MICs for susceptible β-lactam drugs (Tooke et al. 2019).

Overproduction of AmpC not only induces resistance to cephalosporins, cephamycin, and monobactams but is also responsible for carbapenem resistance (Mirsalehian et al. 2014). Overexpressions of AmpC enzymes do not develop resistance to cefepime, cefpirome and are usually resistant to classical β-lactamase inhibitors. Overproduction of AmpC occurs either from temporary transcription induction of AmpC as a reaction to β-lactams exposure or from the failure of the AmpC regulation system due to one of the regulatory genes mutation controlling the AmpC expression (Mizrahi et al. 2020). Similar to other β-lactamases, they can develop mutations of single amino acid, thereby expanding their specificity to various substrates (Beceiro and Bou 2004; Pérez-Pérez and Hanson 2002). The medical importance of class C enzymes is further augmented by the spread of DHA, FOX, and CMY enzymes, to mobile genetic elements in Gram-negative organisms (Tooke et al. 2019).

Plasmids mediated AmpC enzymes exhibit similar biochemical characteristics and resistance as that of chromosome types, and the same active sites are identified by the sequence analysis of amino acids, containing Ser-X-X-Lys at position 64, Lys-Ser/Thr-Gly at position 315–317, and tyrosine residue at position 150. Certain cephalosporins induce AmpC expression leading to its resistance. The resistance genes are mobilized between the chromosome and the plasmid via insertion sequences, integron, and transposon; thus, pAmpC resistance causes more damage than that caused by the chromosome-mediated AmpC (Luan et al. 2015). pAmpC reported are *E. coli* (CMY-4, CMY-9, CMY-7, CMY-6, FOX-4, FOX-2, LAT-3, LAT-4, and BIL-1), *K. oxytoca* (FOX-3 and CMY-5), *Klebsiella pneumoniae* (CMY-1, CMY-8, CMY-2, LAT-1, LAT-2, LAT-2b, MOX-1, MOX-2, ACT-1, MIR-1, FOX-1, FOX-5, and ACC-1), and *Salmonella enteritidis* (DHA-1) (Lee et al. 2003).

6.2 Epidemiology

The worldwide distribution of pAmpC generating isolates has increased (Rizi et al. 2020). pAmpC can pose danger as they are effectively adaptable among species and can cause an increase in nosocomial infections. In the Netherlands, the prevalence was stated to be 0.6% and 1.3% among non-hospitalized individuals (Rensing et al. 2019). Studies performed in Iran (1.5%) (Elham and Sajedeh 2016), Nigeria (15.2%) (Ogefere Ho and Omoregie 2016), Spain (14.2%) (Gómara-Lomero et al. 2018), and

India (37%) (Shivanna 2017) revealed a greater number of AmpC-producing GNB (Tekele et al. 2020). The pAmpC prevalence has been stated to be 0.59% in Tunisia, 0.09% in Canada, 12% in Pakistan. pAmpC prevalence rates have risen globally over the last decade, including rises from 0.07 to 0.4% in New Zealand, 2.6 to 9.3% in China, 0.32 to 13.2% in the USA, and 0.6 to 4.3% in Korea. There are limited details on the occurrence of pAmpC-β-lactamases in Saudi Arabia and the other Gulf States (Abdalhamid et al. 2017). Explanations for these various findings may be linked to the methods of detection used, participants in the research, geographical area, and the difference in prevalence of AmpC genes (Tekele et al. 2020).

At the species level, research in Denmark, the Czech Republic, and France found that 0.06%, 1.3%, and 0.09% of *E. coli* were pAmpC producers, respectively. In Asia, pAmpC-positive strains of *E. coli* range over a wider spectrum (Rensing et al. 2019). The lowest prevalence was recorded 0.12% and 1.7% of *E. coli* in Japan, 2% of *E. coli* in China and Iran, 2.8% of *E. coli* in Iran. 10.9% of *E. coli* isolates were pAmpC positive in a sample in Turkey (Yamasaki et al. 2010). Tekele et al. in Ethiopia observed that the major AmpC generating GNB was *E. coli* (2.2%) and *K. pneumoniae* (7.3%) (Tekele et al. 2020). Similar results were also found in research conducted in Turkey (Yilmaz No et al. 2013) and Spain (Gómara-Lomero et al. 2018). Several other Indian researches have reported 20.7% of Gram-negative species (Aligarh), 20% of *P. aeruginosa* (Delhi), and 47.8% of *E coli*, 13% of *K. pneumoniae*, and 17.3% of *P. aeruginosa* (Kolkata) as AmpC β-lactamase producers (Jamali et al. 2015). This may be linked to the fact that these genes are present in GNB and are horizontally transferred (Tekele et al. 2020).

Geographical dissemination of various pAmpC types indicates CMY-2 type as the most common, especially in Europe (e.g. Italy, Spain, France, Turkey), Argentina, Canada, Tunisia, Korea, and China. These pAmpC enzymes have been found in *Enterobacteriaceae*, in particular in *Klebsiella pneumoniae*, *Escherichia coli*, etc., and even in naturally occurring AmpC producers such as *Enterobacter aerogenes*, *Enterobacter cloacae*, and *C. freundii* (Chérif et al. 2016). In Amsterdam, a study reported the occurrence of pAmpC (blaFOX, blaMOX, blaDHA, blaACT, blaCMY, blaMIR, and blaACC) among community strains. Studies from Iran and Portugal reported blaDHA, blaCMY, and blaCIT pAmpC in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* (Santiago et al. 2020). In India, Gajamer et al. reported that genes encoding CIT enzymes were more prevalent among isolates producing AmpC, while Jean et al. found blaCMY-2 (Gajamer et al. 2020), Govindaswamy et al. observed predominance of FOX gene (21.9%), followed by CIT (9.19%). Similar research conducted by Manoharan et al. (2016) also found FOX (43.7%) among *E. coli* isolates (Govindaswamy et al. 2018). There are studies of pAmpC genes in *Enterobacter* species including DHA-type genes from Malaysia (Mohd Khari et al. 2016), Spain (Pérez-Llarena et al. 2014), and Taiwan (Kao et al. 2010); CIT and ACC genes in Thailand (Kiratisin and Henprasert 2011); and CIT followed by DHA genes identified in Egypt (Rensing et al. 2019). Though CMY-1/MOX enzymes are thought to be originated from the *Aeromonas* species chromosome ampC gene, significant data is missing. Several types of CMY/MOX-family enzymes have been reported from East Asia and Europe

(CMY-1, MOX-1, CMY-8 to CMY-11, CMY-19), Taiwan/Japan/Korea, *K. pneumoniae*, *K. aerogenes*, *E. coli* and *S. marcescens*; Italy, MOX-9, *Citrobacter freundii*; Greece, MOX-2, *K. pneumoniae*; and China, MOX-4, *Aeromonas caviae*), were available (Ebmeyer et al. 2019). While there is a lack of mobility information for others (*Aeromonas* spp. and *A. caviae*, Spain, MOX-3/-5/-6/-7; and *A. caviae*, Thailand, MOX-8), some other enzymes such as CMY-1/MOX-1 are very widespread in East Asia and compromise the β-lactam drug efficiency, while some are found less frequently (MOX-2 to MOX-9) (Ebmeyer et al. 2019). While plasmids are the primary cause of quick spread of ampC genes, we must be aware that the mobilization of these genes may involve conjugative and integrative elements also (Mata et al. 2012).

6.3 Genetic Environment of AmpC Genes

Plasmid-coded AmpCs are now widespread; they emerge from chromosome variants, comprise other regulatory options, and occur together, inducible, and permanently depressed (Hennequin et al. 2018). They have been disseminated through insertion sequence (IS) elements and transposons and are often associated with integrons. These elements (e.g. IS26, ISEcp1, or ISCR1) have been integrated in near proximity or in an integron and form larger complex forms with other mobile genetic elements (Bohm et al. 2020). Integrons are genetic elements consisting of an *intI* genes coding an integrase, flanked by a recombination site *attI* and a powerful promoter gene, whereas mobile gene cassettes, mainly comprising antibiotic resistance factors, can be incorporated or removed by a site-specific recombination system catalyzed by integrase. Integrons carry dissimilar gene cassettes which are reorganized under specific antibiotic pressure (Goudarzi et al. 2016).

Class 1 integrons, from chromosome structures, are rapidly spread because of natural selection and co-selection (Ghaly et al. 2017). Various ampC genes, such as blaDHA-1, blaMOX-1, blaFOX-4, blaCMY-1, and blaCMY-8, have been stated in a specific class 1 integron, primarily defined in In6 and In7, which contains 2 partial copies of the 3′-CS1 and 3′-CS2 conserved segments all-encompassing the common area and the antibiotic resistance gene (Shahid et al. 2009). Other antimicrobial resistance genes were identified comparatively close to the CR of closely related integrons, including blaCTX-M-9 in In60, qnr in In37, dfrA10 in In34, blaCMY-9 in pCMXR1 in *Escherichia coli*, dfrA10 in *Salmonella enterica* serovar *Agona*, and catA2 in pAr-32 in *Aeromonas salmonicida* (Shahid et al. 2009). Partridge and Hall have shown that CR1 containing the open reading frame (ORF) orf513, CR3 (containing orf2), and CR2 (containing orfA) form a genetic element family that is engaged in the mobility of resistance genes in Gram-negative bacteria. It has been suggested that orf513 facilitates the addition of the resistant genes at the 3′ end of the CR (Partridge and Hall 2003). Many existing resistance gene cassettes and associations to transposable elements have made this process feasible, considerably enhanced by usage of antimicrobials and disinfectants (Bohm et al. 2020).

A few class A β-lactamases, such as BEL, KPC, and GES, and certain class D β-lactamase OXA, are quite well documented as resistance gene cassettes. Class B metallo-β-lactamases, such as VIM or IMP, also are ubiquitous in class 1 integron cassettes (Bohm et al. 2020). A study by Bohm et al. published the first explanation for class C β-lactamase, the blaIDC family (IDC-1, IDC-2), expressed as gene cassettes under class 1 integron (Bohm et al. 2020). These two novel gene cassettes are different from the previously identified ampC genes. Although not detected so far in clinical samples, they are present in hospital and municipal wastewater contaminated samples, revealing their ability to disseminate to human pathogens—or that they are previously present but have evaded detection (Böhm et al. 2020).

As plasmids are the main means of disseminating a wide range of resistance genes, their research and analysis are crucial to changing the rising trend in antibiotic resistance rates globally (Mata et al. 2011a). AmpC genes are noticed on plasmids of the size 7–180 kb (Shahid et al. 2009). Many of the plasmid-coded AmpC enzymes have amino acid sequences similar to chromosomal AmpC enzymes. The pAmpC enzymes of subclass 1 g (DHA-1) are quite like *Morganella morganii* AmpC-SLM01; subclass 1h (MIR-1and ACT-1) are alike to AmpC enzymes (AmpC-MHN1, AmpC-P99, and AmpC-Q908R) of *E. Cloacae*; subclass 1j (BIL-1, CMY-2 to CMY-7, LAT-1, LAT-2, and LAT-3) are relatively comparable to *Citrobacter freundii* AmpC-OS60 (Lee et al. 2003).

The most common blaCMY-2 gene has been found all over the world. This gene is observed in a variety of plasmid environments, implying its mobilization as portion of a smaller transferable fragment. The global spread of plasmid CMY-2 among *Enterobacteriaceae* can be directly related to ISEcp1. *Citrobacter freundii* chromosome blaCMY-2 was believed to be mobilized by ISEcp1 (Helmy and Wasfi 2014). Plasmids carrying blaCTX-M or blaCMY β-lactamase genes are being correlated with transferable replicate types IncA/C or IncI1 (Tran et al. 2020).

A high degree of resemblance between CMY-10 and CMY-1 is also seen which suggests that CMY-10 may be derived from CMY-1. There are reports of MOX-1, CMY-1, and CMY-8 to CMY-10 from East Asia (Lee et al. 2003). The presence of numerous plasmid cephamycinase producers increased the likelihood that cephamycin-resistant genes might disperse to the *Enterobacteriaceae* family, which has been proved by the finding of blaCMY-10, a plasmid cephamycinase gene in *Ent. aerogenes* K9911729 (Lee et al. 2003). There are reports stating the horizontal transfer of beta-lactamase genes mediated by IncF and IncK plasmids in bloodstream isolated *E. coli* strains (Xiao et al. 2019). A study by Mata et al. has stated a close correlation between the plasmid involved and each ampC gene. Plasmids relating to the I1 Inc and A/C groups are the most observed blaCMY-2 carriers followed by IncK plasmids (Mata et al. 2011a). A study by Manageiro et al. described two novel CMY-2-type genes in *C. freundii* fragment. Their research of the sequences around the bla CMY-50 and the bla CMY-46 reported the existence of the sugE gene (coding a small MDR protein) and blc gene (coding outer membrane lipoprotein) in the direction of their open reading frames (Manageiro et al. 2015).

DHA-1, first identified in *Salmonella enteritidis*, can hydrolyze broad-spectrum cephalosporin, penicillins, having left healthcare professionals with restricted antimicrobial selections (Ingti et al. 2017). It was the first inducible plasmid-encoded β-lactamase discovered and it can be expressed at a high level. Up to now 24 gene types have been reported from DHA family. The expression of this enzyme is intimately associated with recycling of the cell wall and is regulated by at least three genes: ampG, ampR, and ampD (Ingti et al. 2017). The blaDHA-1 gene was primarily associated with IncFII plasmids and IncL/M replicates (Mata et al. 2011a). DHA-1 enzymes mobilization has been correlated (Mata et al. 2011a) with IS26 or ISCR1 elements. SXT/R391-like mobile genetic elements carrying blaCMY-2 is noticed in *Proteus mirabilis* (Mata et al. 2011b). DHA-2-type in *K. pneumoniae* is a strain with point-mutation of DHA-1 enzyme and has a 99% similarity with *M. morganii* (Luan et al. 2015).

There are evidences that indicate that CMY-3 and CMY-4 are also transposon-mediated: CMY-3 since its gene is positioned on *P. mirabilis* chromosome which lacks the native AmpC gene, and CMY-4 in *E. coli*, a CMY-4 probe is hybridized to both 45-kb and 7-kb plasmids, a twin location that can be described by transposability (Philippon et al. 2002). The MIR-1 gene is found closely related to the transposase insertion sequence, but attempts to establish the transposability of MIR-1 or BIL-1 (CMY-2) have been unsuccessful (Philippon et al. 2002). The *E. cloacae* ampC gene is generally expressed at a low but in an inducible level. Certain pAmpC genes are inducible but in *E. coli*, MIR-1 is not inducible (Jacoby and Tran 1999). *E. coli*, however, lacks the ampR locus which is required for induction. Consequently, pMG230 was moved to *E. coli* SNO_3/pNU311. SNO_3 is an ampC8, so *E. coli* chromosomal β-lactamase cannot be expressed, while pNU311 carries ampR gene of *Citrobacter freundii*. In this strain, MIR-1 was uninducible with imipenem or cefoxitin, signifying that there were few nucleotide dissimilarities between the blaMIR-1 promoter regions and the chromosomal AmpC genes of *E. Cloacae* are responsible for the induction control escape (Jacoby and Tran 1999).

6.4 Clinical Significance of AmpC Producers

The recovery of AmpC isolates from hospitalized patients after several days of hospitalization has been shown in various epidemiological studies. The affected patients have had a long stay (Mohamudha Parveen et al. 2010). AmpC-producing isolates have also been identified in isolates from rehabilitation centers and outpatient departments in hospitals (Cheng et al. 2019). This resistance mechanism is also known to cause hospital acquired infections, appears to increase the occurrence, and warrants further analysis to determine the best alternatives for detection and management (Mohamudha Parveen et al. 2010).

pAmpC β-lactamases are found globally and *E. coli* tends to be less frequently a source of cefoxitin resistance than increased chromosome production of AmpC enzymes (Jacoby 2009). pAmpC β-lactamases encoding organisms can cause both hospital acquired infections and community infections, including blood stream

infections, urinary tract infections, central nervous system infections like meningitis, wound infections, and respiratory tract infections such as pneumonia. Moreover, various outbreaks have been reported worldwide because of pAmpC β-lactamase harboring organisms (Abdalhamid et al. 2017). Patients with pAmpC producers are frequently affected by co-morbidities (chronic renal disease, diabetes mellitus, abdomino-biliary, and neoplastic) and by invasive procedures including urinary catheterization or the insertion of nasogastric tubes or are artificially ventilated (Conen et al. 2015).

Not only do these infections increase morbidity and mortality, but they are also an infection control concern, as these genes are plasmid-mediated and can be transferred from one organism to another (Abdalhamid et al. 2017). All such genes often are associated with several other resistance determinants such as extended-spectrum β-lactamases (ESBLs), aminoglycoside-modifying enzymes (AMEs), and plasmid-mediated quinolone resistance (PMQR) that cause resistance to beta-lactams, aminoglycosides, and fluoroquinolones, respectively (Abdalhamid et al. 2017).

DHA-1, of the DHA family, has extremely been found among *Enterobacteriaceae* globally and has been extremely worried in the field of medicine as it leads to therapeutic failure (Ingti et al. 2017). DHA-1 is originating from the *M. Morganii* AmpC chromosomal gene. Research conducted by Moland et al. has shown that the mortality rate of patients infected with DHA-1 producing organisms is high when compared to the patients infected with CMY-1 producing pathogens and that there is a concern about the dissemination of this inducible enzyme (Helmy and Wasfi 2014; Moland et al. 2008).

Several risk factors for AmpC *Enterobacteriaceae* blood stream infections (BSI) are being recognized, such as hospital stay, prior antibiotic use, in particular fluoroquinolones, renal transplantation, improper empiric therapy, and presence of vascular and urinary catheters (Chavada et al. 2018). Chaubey et al. observed that patients previously treated with oxyimino-cephalosporins had poorer outcomes when the same drugs were used as empiric therapy for AmpC Blood Stream Infections (Chaubey et al. 2014). Related studies in Europe have noticed *E. coli* producing blaCMY–2 in clinical bloodstream infections, whereas Xiao et al. detected and proved a case of *E. coli* producing $bla_{ADC-162}$ from clinical blood stream infections (Xiao et al. 2019). In a study from Taiwan, the authors reported a clonal spread of *Klebsiella pneumoniae* producing CMY-2 in surgical ICU and that the transmission risk increased due to surgeries (Ko et al. 2009).

Akinyami et al. reported the highest incidence of AmpC fox genes (43.8%) in *S. typhi* strains followed by *S. typhimurium* (25%) (Akinyemi et al. 2017). They also noticed varying capacity of *Salmonella* serotypes to express FOX genes. To give an example, fox gene was found in three ESBL producing *S. typhi* strains, but not expressed. And the same was also noticed in two ESBL producing *S. typhimurium* isolates. These findings may be due to other mechanisms or AmpC types that mediate resistance to cefoxitin in *Salmonella* isolates (Akinyemi et al. 2017). Another study found that 30.4% of AmpC producers had fox genes, 73.9% had MOX genes (including CYM-1), and 56.5% had CIT genes (including CMY-2) in clinical strains of *Enterobacteriaceae* (El-Hady and Adel 2015).

Urinary tract infections are the most common community acquired infections. The prevalence of AmpC genes among *Enterobacteriaceae* is reported in the USA, Korea, India, China, etc., ranging between 1.2% and 2.79% (Lee et al. 2015). Lee et al. observed that the prior history of cerebrovascular accidents and previous use of fluoroquinolones and cephamycins were related with the acquirement of plasmid-mediated *Enterobacteriaceae*-producing AmpCs in urinary tract infections. The most frequently identified pAmpC gene was blaCIT, followed by blaDHA, blaEBC, and blaMOX (Lee et al. 2015). Several studies from Japan, Spain, and Thailand reported CMY-2 as the predominant gene among *E. coli* AmpC producers (Lee et al. 2015).

Multiple drug-resistant organisms carrying such plasmids are major concern as these plasmids may be transmitted between organisms and patients in hospital settings that cause nosocomial outbreaks and pose significant challenges to infection control (Jacoby 2009). These challenges are becoming more difficult, given the fact that there are no standardized procedures for the detection of pAmpC-borne organisms. Adding on, these multiple drug-resistant organisms have also been isolated from farm animals, such as dogs, food products, drinking water supplies and rivers beaches. These sources may be reservoirs for organisms encoding pAmpC, which contribute to their spread and acquisition in both the community and hospitals (Abdalhamid et al. 2017).

6.5 Laboratory Detection of AmpC Producers

The identification of pAmpC is useful for the hospital infection control, as well as for epidemiological studies to prevent hospital acquired infection outbreaks (Rizi et al. 2020). Technical methods to identify AmpC producers are not yet standardized for the laboratories; hence the resistance mechanism is underestimated (Gupta et al. 2014). Reporting of antimicrobial susceptibility results for *Enterobacteriaceae* including *Enterobacter spp*., *Citrobacter* spp., and *Serratia* spp. is indicated by the Clinical and Laboratory Standard Institute (CLSI) strategies depending on phenotypic tests (Cheng et al. 2019). Although these species may become resistant after treatment initiation, initial supplemental tests for inducible resistance are not recommended by the CLSI guidelines (CLSI 2019). Isolates that yield a zone diameter of $\leq$18 mm for cefoxitin by Kirby Bauer method are considered to be possible AmpC producers (Pal et al. 2016) and further proved by phenotype methods (inhibitor-based assay, disc approximation test, AmpC disk test, modified three-dimensional tests (M3DT)) and molecular methods.

6.5.1 Antibiotic Susceptibility Testing

Initial screening for AmpC is done by the Kirby Bauer method. Susceptibility testing is performed on Mueller-Hinton agar (MHA). The bacterial inoculum is swabbed on MHA plate and cefoxitin (30 μg/disk) and cefotetan (30 μg/disk) discs are placed on

the agar plate. Incubate the plate at 35 °C for 16–18 h. For cefoxitin and cefotetan, screening cutoffs of ≤18 and ≤16 mm, respectively, are used as per Clinical and Laboratory Standards Institute (CLSI) guidelines (Polsfuss et al. 2011).

6.5.2 Inhibitor-Based Assay

MHA plates are swabbed with the test isolate. Cefoxitin-boronic acid disks are prepared as mentioned by Coudron (2005). Cefoxitin and cefoxitin with boronic acid discs are placed on the inoculated plates followed by incubation of the plates at 37 °C for 16–18 h. An AmpC producer is a test strain that has a zone diameter of ≥5 mm in the presence of boronic acid when compared to cefoxitin alone (Gupta et al. 2014).

6.5.3 Disc Approximation Test

MHA plates are swabbed with the test strains. In the center of the swabbed plate, a ceftazidime disk (30 μg) is placed. Imipenem (10 μg), cefoxitin (30 μg), and amoxicillin/clavulanate (20/10 μg) disks are then placed at 20 mm from the center of the ceftazidime disk. The inoculated plates are incubated at 37 °C for 16–18 h. Following incubation, if the test isolate shows flattening or an obvious blunting of the zone of inhibition between the ceftazidime disk and the inducing substrates, the isolate is classified as an AmpC producer (El-Hady and Adel 2015).

6.5.4 Modified Three-Dimensional Tests (M3DT)

With the test strain, MHA plates are inoculated and the cefoxitin 30 μg disc is placed in the center of the plate. Using a sterile blade, a 3 cm linear slit is cut, 3 mm away from the cefoxitin disc. At the other end of the slit, a small circular well is rendered using the No-18 shaft needle. The AmpC β-lactamases enzyme extract is prepared by freezing and thawing the test strain 7-8 times and then by centrifugation (2000 rpm for 15 min). This releases the enzymes into the fluid that suspends them. In the well, a total of 20–30 μl of supernatant containing the extract is loaded. To allow the enzyme extract to seep and disperse into the slit, the plates are held for 5-10 min and then incubated at 37 °C for 24 h. The positive result is interpreted as a small heart-shaped indentation towards the cephalosporin disc seen at the split junction along the inhibition axis (Maraskolhe et al. 2014).

6.5.5 AmpC Disk Test

Preceding use, AmpC disks are rehydrated with saline (20 μl) and many colonies of the test isolates are added to the disk. A cefoxitin disc (30 μg) is placed on the

swabbed MHA plate. The inoculated AmpC disk is then placed in contact with the antibiotic disk. The plate is incubated at 35 ° C overnight. After incubation, plates are inspected for indentation or flattening of the zone of inhibition, indicating AmpC positive isolate or for lack of distortion, indicating AmpC negative strain (Black et al. 2005).

6.5.6 Molecular Detection of pAmpC Genes

Multiplex polymerase chain reactions (PCR) are used to identify the pAmpC genes: ACC (expected base pair amplicon size 346), FOX (expected base pair amplicon size 190), MOX (expected base pair amplicon size 520), DHA (expected base pair amplicon size 405), CIT (expected base pair amplicon size 462), and EBC (expected base pair amplicon size 302). Table 6.2 gives the set of PCR primers that are unique to the respective organisms (Pérez-Pérez and Hanson 2002).

DNA template preparation: From a blood agar plate, a colony is inoculated into Luria-Bertani broth (5 ml) and incubated with shaking for 20 h at 37 °C. At centrifugation of 17,310 × g for 5 min, cells from 1.5 ml of overnight culture are collected. The pellet is suspended again into distilled water (500 µl). The cells are lysed at 95 °C for 10 min, and the cellular debris is extracted by centrifugation (17,310 × g) for 5 min. Two microliter supernatant is used as the amplification template source (Pérez-Pérez and Hanson 2002).

PCR protocol: PCR is performed in thin-walled tubes (0.5 ml) with 50 µl final volume. Each reaction includes 20 mM Tris-HCl; 0.2 mM deoxynucleoside triphosphate; 1.5 mM $MgCl_2$; 50 mM KCl; 0.4 µM (FOXMF and FOXMR primers); 0.5 µM (EBCMF, EBCMR, ACCMF, and ACCMR primers); 0.6 µM (MOXMF, MOXMR, CITMF, CITMR, DHAMF, and DHAMR primers); and 1.25 U of Taq DNA polymerase primers. In 48 µl of the master mixture, template DNA (2 µl) is added and then covered with mineral oil. The PCR program consists of an initial

Table 6.2 Polymerase chain reaction primers for amplification of AmpC genes (Pérez-Pérez and Hanson 2002)

Primers	Amplicon size (bp)	Sequence (5′–3′)
MOX	520	Forward: GCT GCT CAA GGA GCA CAG GAT Reverse: CAC ATT GAC ATA GGT GTG GTG C
CIT	462	Forward: TGG CCA GAA CTG ACA GGC AAA Reverse: TTT CTC CTG AAC GTG GCT GGC
DHA	405	Forward: AAC TTT CAC AGG TGT GCT GGG T Reverse: CCG TAC GCA TAC TGG CTT TGC
ACC	346	Forward: AAC AGC CTC AGC AGC CGG TTA Reverse: TTC GCC GCA ATC ATC CCT AGC
EBC	302	Forward: TCG GTA AAG CCG ATG TTG CGG Reverse: CTT CCA CTG CGG CTG CCA GTT
FOX	190	Forward: AAC ATG GGG TAT CAG GGA GAT G Reverse: CAA AGC GCG TAA CCG GAT TGG

denaturation for 3 min at 94 °C; 25 DNA denaturation cycles at 94 °C for 30 s; primer annealing for 30 s at 64 °C; primer extension for 1 min at 72 °C; and a final extension phase at 72 °C for 7 min. The PCR product (5 μl) is analyzed by gel electrophoresis using 2% agarose. The gels are stained with ethidium bromide (10 μg/ml) and visualized by UV light. As a marker, a 100-bp DNA is used. Water is used as the negative control (Pérez-Pérez and Hanson 2002). PCR amplicon sequence analysis can be conducted and analyzed using the GenBank database.

PCR amplicons can be sequenced with the amplification primers and the sequences can be analyzed for similarities by using the GenBank database, the National Center for Biotechnology Information (NCBI) (Polsfuss et al. 2011).

6.6 WAVE DNA Fragment Analysis

It is a nucleic acid analysis technology which is based on high-pressure liquid chromatography used to decrease the total analytical time required without losing sensitivity or specificity. It detects six amplicons in a single multiplex PCR sample. Peaks are observed here, with the retention time of each peak equal to that observed in a single template amplification, and thus the PCR products can be correlated with the chromatogram in the WAVE analysis (Pérez-Pérez and Hanson 2002).

6.7 Multiplex Asymmetric PCR-Based Oligonucleotide Microarray (MAPCR)

Microarray technology enables the continuous processing of a large amount of genetic data in a single assay and eliminates the necessity of gel electrophoresis for analysis of fragment size in order to detect gene variants (Zhu et al. 2007). MAPCR developed by Zhu et al. detected ten known ESBLs and pAmpC in Gram-negative bacteria (Zhu et al. 2007). MAPCR promotes the buildup of single stranded amplifiers suitable for microarray hybridization (Shahid et al. 2009). The simplicity, processing rate, and consistency of this assay make it a valuable tool for major research in epidemiology of pAmpC and ESBL enzymes and can also be a beneficial instrument to support phenotypic testing in clinical laboratories (Zhu et al. 2007).

6.8 Optimal Treatment of Infections Caused by AmpC Producers

A significant alarm has been raised in several studies for the treatment of infections caused by AmpC producers (Rizi et al. 2020). Concerns about persistent antibiotic efficacy against high-level species or the possibility for high-level expression of AmpC make treatment decisions difficult and worrisome (Tamma et al. 2019). If the AmpC production is repressed, the isolates will test as susceptible to cephalosporins but resistance may develop during treatment with these drugs (Jacoby 2009).

pAmpC pose a new threat as they impart cephamycin resistance but do not effectively hydrolyze cefepime (Mohamudha Parveen et al. 2010; Rodríguez-Baño et al. 2018). For AmpC beta-lactamases, carbapenems are the drug of choice including alternatives like the cefepime and beta-lactam/beta-lactamase inhibitors (Rodríguez-Baño et al. 2018).

Carbapenems are stable and are less likely to induce AmpC enzymes, they are considered as the drug of choice for AmpC-producing *Enterobacteriaceae* (Tan et al. 2017). This has contributed to a substantial rise in the worldwide intake of carbapenem, which could be partially attributed to the subsequent dissemination of carbapenem resistance (Van Boeckel et al. 2014). Certain species exhibit carbapenem resistance due to mutations that reduce influx or increase efflux (Jacoby 2009). pAmpC is frequently associated with multidrug resistance and when combined with porin loss can result in carbapenem resistance. As a result, *Enterobacteriaceae*-producing pAmpC-infections have high rates of therapy failure and mortality (Rizi et al. 2020). Concerning *Enterobacteriaceae* containing chromosomal AmpC, recent research reported that carbapenem activity was similar to fluoroquinolones, beta-lactam beta-lactamase inhibitors, or cefepime (Harris et al. 2016). Increasing prevalence of carbapenem resistance worldwide implies a need to look for potential alternative antibiotics (Rodríguez-Baño et al. 2018).

Cefepime is a zwitter ion. It can rapidly penetrate the bacterial outer membranes and is found to be more stable against AmpC enzymes (Cheng et al. 2017). A study by Tamma et al. has stated that the efficacy of cefepime in *Enterobacter* spp. bacteremia is comparable to carbapenems. On comparing cefepime with carbapenem use, they found no difference in the duration of the disease, mortality, or duration of hospital stay (Tamma et al. 2019). Another study on the meta-analysis of bloodstream infections (BSI) caused by *Enterobacteriaceae* harboring chromosomal AmpC found no significant changes in patient outcomes when treated with cefepime or carbapenems (Harris et al. 2016). D'Angelo et al. have also reported the clinically use of cefepime as a carbapenem-sparing substitute. They stated that both carbapenems and cefepime have similar structural characteristics with respect to AmpC β-lactamases susceptibility (D'Angelo et al. 2016). In summary, cefepime appears to be a fair alternative to carbapenems for the treatment of chromosomally mediated AmpC *Enterobacteriaceae* infections. There is very little understanding of cefepime efficacy against pAmpC producers (Rodríguez-Baño et al. 2018).

Broad-spectrum Beta-lactam/Beta-lactamase inhibitors (BLBLI) have been proposed as a substitute in the treatment of AmpC producers. A survey has showed a treatment preference with carbapenems (58%) and cefepime (19%) in *Enterobacter* spp. bacteremia, and a small minority prescribed piperacillin-tazobactam (Harris et al. 2016). In a retrospective study, Cheng et al. found no substantial difference in the mortality rate between patients treated with piperacillin-tazobactam and also with cefepime or meropenem, in *Enterobacteriaceae*-infected cases. These reports support the clinical use of piperacillin-tazobactam as a therapeutic choice for treating bloodstream infections by AmpC strains (Cheng et al. 2017). Since Piperacillin-tazobactam is a weak AmpC inducer, they can be used as a potential alternative.

Nevertheless, the efficacy of this combination has not been fully explained (Schwaber et al. 2003; Cheng et al. 2017).

Temocillin, a 6-α-methoxy derivative of ticarcillin, available only in some parts of the world is also active in vitro against *Enterobacteriaceae (*AmpC producers) (Jacoby 2009). No clinical trials have been found to equate temocillin with carbapenems or other antimicrobials in AmpC-producing *Enterobacteriaceae* infections (Rodríguez-Baño et al. 2018).

Tigecycline, a glycylcycline, is another alternative drug which has exhibited strong in vitro activity against AmpC-hyper-producing strains of *E. coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. from the UK (Jacoby 2009). Importantly, both the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) provided alerts that the medication was associated with clinical failure and an increased risk of mortality in randomized trial meta-analysis. Tigecycline is therefore recommended only if other solutions are not suitable or not available (Rodríguez-Baño et al. 2018; Shen et al. 2015).

In summary, AmpC producers play a significant role in therapeutic decisions. There is no Clinical Laboratory Standards Institute (CLSI) guidelines or other accepted criteria for AmpC detection. Therefore, lab diagnosis and treatment should be performed with caution. While there are no persuading clinical evidence recommending that carbapenems are better than other elective medications for infections caused by AmpC producers, carbapenem treatment could be a powerful treatment of decision dependent upon the patients' illness.

References

Abdalhamid B, Albunayan S, Shaikh A, Elhadi N, Aljindan R (2017) Prevalence study of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking inducible ampC from Saudi hospitals. J Med Microbiol 66:1286–1290

Akinyemi IB, Oyefolu A, Fakorede C (2017) Occurrence of extended-spectrum and AmpC β-lactamases in multiple drug resistant Salmonella isolates from clinical samples in Lagos. Infect Drug Resist 10:19–25

Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z (2018) Antibiotic resistance: a rundown of a global crisis. Inf Drug Resist 11:1645–1658

Beceiro A, Bou G (2004) Class C β-Lactamases: an increasing problem worldwide. Rev Med Microbiol 15:141–152

Black JA, Moland ES, Thomson KS (2005) AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in Enterobacteriaceae lacking chromosomal AmpC beta-lactamases. J Clin Microbiol 43:3110–3113

Böhm M-E, Razavi M, Marathe NP, Flach C-F, Larsson DGJ (2020) Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities. Microbiome 8:41

Bohm ME, Razavi M, Flach CF, Larsson DGJ (2020) A novel, integron-regulated, class C beta-Lactamase. Antibiotics 9:123

Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211–1233

Chaubey VP, Pitout JDD, Dalton B, Gregson DB, Ross T, Laupland KB (2014) Clinical and microbiological characteristics of bloodstream infections due to AmpC β-lactamase producing Enterobacteriaceae: an active surveillance cohort in a large centralized Canadian region. BMC Infect Dis 14:647–647

Chavada R, Tong D, Maley M (2018) In-hospital surgery as a risk factor for onset of AmpC-producing escherichia coli blood stream infections. Diseases 6:71

Cheng L, Nelson BC, Mehta M, Seval N, Park S, Giddins MJ, Shi Q, Whittier S, Gomez-Simmonds A, Uhlemann A-C (2017) Piperacillin-tazobactam versus other antibacterial agents for treatment of bloodstream infections due to AmpC β-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother 61:e00276

Cheng MP, Lee RS, Cheng AP, De L'étoile-Morel S, Demir K, Yansouni CP, Harris P, Mcdonald EG, Lee TC (2019) Beta-lactam/beta-lactamase inhibitor therapy for potential ampc-producing organisms: a systematic review and meta-analysis. Open Forum Infect Dis 6:248

Chérif T, Saidani M, Decré D, Boutiba-Ben Boubaker I, Arlet G (2016) Cooccurrence of multiple AmpC β-lactamases in Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis in Tunisia. Antimicrob Agents Chemother 60:44–51

CLSI (2019) Performance standards for antimicrobial susceptibility testing, 29th edn. Clinical and Laboratory Standards Institute, Wayne

Conen A, Frei R, Adler H, Dangel M, Fux CA, Widmer AF (2015) Microbiological screening is necessary to distinguish carriers of plasmid-mediated AmpC beta-lactamase-producing Enterobacteriaceae and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae because of clinical similarity. PLoS One 10:e0120688

Coudron PE (2005) Inhibitor-based methods for detection of plasmid-mediated AmpC beta-lactamases in Klebsiella spp., Escherichia coli, and Proteus mirabilis. J Clin Microbiol 43: 4163–4167

D'angelo RG, Johnson JK, Bork JT, Heil EL (2016) Treatment options for extended-spectrum beta-lactamase (ESBL) and AmpC-producing bacteria. Expert Opin Pharmacother 17:953–967

Ebmeyer S, Kristiansson E, Larsson DGJ (2019) CMY-1/MOX-family AmpC β-lactamases MOX-1, MOX-2 and MOX-9 were mobilized independently from three Aeromonas species. J Antimicrob Chemother 74:1202–1206

El-Hady SA, Adel LA (2015) Occurrence and detection of AmpC β-lactamases among Enterobacteriaceae isolates from patients at Ain Shams University Hospital. Egypt J Med Hum Genet 16:239–244

Elham F, Sajedeh K (2016) Prevalence of AmpC type extended spectrum beta lactamases genes in clinical Samples of E. coli isolated from poultry and humans. Int J Med Res Health Sci 5:83–93

Eriksson-Grennberg KG, Boman HG, Jansson J, Thorén S (1965) Resistance of Escherichia coli to Penicillins I. Genetic study of some ampicillin-resistant mutants. J Bacteriol 90:54–62

Gajamer VR, Bhattacharjee A, Paul D, Ingti B, Sarkar A, Kapil J, Singh AK, Pradhan N, Tiwari HK (2020) High prevalence of carbapenemase, AmpC β-lactamase and aminoglycoside resistance genes in extended-spectrum β-lactamase-positive uropathogens from Northern India. J Glob Antimicrob Resist 20:197–203

Ghaly TM, Chow L, Asher AJ, Waldron LS, Gillings MR (2017) Evolution of class 1 integrons: mobilization and dispersal via food-borne bacteria. PLoS ONE 12:e0179169

Gómara-Lomero A, Vela Iglesia BM, Cerón I, López A, Pinilla M (2018) Detection of carbapenemases and other mechanisms of enzymatic resistance to beta-lactams in Enterobacteriaceae with diminished susceptibility to carbapenems in a tertiary care hospital. Enferm Infecc Microbiol Clin 36:296–301

Goudarzi H, Azad M, Seyedjavadi SS, Azimi H, Salimi Chirani A, Fallah Omrani V, Goudarzi M (2016) Characterization of integrons and associated gene cassettes in Acinetobacter baumannii strains isolated from intensive care unit in Tehran, Iran. J Acute Dis 5:386–392

Govindaswamy A, Bajpai V, Batra P, Malhotra R, Mathur P (2018) Phenotypic and molecular characterization of extended spectrum beta lactamase and AmpC beta lactamases in Escherichia coli from a tertiary care centre in India. J Patient Saf Inf Control 6:54–58

Gupta G, Tak V, Mathur P (2014) Detection of AmpC β lactamases in gram-negative bacteria. J Lab Phys 6:1–6

Halat DH, Sarkis DK, Moubareck CA (2016) Chapter 5 - carbapenem-resistant, gram-negative bacilli: the state of the art. In: Kon K, Rai M (eds) Antibiotic Resistance. Academic Press, Cambridge

Harris PN, Wei JY, Shen AW, Abdile AA, Paynter S, Huxley RR, Pandeya N, Doi Y, Huh K, O'neal CS, Talbot TR, Paterson DL (2016) Carbapenems versus alternative antibiotics for the treatment of bloodstream infections caused by Enterobacter, Citrobacter or Serratia species: a systematic review with meta-analysis. J Antimicrob Chemother 71:296–306

Helmy MM, Wasfi R (2014) Phenotypic and molecular characterization of plasmid mediated AmpC β-lactamases among Escherichia coli, Klebsiella spp., and Proteus mirabilis isolated from urinary tract infections in Egyptian hospitals. Biomed Res Int 2014:171548–171548

Hennequin C, Ravet V, Robin F (2018) Plasmids carrying DHA-1 β-lactamases. Eur J Clin Microbiol Infect Dis 37:1197–1209

Ingti B, Paul D, Maurya AP, Bora D, Chanda DD, Chakravarty A, Bhattacharjee A (2017) Occurrence of bla (DHA-1) mediated cephalosporin resistance in Escherichia coli and their transcriptional response against cephalosporin stress: a report from India. Ann Clin Microbiol Antimicrob 16:13–13

Jacobs C, Joris B, Jamin M, Klarsov K, Van Beeumen J, Mengin-Lecreulx D, Van Heijenoort J, Park JT, Normark S, Frère JM (1995) AmpD, essential for both β-lactamase regulation and cell wall recycling, is a novel cytosolic N-acetylmuramyl-L-alanine amidase. Mol Microbiol 15: 553–559

Jacoby GA (2009) AmpC β-Lactamases. Clin Microbiol Rev 22:161–182

Jacoby GA, Tran J (1999) Sequence of the MIR-1 β-lactamase gene. Antimicrob Agents Chemother 43:1759–1760

Jamali S, Sobia F, Singh A, Khan H (2015) AmpC β-lactamases among Pseudomonas and Acinetobacter species isolates, from a tertiary hospital of North India. Int J Adv Res 3:361–367

Juan C, Moyá B, Pérez JL, Oliver A (2006) Stepwise upregulation of the *Pseudomonas aeruginosa* chromosomal cephalosporinase conferring high-level β-lactam resistance involves three AmpD homologues. Antimicrob Agents Chemother 50:1780–1787

Kao C-C, Liu M-F, Lin C-F, Huang Y-C, Liu P-Y, Chang C-W, Shi Z-Y (2010) Antimicrobial susceptibility and multiplex PCR screening of AmpC genes from isolates of Enterobacter cloacae, Citrobacter freundii, and Serratia marcescens. J Microbiol Immunol Infect 43:180–187

Kiratisin P, Henprasert A (2011) Resistance phenotype-genotype correlation and molecular epidemiology of Citrobacter, Enterobacter, Proteus, Providencia, Salmonella and Serratia that carry extended-spectrum β-lactamases with or without plasmid-mediated AmpC β-lactamase genes in Thailand. Trans R Soc Trop Med Hyg 105:46–51

Ko WC, Lee HC, Yan JJ, Wu JJ, Chang CM, Wu CJ, Lee NY, Chen PL, Lee CC (2009) Clonal spread of Klebsiella pneumoniae producing CMY-2 AmpC-type beta-lactamase in surgical intensive care units. J Microbiol Immunol Infect 42:479–487

Lee SH, Jeong SH, Park Y-M (2003) Characterization of blaCMY-10 a novel, plasmid-encoded AmpC-type β-lactamase gene in a clinical isolate of Enterobacter aerogenes. J Appl Microbiol 95:744–752

Lee C-H, Lee Y-T, Kung C-H, Ku W-W, Kuo S-C, Chen T-L, Fung C-P (2015) Risk factors of community-onset urinary tract infections caused by plasmid-mediated AmpC β-lactamase-producing Enterobacteriaceae. J Microbiol Immunol Infect 48:269–275

Luan Y, Li G-L, Duo L-B, Wang W-P, Wang C-Y, Zhang H-G, He F, He X, Chen S-J, Luo D-T (2015) DHA-1 plasmid-mediated AmpC β-lactamase expression and regulation of Klebsiella pnuemoniae isolates. Mol Med Rep 11:3069–3077

Manageiro V, Ferreira E, Pinto M, Fonseca F, Ferreira M, Bonnet R, Caniça M (2015) Two novel CMY-2-type β-lactamases encountered in clinical Escherichia coli isolates. Ann Clin Microbiol Antimicrob 14:12

Manoharan A, Barla GS, Peter R, Sugumar M, Mathai D (2016) Multidrug resistance mediated by co-carriage of extended-spectrum beta-lactamases, AmpC and New Delhi metallo-beta-lactamase-1 genes among carbapenem-resistant Enterobacteriaceae at five Indian medical centres. Indian J Med Microbiol 34:359–361

Maraskolhe DL, Deotale VS, Mendiratta DK, Narang P (2014) Comparision of three laboratory tests for detection of AmpC β lactamases in Klebsiella species and E. coli. JCDR 8:8

Mata C, Miró E, Alvarado A, Garcillán-Barcia MP, Toleman M, Walsh TR, De La Cruz F, Navarro F (2011a) Plasmid typing and genetic context of AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes: findings from a Spanish hospital 1999–2007. J Antimicrob Chemother 67:115–122

Mata C, Navarro F, Miró E, Walsh TR, Mirelis B, Toleman M (2011b) Prevalence of SXT/R391-like integrative and conjugative elements carrying blaCMY-2 in Proteus mirabilis. J Antimicrob Chemother 66:2266–2270

Mata C, Miró E, Alvarado A, Garcillán-Barcia M, Toleman M, Walsh T, Cruz F, Navarro F (2012) Plasmid typing and genetic context of AmpC-lactamases in Enterobacteriaceae lacking inducible chromosomal ampC genes: findings from a Spanish hospital 1999-2007. J Antimicrob Chemother 67:115–122

Mirsalehian A, Kalantar-Neyestanaki D, Nourijelyani K, Asadollahi K, Taherikalani M, Emaneini M, Jabalameli F (2014) Detection of AmpC-β-lactamases producing isolates among carbapenem resistant P. aeruginosa isolated from burn patient. Iran J Microbiol 6:306–310

Mizrahi A, Delerue T, Morel H, Le Monnier A, Carbonnelle E, Pilmis B, Zahar JR (2020) Infections caused by naturally AmpC-producing Enterobacteriaceae: can we use third-generation cephalosporins? A narrative review. Int J Antimicrob Agents 55:105834

Mohamudha Parveen R, Harish BN, Parija SC (2010) Ampc beta lactamases among gram negative clinical isolates from a tertiary hospital, South India. Braz J Microbiol 41:596–602

Mohd Khari FI, Karunakaran R, Rosli R, Tee Tay S (2016) Genotypic and phenotypic detection of AmpC β-lactamases in Enterobacter spp. isolated from a teaching hospital in Malaysia. PLoS One 11:e0150643

Moland ES, Kim S-Y, Hong SG, Thomson KS (2008) Newer β-lactamases: clinical and laboratory implications, part I* *Editor's note: part II of this article will be published in the June 1, 2008 issue of CMN (Vol. 30, No. 11). Clin Microbiol Newsl 30:71–77

Ogefere Ho OJ, Omoregie R (2016) Prevalence of AmpC beta-lactamase among gram negative bacteria recovered from clinical specimens in Benin City, Nigeria. Pharm Res 15:1947

Oliveira C, Amador P, Prudêncio C, Tomaz CT, Tavares-Ratado P, Fernandes R (2019) ESBL and AmpC β-lactamases in clinical strains of Escherichia coli from Serra da Estrela, Portugal. Medicina 55:272

Pal N, Hooja S, Sharma R, Maheshwari RK (2016) Phenotypic detection and antibiogram of β-lactamase-producing proteus species in a tertiary care hospital, India. Ann Med Health Sci Res 6:267–273

Partridge SR, Hall RM (2003) In34, a complex In5 family class 1 integron containing orf513 and dfrA10. Antimicrob Agents Chemother 47:342–349

Pérez-Llarena FJ, Zamorano L, Kerff F, Beceiro A, García P, Miró E, Larrosa N, Gómez-Bertomeu F, Méndez JA, González-López JJ, Oliver A, Galleni M, Navarro F, Bou G (2014) Genetic and kinetic characterization of the novel AmpC β-Lactamases DHA-6 and DHA-7. Antimicrob Agents Chemother 58:6544–6549

Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162

Philippon A, Arlet G, Jacoby GA (2002) Plasmid-determined AmpC-type β-Lactamases. Antimicrob Agents Chemother 46:1–11

Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M (2011) Practical approach for reliable detection of AmpC beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol 49:2798–2803

Rensing KL, Abdallah HM, Koek A, Elmowalid GA, Vandenbroucke-Grauls CMJE, Al Naiemi N, Van Dijk K (2019) Prevalence of plasmid-mediated AmpC in Enterobacteriaceae isolated from humans and from retail meat in Zagazig, Egypt. Antimicrob Resist Infect Control 8:45

Rizi KS, Mosavat A, Youssefi M, Jamehdar SA, Ghazvini K, Safdari H, Amini Y, Farsiani H (2020) High prevalence of blaCMY AmpC beta-lactamase in ESBL co-producing Escherichia coli and Klebsiella spp. clinical isolates in the northeast of Iran. J Glob Antimicrob Resist 22:477–482

Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A (2018) Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clin Microbiol Rev 31:e00079

Santiago GS, Gonçalves D, Da Silva Coelho I, De Oliveira Coelho S, Neto Ferreira H (2020) Conjugative plasmidic AmpC detected in Escherichia coli, Proteus mirabilis and Klebsiella pneumoniae human clinical isolates from Portugal. Braz J Microbiol 51(4):1807–1812

Schwaber MJ, Graham CS, Sands BE, Gold HS, Carmeli Y (2003) Treatment with a broad-spectrum cephalosporin versus piperacillin-tazobactam and the risk for isolation of broad-spectrum cephalosporin-resistant Enterobacter species. Antimicrob Agents Chemother 47: 1882–1886

Shahid M, Sobia F, Singh A, Khan H, Hawkey P, Huq A, Khardori N (2009) AmpC bete-lactamases and bacterial resistance: an updated mini review. Rev Med Microbiol 20(41):55

Shen F, Han Q, Xie D, Fang M, Zeng H, Deng Y (2015) Efficacy and safety of tigecycline for the treatment of severe infectious diseases: an updated meta-analysis of RCTs. Int J Infect Dis 39: 25–33

Shivanna V (2017) Detection of co-existence of beta-lactamases in gram negative bacteria using disc potentiation tests. Indian J Microbiol Res 4:64–67

Tamma PD, Doi Y, Bonomo RA, Johnson JK, Simner PJ (2019) A Primer on AmpC beta-Lactamases: Necessary Knowledge for an Increasingly Multidrug-resistant World. Clin Infect Dis 69:1446–1455

Tan S, Yap M, Cheok S, Ng T, Lye D (2017) Treatment of AmpC beta-lactamase-producing Enterobacteriaceae bacteraemia - analysis of effects of empirical piperacillin-tazobactam versus carbapenems. Available: https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=41376. Accessed 22 November 2020

Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH (2020) Extended-spectrum Beta-lactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. PLoS One 15:e0241984

Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J (2019) Beta-lactamases and beta-lactamase inhibitors in the 21st century. J Mol Biol 431:3472–3500

Tran T, Checkley S, Caffrey N, Cassis R, Mainali C, Gow S, Agunos A, Liljebjelke K (2020) Genetic characterization of AmpC and extended-spectrum beta-lactamase (ESBL) phenotypes in Escherichia coli and Salmonella from Alberta poultry. bioRxiv, 2020.08.11.246645

Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis 14:742–750

Xiao L, Wang X, Kong N, Zhang L, Cao M, Sun M, Wei Q, Liu W (2019) Characterization of Beta-Lactamases in Bloodstream-Infection Escherichia coli: Dissemination of blaADC–162 and blaCMY–2 Among Bacteria via an IncF Plasmid. Front Microbiol 10:02175

Yamasaki K, Komatsu M, Abe N, Fukuda S, Miyamoto Y, Higuchi T, Ono T, Nishio H, Sueyoshi N, Kida K, Satoh K, Toyokawa M, Nishi I, Sakamoto M, Akagi M, Nakai I, Kofuku T, Orita T, Wada Y, Yamamoto Y (2010) Laboratory surveillance for prospective plasmid-mediated AmpC-lactamases in the Kinki Region of Japan. J Clin Microbiol 48:3267–3273

Yilmaz No AN, Bozcal E, Oner O, Uzel A (2013) Detection of plasmid-mediated AmpC β-lactamase in Escherichia coli and Klebsiella pneumoniae. Indian J Med Microbiol 31:53–59

Zhu L-X, Zhang Z-W, Liang D, Jiang D, Wang C, Du N, Zhang Q, Mitchelson K, Cheng J (2007) Multiplex asymmetric PCR-based oligonucleotide microarray for detection of drug resistance genes containing single mutations in Enterobacteriaceae. Antimicrob Agents Chemother 51: 3707–3713

Class D Type Beta-Lactamases

7

Fatima Khan, Bhanu Chaudhary, and Asad U. Khan

Abstract

Among various β-lactam hydrolyzing enzymes, classified from group A to D, the most genetically as well as biochemically diverse is the class D β-lactamases (DBL), few of which can incapacitate the complete spectrum of β-lactamases. DBLs, like class A and C, are active serine site enzymes, differing from them in amino acid structure. The DBLs form an enzyme substrate complex with β-lactam antibiotics in the periplasmic space leading to their hydrolysis with Ser70 serving as the active site. DBLs can be acquired and natural. Acquired DBLs are classified into narrow spectrum, extended spectrum, and carbapenem-hydrolyzing β-lactamases (CHDLs). Detection of class D β-lactamases is crucial yet challenging due to the lack of appropriate and standardized phenotypic assays. However, currently, molecular detection of the DBL genes is the only standardized method of identification of class D β-lactamases. Intensive research is required for developing rapid and easy detection tools for DBLs and for the discovery of class D specific inhibitors.

Keywords

Class D β-lactamases · Antimicrobials · Resistance

F. Khan · B. Chaudhary
Microbiology Department, Faculty of Medicine, Aligarh Muslim University, Aligarh, India

A. U. Khan (✉)
Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_7

7.1 Introduction

β lactam antibiotics have had been the vanguard of antimicrobial chemotherapy despite their use of over half a century. Of late, majority of these antibiotics are rendered ineffective due to the increasing antimicrobial resistance. Of the various mechanisms of the antimicrobial resistance, presence of bacterial enzymes remains the smartest and traditional mechanism of acquired as well as innate resistance. Among the various β lactam hydrolyzing enzymes, classified from group A to D, the most genetically as well as biochemically diverse is the class D β lactamases (DBLs), few of which can incapacitate the complete spectrum of β lactamases (i.e., penicillins, cephalosporins, and carbapenems) (Leonard et al. 2012; Poirel et al. 2010). Clinically they may be signified into narrow spectrum (effective against earliest generation penicillins and cephalosporins), extended spectrum (hydrolyze later generation cephalosporins), and the most concerning are the DBLs that hydrolyze clinically important carbapenems (e.g., imipenem). They are the largest growing family of β lactamases based on the percentage of new enzymes and their variants (Bush 2013). Their genes may be chromosomal or may be located on plasmids of gram-negative pathogens like *Acinetobacter*, *Shewanella*, *Pseudomonas*, *Burkholderia*, and few gram-positive microbes also (Sanschagrin et al. 1995; Poirel et al. 2010; Toth et al. 2016). These enzymes are easily transferred between the species due to their association with integrons, insertion sequences, and transposons and are a formidable threat to hospitalized patients (Naas and Nordmann 1999). When associated with other enzymes on the same plasmid they result in synergized phenotypic resistance spectrum narrowing the treatment options (Mendes et al. 2009).

7.2 General Properties

DBLs, similar to class A and C, are active serine site enzymes differing from them in amino acid structure. In contrast, class B β lactamases have a Zn^{2+} ion(s) at the active site and are considered as metallo-enzymes (Ambler 1980; Jaurin and Grundstrom 1981; Lamotte-Brasseur et al. 1994). DBLs are highly diverse in sequence and show less than 20% homology with class A and C enzymes (Couture et al. 1992). However, the topological fold is preserved among the three classes and more so within class D (Fisher et al. 2005).

Also known as OXA-type enzymes or oxacillinases, DBLs include more than 400 genetically diverse enzymes described (Bush 2013) predominantly in gram-negative pathogens (e.g., *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Enterobacterales*), along with the gram-positive pathogens (Walther-Rasmussen and Høiby 2006; Bush and Fisher 2011).

Majority DBLs hydrolyze cloxacillin or oxacillin at a rate of more than 50% higher than that for benzylpenicillin, although this generalization is no longer valid (Fisher et al. 2005; Walther-Rasmussen and Høiby 2006). They are characteristically not inhibited by β-lactamase inhibitors such as clavulanic acid, tazobactam, and

sulbactam (Payne et al. 1994; Bush et al. 1995), with a few exceptions, such as OXA-2, OXA-29, and OXA-32 inhibited by tazobactam, and OXA-53, inhibited by clavulanic acid (Franceschini et al. 2001; Mulvey 2004; Naas and Nordmann 1999). However, they are susceptible towards the recently developed inhibitors like avibactum and vaborbactam (Voha et al. 2006; Schneider et al. 2006).

7.3 Occurrence of DBLs

DBLs genes have been shown to be acquired as well as are naturally present in pathogens as well as environmental microbes (Poirel et al. 2010). The naturally occurring OXA β lactamases are found as cluster in bacterial species (Yoon and Jeong 2020). Few notable innate DBLs include OXA-22 in *Ralstonia* species (Nordmann et al. 2000; Jiang et al. 2020), OXA-42 like enzymes in *Burkholderia pseudomallei* (Niumsup and Wuthiekanun 2002), OXA-12 like subfamilies in *Aeromonas* species (Walsh et al. 1995), OXA-22 subfamilies in *Acinetobacter* species (Tian et al. 2018; Périchon et al. 2013). Mostly the innate DBLs occur as survival machinery in environmental bacteria (Yoon and Jeong 2020).

Rampant antimicrobial use/misuse/overuse creates a castigatory environment for the clinical bacterial isolates, which thus acquire various resistance mechanisms. The attainment of resistance genes for DBLs is mostly through mobile genetic elements like ISs using transposons or integrons and less commonly by homologous recombination (Yoon and Jeong 2020). The genes for NS and ES DBLs are frequently found as gene cassettes on class 1 integrons or less commonly on class 3 integrons, whereas genes for CHDLs are usually associated with ISs associated with transposons (Yoon and Jeong 2020).

7.4 Mechanism of Action

The DBLs form an enzyme substrate complex with β-lactam antibiotics in the periplasmic space leading to their hydrolysis. Like other beta-lactamases OXA-β lactamases or DBLs also have Ser70 that serves as the active site. However, DBL has a special hydrophobic active site compared to other β-lactamases.

Lys73 present in the Ser70-X-X-Lys motif in the DBL undergoes N-carboxylation post-translationally to become carbamylated lysine. A strongly hydrophobic active site helps create the conditions that allow the lysine to combine with CO_2, and the resulting carbamate is stabilized by a number of hydrogen bonds (Leonard et al. 2012). Trp158 interacts with the carboxylate group of carbamylated Lys73 to form the active site channel. The Ser70 active site in DBL undergoes transient acylation and mimics the penicillin binding proteins (PBPs). The lysine carbamate is essential in acetylation and diacylation step in DBL catalysis, it serves as a general base to activate the serine nucleophile in the acylation reaction and the deacylating water in the second step.

7.5 Classification

The two classic and most frequently schemes for classification of β lactamases include molecular structure classification using the Ambler method (Ambler 1980) and functional classification using the Bush–Jacoby–Medeiros method (Bush 2013; Bush et al. 1995). β-lactamases are divided into four classes A, B, C, and D in the Ambler classification, by motifs composed of primary sequences constituting the protein molecules. Class A, C, and D β-lactamases use serine at the enzyme active center, whereas class B β-lactamases use metal zinc ions. In functional classification using the Bush–Jacoby–Medeiros method, β-lactamases are classified into groups 1–3 based on the hydrolysis of β-lactam substrates and the effect of the inhibitor.

According to the Bush–Jacoby classification based on substrate hydrolysis, DBLs are classified into group 2d. Those hydrolyzing extended spectrum cephalosporins into 2de, carbapenems into 2df and those hydrolyzing both extended spectrum cephalosporins and carbapenems in group 2def (Bush 2013).

DBLs can be divided into acquired and natural. Acquired DBLs are classified into narrow spectrum, extended spectrum, and carbapenem hydrolyzing β lactamases (CHDLs).

1. Acquired narrow spectrum class D β lactamases (NS-DBL): Important examples include OXA 1, OXA 2, OXA 10 (Poirel et al. 2010). Others acquired narrow spectrum DBLs include OXA 9, 18, 12, 20, LCR 1, NPS 1 (Poirel et al. 2010). OXA 30 and 1 are the same, due to an original sequencing error during identification leading to a mistake (Boyd and Mulvey 2006).
   OXA 1 has less than 30% homology with plasmid and chromosomal DBLs (Antunes et al. 2014). Since it is a narrow spectrum DBL it hydrolyses amino and ureidopenicillins and decreases the susceptibility to cephalothin, cefotaxime, and cefepime. However, it has no effect on carbapenems and ceftazidime. OXA 1 and OXA 31, which differ from it by two amino acid sequences, possess the ability to hydrolyze cefepime and cefpirome slightly. These can be considered to be extended spectrum DBLs for bacterial species with high level intrinsic impermeability (e.g. *Pseudomonas* species) and not on bacterial species with low level intrinsic permeability (e.g. *E. coli*) (Poirel et al. 2010). The OXA 1 gene is allied with class 1 integrons and is surrounded by the integrase and aminoglycoside aminoacyl transferase gene (Siu et al. 2000; Moura et al. 2012).

   OXA 2 shares another cluster with its derivatives OXA 3, OXA 15, OXA 21, OXA 32, OXA 34, OXA 36, and OXA 53 and has 30% homology with OXA 1 (Kratz et al. 1983). OXA 2 has been identified in varying clinical species like *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Morganella morganii*, *Klebsiella pneumoniae*, *Bordetella bronchiseptica*, *Aeromonas hydrophila*, and even gram-positive microbes like *Corynebacterium amycolatum.* OXA 2 is historical and can be tracked back to 1970s, characterized by hydrolysis of oxacillin many times higher than for benzylpenicillin (Suzuki et al. 2015). Although grouped with the narrow spectrum DBLs, studies have shown that OXA 2 is a

CHDL (Antunes et al. 2014). Unlike other DBLs, it is inhibited by clavulanic acid and tazobactam.

OXA 10 The OXA 10 DBL (formerly known as PSE-2), originally found in Pseudomonas (Matthew and Sykes 1977), is now detected in a wide variety of gram-negative bacterial pathogens (Fournier et al. 2006; Centron and Roy 2002; Kumar and Thomas 2011). It hydrolyses cephalosporins including cefotaxime, ceftriaxone, aztreonam, but not ceftazidime, cephamycins, and carbapenems (Huovinen et al. 1988). Point mutation derivatives of OXA 10 (OXA 11, 13, 16, 28, 35, and 74) show extended spectrum of activity against cephalosporins (Poirel et al. 2010).

Other narrow spectrum DBLs include LCR-1, NPS-1, OXA 20, and OXA 46.

2. Extended spectrum class D β lactamases (ES-DBL): These are mostly point mutation (clustered around the active site tryptophan) derivatives of the narrow spectrum DBLs and obviously pose a greater clinical challenge as they hydrolyze later generation cephalosporins that contain bulkier side chain constituents (e.g. cefotaxime, ceftazidime, and cefepime) (Leonard et al. 2012). Generally, members of the NS-OXA and CHDL transform their substrate profile to that of ES-DBLs. OXA-2 like and OXA-10 like subfamilies primarily consist of ES-DBLs (Yoon and Jeong 2020). OXA 15, derivative of OXA 2, was the first ES-DBL described (Gly replacing Asp at 150 position in the DBL numbering system) (Danel et al. 1997). OXA 32 is another derivative of OXA 2 (Leu 169 Ile substitution) (Poirel et al. 2002). A number of ES-DBL variants of OXA-10 have been identified, which include OXA 11 (with two substitutions at 146 and 167 BDL numbering system) (Hall et al. 1993), OXA 14 (Gly 167 Asp change) (Danel et al. 1995), OXA 16 (Ala114Thr and Gly167 Asp changes) (Danel et al. 1998), OXA 17 (Asn 76 Ser change) (Danel et al. 1999).
   Other ES-DBLs like OXA 18, OXA-45 and OXA- 53 are extended spectrum β lactamases which are not structurally related to narrow spectrum OXAs. OXA 18 displays resistance to high level cephalosporins, but not cephamycins and carbapenems and unlike classic OXA DBLs are inhibited by clavulanic acid (Philippon et al. 1997). OXA 45 and OXA 53, similar to OXA 18 confer resistance to wide range of cephalosporins and are inhibited by clavulanic acid. OXA 18 is chromosomal (Naas et al. 2008), OXA 45 plasmid, while OXA 53 gene is plasmid and integron borne (Mulvey 2004).
3. Acquired carbapenem hydrolyzing class D β lactamases (CHDLs): Of most concern are DBLs with the ability to hydrolyze carbapenems leading to treatment failures. Most of the CHDLs are found in Acinetobacter species. Of note is these carbapenem hydrolyzing CHDLs is the inability or low capacity to hydrolyze expanded spectrum cephalosporins (Poirel et al. 2010). OXA 23 (also known as ARI-1) was the first reported CHDL, detected in *Acinetobacter baumannii* isolate from Scotland and was found to be plasmid mediated (Dortet et al. 2008).
   The CHDLs are divided into four subfamilies (OXA 23 like, OXA 24 like, OXA 48 like, and OXA 58 like) based on their phylogenetic origin, and they cluster according to their source of bacterial genera. These CHDLs are encoded as mobile gene in plasmids as identified in clinical strains, whereas the other CHDLs

are generally immobile (Yoon and Jeong 2020). Clinically challenging bacteria possessing CHDLs include OXA 23 producing *Acinetobacter baumannii*, OXA 24 producing *Acinetobacter baumannii*, OXA 48 producing *Enterobacterales*, and OXA 58 producing *Acinetobacter* species (Yoon and Jeong 2020).

a. *OXA 23 like subfamily*: This subfamily consists of 41 members, most of which are carbapenemases, with the exception of OXA 105 and OXA 481, which are yet to be described (Yoon and Jeong 2020). OXA 23 was the first CHDL to be identified as mentioned above. The other significant member of the first group of CHDLs is OXA 27, identified from Singapore in *Acinetobacter baumannii* isolate (Afzal-Shah et al. 2001). OXA 27 has been identified in a single isolate as of yet, whereas OXA 23 is widespread clinically in *Acinetobacter* isolates and has been reported from different parts of the world (Corvec et al. 2007; Stoeva et al. 2008; Feizabadi et al. 2008; Mugnier et al. 2008; Mansour et al. 2008; Dalla-Costa et al. 2003; Valenzuela et al. 2007). Despite the widespread resistance in *Acinetobacter* species, the inadequacy of OXA-23 to confer resistance to carbapenems in *Enterobacterales* may be due to their low turnover and high affinity for carbapenems, resulting in weak hydrolysis (Antunes et al. 2014).
b. *OXA 24 like subfamily*: The group consists of 18 members, all of which have been identified as CHDLs. Few significant enzymes of the group include, OXA-24 (now OXA 40), OXA 25, OXA 26, OXA 72 (Poirel et al. 2010). An original sequencing error in the index type OXA 24 identified later makes it now OXA 40 (Lopez-Otsoa et al. 2002). In contrast to other subfamilies, the genes for OXA 24 like subfamily are not associated with the ISs or integron associated components but are flanked by inverted repeats homologous to the *Xer*C/*Xer*D binding sites, signifying mobilization of gene by site specific recombination (Merino et al. 2010; D'Andrea et al. 2009). OXA 24 like producing isolates are found to be endemic in Portugal since the mid-1990s (Grosso et al. 2011); however, recently they have been disseminated in other regions of the world leading to clinical concerns (Dortet et al. 2016; Pagano et al. 2017).
c. *OXA*-58 *like subfamily*: The third identified group, with a total of seven carbapenem members, also found only in *Acinetobacter* species has OXA-58 as its prototype and has been often associated with hospital outbreaks. All the seven members are CHDLs. OXA 58 hydrolyses penicillins and carbapenems, but not cefepime, ceftazidime, and cefotaxime, whereas cefpirome hydrolyzed only weakly (Poirel et al. 2005). OXA-58 producing isolates have been isolated from different regions among different bacterial species, with *Acinetobacter baumannii* global clone 2 being the major host carrying genes for OXA-58 like enzyme (Hamidian and Nigro 2019; Lowe et al. 2018; Taşbent and Özdemir 2015; Higgins et al. 2010).
d. *OXA-48 like subfamily*: OXA-48 was first identified in plasmid carried gene, in a carbapenem resistant *Klebsiella pneumoniae* isolate from Istanbul, Turkey in 2001 (Poirel et al. 2004). The OXA-48 subfamily has been merged with

OXA-548 subfamily and together comprises 101 enzymes (Yoon and Jeong 2020). OXA-48 is a DBL with highest catalytic activity against imipenem, but is unable to hydrolyze extended spectrum cephalosporins (Zong 2012). OXA-48 occurs primarily in *Enterobacterales*. Nonetheless, occurrence of chromosomal OXA-48 in *Shewanella* species is intrinsic (Zong 2012). OXA-48 has been reported in various hospital outbreaks and is reported frequently with NDM-1 producing *Enterobacterales* (Balm et al. 2013; Avolio et al. 2017).

7.6 Naturally Occurring Class D β Lactamases

Naturally occurring chromosomal class D β lactamase genes have been described in several species, first one being identified in *Aeromonas jandaei* (Poirel et al. 2010). OXA-12 (inducible) and AmpS are the two DBLs produced by *Aeromonas jandaei* (Rasmussen et al. 1994; Walsh et al. 1995). Chromosomally located OXA-22 is found in *Ralstonia pickettii*, leading to intrinsic resistance to penicillins, narrow spectrum cephalosporins, ceftazidime, and aztreonam (Nordmann et al. 2000). OXA-61 is identified in chromosome of *Campylobacter jejuni*, OXA-62 in *Pandoraea* species, and OXA-42 in *Burkholderia pseudomallei* (Walsh et al. 1995; Alfredson and Korolik 2005; Nordmann et al. 2000). A number of other naturally occurring DBLs are reported across several bacterial species and are considered as their survival mechanism against the environment.

7.7 Class D β lactamases in Gram-Positive Organisms

DBLs are occur frequently in the *Bacillaceae* family and the environmental isolates of family *Clostridiaceae* and *Eubacteriaceae* (Toth et al. 2016). Due to the lack of an arginine residue conserved in all known serine β lactamases, the DBLs in gram-positive organisms engage a unique substitute binding mode. This binding mode differentiates them not only from the DBLs of gram-negative bacteria but also from class A and C enzymes (Toth et al. 2016). DBLs among gram-positive cocci are not yet reported.

7.8 Detection of Class D β lactamases

Detection of class D β lactamases is crucial yet challenging due to the lack of appropriate and standardized phenotypic assays unlike various rapid and easy tests available for class A, B, and C enzymes. However, few properties of DBLs can be utilized for their early detection.

1. Inhibition of OXA-13 and its variant OXA-19 by imipenem: placing an imipenem disc in proximity to cefsulodin (which is easily hydrolyzed by OXA-13 in the absence of imipenem), decreasing the zone of inhibition of cefsulodin can be used for identification of these DBLs (Mugnier et al. 1998). This feature is also shown by other DBLs like OXA-10 and can be utilized for their identification (Poirel et al. 2010).
2. Synergy tests using clavulanic acid discs: DBLs whose activity is inhibited by clavulanic acid or tazobactam (OXA-12, 18, 45, 46) may be identified by synergy tests using clavulanic acid containing discs (Poirel et al. 2010). Nevertheless, differentiating these from class A ESBL producers is vital.
3. Spectrophotometric analysis: Well-equipped laboratories can utilize crude extracts and UV spectrophotometry to assess the capacity to hydrolyze oxacillin. NaCl inhibition property can be evaluated with a reference substrate like benzylpenicillin.
   However, various drawbacks of this methods are that all DBLs do not hydrolyze oxacillin, in vitro inhibition of OXA enzymes activity is difficult, coproduction of other enzymes which interfere in correct identification.

   Currently molecular detection of the DBL genes is the only standardized method of identification of class D β lactamases.

7.9 Treatment of Class D β-lactamases

In contrast to other class of β-lactamases, no specific inhibitor has had been identified for DBLs. Nevertheless, a number of potential candidates have shown inhibitor activity and can be utilized for degrading these enzymes.

Potential class D β-lactamases inhibitors can be classified into those:

1. Derived from β-lactams includes methylidene penems, penicillin sulfones and
2. Non-β lactams derived include avibactam, phosphonates, boronic acid.

Methylidene penems are potent inhibitors of OXA 1 and given with β-lactams.

Penicillin sulfones (Drawz et al. 2010) are active against OXA1, extended spectrum β-lactamases (OXA10, OXA14, OXA17), and OXA24/40. These inhibitors act by preventing the attack of deacylating water molecule. Their negatively charged sulfinate group mimics C3/C4 carboxylate group of penicillins and interact with a carboxylate recognizing residue on DBL. Studies show that C2 substituted 6-alkylidine penicillins were better than C3 substituted 7-alkylidene cephalosporins sulfones (Pattanaik et al. 2009).

Avibactam has activity against OXA48, given in combination with ceftazidime, ceftaroline, aztreonam (Livermore et al. 2011; Mushtaq et al. 2010) it forms a covalent complex in complex with OXA10 and OXA48 and undergoes ring opening reaction (Docquir et al. 2010).

Phosphonates and boronic acid are novel inhibitors of DBL that do not resemble β-lactams (Antunes and Fisher, 2014). Thiophenyl oxime derived phosphonates and

4,7-dichloro-1-benzothien-2yl-sulfonyl-aminomethyl boronic acid are inhibitors of OXA24/40 (Majumdar et al. 2005; Tan et al. 2011). They work by acylating the enzyme by acting as transition state analogue inhibitors and forming a reversible covalent bond with catalytic serine of enzyme with their phosphorus and boron atom, respectively. Thiophenyl oxime exhibits synergy in combination with imipenem (Tan et al. 2010).

Polycarboxylates are active against OXA46. They work by forming hydrogen bond with active site residues on enzyme, one of the carboxylates also makes ionic interaction with a residue that recognize C3/C4 carboxylate group of β-lactams. Other polycarboxylates, lipophilic aminocitrate, and aminoisocitrate derivatives also inhibit OXA10 (Beck et al. 2009).

Yet, none of the compounds is able to inhibit the entire class D enzymes. This can be attributed to the magnanimous size of the family and the diversity of the members of the group. More research is thus required, to explore inhibitors for the subfamilies existing in the class, if not for the entire family.

7.10 Conclusion

Class D β lactamases are the largest and most diverse, yet most neglected group of β lactamases. Clinically they should be considered a threat similar to or even greater than other β lactamases, since lack of detection may augment their unseen and rapid spread among the clinical settings. Intensive research is required for developing rapid and easy detection tools for DBLs and also for the discovery of class D specific inhibitors.

Seeing the magnanimous and diverse range of members of this group, and for the unification of the subfamilies, new scheme for their classification should be considered.

References

Afzal-Shah M, Woodford N, Livermore DM (2001) Characterization of OXA-25, OXA-26, and OXA-27, molecular class D β-lactamases associated with carbapenem resistance in clinical isolates of Acinetobacter baumannii. Antimicrob Agents Chemother 45(2):583–588. https://doi.org/10.1128/aac.45.2.583-588.2001

Alfredson DA, Korolik V (2005) Isolation and expression of a novel molecular class D β-lactamase, OXA-61, from Campylobacter jejuni. Antimicrob Agents Chemother 49:2515–2518

Ambler RP (1980) The structure of β-lactamases. Philos Trans R Soc Lond B Biol Sci 289(1036): 321–331. https://doi.org/10.1098/rstb.1980.0049

Antunes N, Fisher J (2014) Acquired class D β-lactamases. Antibiotics 3(3):398–434. https://doi.org/10.3390/antibiotics3030398

Antunes NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB (2014) Class D β-lactamases: are they all carbapenemases? Antimicrob Agents Chemother 58(4):2119–2125. https://doi.org/10.1128/aac.02522-13

Avolio M, Vignaroli C, Crapis M, Camporese A (2017) Co-production of NDM-1 and OXA-232 by ST16 Klebsiella pneumoniae, Italy, 2016. Future Microbiol 12(13):1119–1122. https://doi.org/10.2217/fmb-2017-0041

Balm M, La MV, Krishnan P, Jureen R, Lin R, Teo J (2013) Emergence of Klebsiella pneumoniae co-producing NDM-type and OXA-181 carbapenemases. Clin Microbiol Infect 19(9):E421–E423. https://doi.org/10.1111/1469-0691.12247

Beck J, Vercheval L, Bebrone C, Herteg-Fernea A, Lassaux P, Marchnd-Brynaert J (2009) Discovery of novel lipophilic inhibitors of OXA-10 enzyme (Class D β-lactamase) by screening amino analogs and homologs of citrate and isocitrate. Bioorg Med Chem Lett 19:3593–3597

Boyd DA, Mulvey MR (2006) OXA-1 is OXA-30 is OXA-1. J Antimicrob Chemother 58:224–225

Bush K (2013) Proliferation and significance of clinically relevant β-lactamases. Ann N Y Acad Sci 1277(1):84–90. https://doi.org/10.1111/nyas.12023

Bush K, Fisher JF (2011) Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-Negative bacteria. Annu Rev Microbiol 65(1):455–478. https://doi.org/10.1146/annurev-micro-090110-102911

Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39(6):1211–1233. https://doi.org/10.1128/aac.39.6.1211

Centron D, Roy PH (2002) Presence of a group II intron in a multiresistant Serratia marcescens strain that harbors three integrons and a novel gene fusion. Antimicrob Agents Chemother 46:1402–1409

Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P (2007) Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in Acinetobacter baumannii. Antimicrob Agents Chemother 51(4):1530–1533. https://doi.org/10.1128/aac.01132-06

Couture F, Lachapelle J, Levesque RC (1992) Phylogeny of LCR-1 and OXA-5 with class A and class D β-lactamases. Mol Microbiol 6(12):1693–1705. https://doi.org/10.1111/j.1365-2958.1992.tb00894.x

D'Andrea MM, Giani T, D'Arezzo S, Capone A, Petrosillo N, Visca P, Luzzaro F, Rossolini GM (2009) Characterization of pABVA01, a plasmid encoding the OXA-24 Carbapenemase from Italian isolates of Acinetobacter baumannii. Antimicrob Agents Chemother 53(8):3528–3533. https://doi.org/10.1128/aac.00178-09

Dalla-Costa LM, Coelho JM, Souza HAPHM, Castro MES, Stier CJN, Bragagnolo KL, Rea-Neto A, Penteado-Filho SR, Livermore DM, Woodford N (2003) Outbreak of carbapenem-resistant Acinetobacter baumannii producing the OXA-23 enzyme in Curitiba, Brazil. J Clin Microbiol 41(7):3403–3406. https://doi.org/10.1128/jcm.41.7.3403-3406.2003

Danel F, Hall LM, Gur D, Livermore DM (1995) OXA-14, another extended-spectrum variant of OXA-10 (PSE-2) beta- lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 39(8):1881–1884. https://doi.org/10.1128/aac.39.8.1881

Danel F, Hall LM, Gur D, Livermore DM (1997) OXA-15, an extended-spectrum variant of OXA-2 beta-lactamase, isolated from a Pseudomonas aeruginosa strain. Antimicrob Agents Chemother 41(4):785–790. https://doi.org/10.1128/aac.41.4.785

Danel F, Hall LMC, Gur D, Livermore DM (1998) OXA-16, a further extended-spectrum variant of OXA-10 β-lactamase, from two Pseudomonas aeruginosa Isolates. Antimicrob Agents Chemother 42(12):3117–3122. https://doi.org/10.1128/aac.42.12.3117

Danel F, Hall LMC, Duke B, Gur D, Livermore DM (1999) OXA-17, a further extended-spectrum variant of OXA-10 β-lactamase, isolated from Pseudomonas aeruginosa. Antimicrob Agents Chemother 43(6):1362–1366. https://doi.org/10.1128/aac.43.6.1362

Docquir JD, Benvenuti M, Luca DF, Rossolini GM, Mangini S, Miossec C, Black MT (2010) X ray crystal structure of the Klebsiella pneumoniae OXA-48 class D carbepenemase inhibited by NXL 104. In 50th interscience conference of antimicrobial agents and chemotherapy, abstracts C1-640, Boston, MA

Dortet L, Radu I, Gautier V, Blot F, Chachaty E, Arlet G (2008) Intercontinental travels of patients and dissemination of plasmid-mediated carbapenemase KPC-3 associated with OXA-9 and TEM-1. J Antimicrob Chemother 61(2):455–457. https://doi.org/10.1093/jac/dkm455

Dortet L, Bonnin RA, Bernabeu S, Escaut L, Vittecoq D, Girlich D, Imanci D, Fortineau N, Naas T (2016) First Occurrence of OXA-72-Producing Acinetobacter baumannii in Serbia. Antimicrob Agents Chemother 60(10):5724–5730. https://doi.org/10.1128/aac.01016-16

Drawz SM, Bethel CR, Doppalapudi VR, Sheri A, Pagadala SR, Hujer AM et al (2010) Penicillin sulfone inhibitors of class D β-lactamases. Antimicrob Agents Chemother 54:1414–1424

Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghifard N, Aligholi M, Soroush S, Mohammadi-Yegane S (2008) Antimicrobial susceptibility patterns and distribution of blaOXA genes among Acinetobacter spp. Isolated from patients at Tehran hospitals. Jpn J Infect Dis 61(4):274–278

Fisher JF, Meroueh SO, Mobashery S (2005) Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. Chem Rev 105(2):395–424. https://doi.org/10.1021/cr030102i

Fournier PE, Vallenet D, Barbe V et al (2006) Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genet 2:e7

Franceschini N, Boschi L, Pollini S, Herman R, Perilli M, Galleni M, Frère JM, Amicosante G, Rossolini GM (2001) Characterization of OXA-29 from Legionella (Fluoribacter) gormanii: molecular class D β-lactamase with unusual properties. Antimicrob Agents Chemother 45(12): 3509–3516. https://doi.org/10.1128/aac.45.12.3509-3516.2001

Grosso F, Quinteira S, Peixe L (2011) Understanding the dynamics of imipenem-resistant Acinetobacter baumannii lineages within Portugal. Clin Microbiol Infect 17(8):1275–1279. https://doi.org/10.1111/j.1469-0691.2011.03469.x

Hall LM, Livermore DM, Gur D, Akova M, Akalin HE (1993) OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) beta-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 37(8):1637–1644. https://doi.org/10.1128/aac.37.8.1637

Hamidian M, Nigro SJ (2019) Emergence, molecular mechanisms and global spread of carbapenem-resistant Acinetobacter baumannii. Microb Genomics 5(10):e000306. https://doi.org/10.1099/mgen.0.000306

Higgins PG, Dammhayn C, Hackel M, Seifert H (2010) Global spread of carbapenem-resistant Acinetobacter baumannii. J Antimicrob Chemother 65(2):233–238. https://doi.org/10.1093/jac/dkp428

Huovinen P, Huovinen S, Jacoby GA (1988) Sequence of PSE-2 beta-lactamase. Antimicrob Agents Chemother 32(1):134–136. https://doi.org/10.1128/aac.32.1.134

Jaurin B, Grundstrom T (1981) ampC cephalosporinase of Escherichia coli K-12 has a different evolutionary origin from that of beta-lactamases of the penicillinase type. Proc Natl Acad Sci 78(8):4897–4901. https://doi.org/10.1073/pnas.78.8.4897

Jiang T, Xu J, He F (2020) Genotypic and phylogenetic characterisation of a clinical Ralstonia pickettii strain carrying two novel OXA allelic variants, blaOXA-898 and blaOXA-899, isolated from a bloodstream infection in China. J Glob Antimicrob Resist 21:46–48. https://doi.org/10.1016/j.jgar.2020.02.020

Kratz J, Schmidt F, Wiedemann B (1983) Transposition of a gene encoding OXA-2 -lactamase. J Gen Microbiol 129(9):2951–2957. https://doi.org/10.1099/00221287-129-9-2951

Kumar P, Thomas S (2011) Presence of qnrVC3 gene cassette in SXT and class1 integrons of Vibrio cholerae. Int J Antimicrob Agents 37:280–281

Lamotte-Brasseur J, Knox J, Kelly JA, Charlier P, Fonzé E, Dideberg O, Frère JM (1994) The structures and catalytic mechanisms of active-site serine β-lactamases. Biotechnol Genet Eng Rev 12(1):189–230. https://doi.org/10.1080/02648725.1994.10647912

Leonard DA, Bonomo RA, Powers RA (2012) Class D β-lactamases: a reappraisal after five decades. Acc Chem Res 46(11):2407–2415. https://doi.org/10.1021/ar300327a

Livermore D, Mushtaq S, Warner M et al (2011) Activities of NXL 104 combinations with ceftazidime and aztreonam against carbepenemase producing Enterobacteriaceae. Antimicrob Agents Chemother 55:390–394

Lopez-Otsoa F, Gallego L, Towner KJ, Tysall L, Woodford N, Livermore DM (2002) Endemic carbapenem resistance associated with OXA-40 carbapenemase among Acinetobacter baumannii isolates from a hospital in Northern Spain. J Clin Microbiol 40(12):4741–4743. https://doi.org/10.1128/jcm.40.12.4741-4743.2002

Lowe M, Ehlers MM, Ismail F, Peirano G, Becker PJ, Pitout J, Kock MM (2018) Acinetobacter baumannii: epidemiological and beta-lactamase data from two tertiary academic hospitals in Tshwane, South Africa. Front Microbiol 9:1280. https://doi.org/10.3389/fmicb.2018.01280

Majumdar S, Adediran SA, Nukaga M, Pratt RF (2005) Inhibitors of class D β-lactamases by diacyl phosphates. Biochemistry 44:16121–16129

Mansour W, Poirel L, Bettaieb D, Bouallegue O, Boujaafar N, Nordmann P (2008) Dissemination of OXA-23–producing and carbapenem-resistant Acinetobacter baumannii in a University Hospital in Tunisia. Microb Drug Resist 14(4):289–292. https://doi.org/10.1089/mdr.2008.0838

Matthew M, Sykes RB (1977) Properties of the beta-lactamase specified by the Pseudomonas plasmid RPL11. J Bacteriol 132(1):341–345. https://doi.org/10.1128/jb.132.1.341-345.1977

Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN (2009) Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among Acinetobacter spp. In Asia-Pacific nations: report from the SENTRY surveillance program. J Antimicrob Chemother 63(1):55–59. https://doi.org/10.1093/jac/dkn434

Merino M, Acosta J, Poza M, Sanz F, Beceiro A, Chaves F, Bou G (2010) OXA-24 carbapenemase gene flanked by XerC/XerD-Like recombination sites in different plasmids from different Acinetobacter species isolated during a nosocomial outbreak. Antimicrob Agents Chemother 54(6):2724–2727. https://doi.org/10.1128/aac.01674-09

Moura A, Pereira C, Henriques I et al (2012) Novel gene cassettes and integrons in antibiotic-resistant bacteria isolated from urban wastewaters. Res Microbiol 163:92–100

Mugnier P, Casin I, Bouthors A-T, Collatz E (1998) Novel OXA-10-derived extended-spectrum β -lactamases selected in vivo or in vitro. Antimicrob Agents Chemother 42:3113–3116

Mugnier P, Poirel L, Pitout M, Nordmann P (2008) Carbapenem-resistant and OXA-23-producing Acinetobacter baumannii isolates in the United Arab Emirates. Clin Microbiol Infect 14(9):879–882. https://doi.org/10.1111/j.1469-0691.2008.02056.x

Mulvey MR (2004) Characterization of a Salmonella enterica serovar Agona strain harbouring a class 1 integron containing novel OXA-type -lactamase (blaOXA-53) and 6'-N-aminoglycoside acetyltransferase genes [aac(6')-I30]. J Antimicrob Chemother 54(2):354–359. https://doi.org/10.1093/jac/dkh347

Mushtaq S, Warner M, Williams G, Critchley I, Livermore D (2010) J Antimicrob Chemother 65:1428–1432

Naas T, Nordmann P (1999) OXA-type beta-lactamases. Curr Pharm Des 5(11):865–879

Naas T, Namdari F, Bogaerts P, Huang TD, Glupczynski Y, Nordmann P (2008) Genetic structure associated with blaOXA-18, encoding a clavulanic Acid-Inhibited Extended-Spectrum oxacillinase. Antimicrob Agents Chemother 52(11):3898–3904. https://doi.org/10.1128/aac.00403-08

Niumsup P, Wuthiekanun V (2002) Cloning of the class D beta-lactamase gene from Burkholderia pseudomallei and studies on its expression in ceftazidime-susceptible and -resistant strains. J Antimicrob Chemother 50(4):445–455. https://doi.org/10.1093/jac/dkf165

Nordmann P, Poirel L, Kubina M, Casetta A, Naas T (2000) Biochemical-genetic characterization and distribution of OXA-22, a chromosomal and inducible class D β -lactamase from Ralstonia (Pseudomonas) pickettii. Antimicrob Agents Chemother 44:2201–2204

Pagano M, Rocha L, Sampaio JL, Martins AF, Barth AL (2017) Emergence of OXA-72-producing Acinetobacter baumannii belonging to high-risk clones (CC15 and CC79) in different Brazilian States. Infect Control Hosp Epidemiol 38(2):252–254. https://doi.org/10.1017/ice.2016.287

Pattanaik P, Bethel CR, Hujer AM, Hujer KM et al (2009) Strategic design of an effective β-lactamase inhibitor. J Biol Chem 284:945–953

Payne DJ, Cramp R, Winstanley DJ, Knowles DJ (1994) Comparative activities of clavulanic acid, sulbactam, and tazobactam against clinically important beta-lactamases. Antimicrob Agents Chemother 38(4):767–772. https://doi.org/10.1128/aac.38.4.767

Périchon B, Goussard S, Walewski V, Krizova L, Cerqueira G, Murphy C, Feldgarden M, Wortman J, Clermont D, Nemec A, Courvalin P (2013) Identification of 50 class D β-lactamases and 65 acinetobacter-derived cephalosporinases in Acinetobacter spp. Antimicrob Agents Chemother 58(2):936–949. https://doi.org/10.1128/aac.01261-13

Philippon LN, Naas T, Bouthors AT, Barakett V, Nordmann P (1997) OXA-18, a class D clavulanic acid-inhibited extended-spectrum beta-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 41(10):2188–2195. https://doi.org/10.1128/aac.41.10.2188

Poirel L, Gerome P, De Champs C, Stephanazzi J, Naas T, Nordmann P (2002) Integron-located oxa-32 gene cassette encoding an extended-spectrum variant of OXA-2 β-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 46(2):566–569. https://doi.org/10.1128/aac.46.2.566-569.2002

Poirel L, Héritier C, Tolün V, Nordmann P (2004) Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 48(1):15–22. https://doi.org/10.1128/aac.48.1.15-22.2004

Poirel L, Marqué S, Héritier C, Segonds C, Chabanon G, Nordmann P (2005) OXA-58, a novel class D β-lactamase involved in resistance to carbapenems in Acinetobacter baumannii. Antimicrob Agents Chemother 49(1):202–208. https://doi.org/10.1128/aac.49.1.202-208.2005

Poirel L, Naas T, Nordmann P (2010) Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54(1):24–38. https://doi.org/10.1128/AAC.01512-08

Rasmussen BA, Keeney D, Yang Y, Bush K (1994) Cloning and expression of a cloxacillin-hydrolyzing enzyme and a cephalosporinase from Aeromonas sobria AER 14M in Escherichia coli: requirement for an E. coli chromosomal mutation for efficient expression of the class D enzyme. Antimicrob Agents Chemother 38(9):2078–2085. https://doi.org/10.1128/aac.38.9.2078

Sanschagrin F, Couture F, Levesque RC (1995) Primary structure of OXA-3 and phylogeny of oxacillin-hydrolyzing class D beta-lactamases. Antimicrob Agents Chemother 39(4):887–893. https://doi.org/10.1128/aac.39.4.887

Schneider I, Queenan AM, Bauernfeind A (2006) Novel carbapenem-hydrolyzing oxacillinase OXA-62 from Pandoraea pnomenusa. Antimicrob Agents Chemother 50(4):1330–1335. https://doi.org/10.1128/aac.50.4.1330-1335.2006

Siu LK, Lo JY, Yuen KY et al (2000) β -Lactamases in Shigella flexneri isolates from Hong Kong and Shanghai and a novel OXA-1-like b-lactamase, OXA-30. Antimicrob Agents Chemother 44:2034–2038

Stoeva T, Higgins P, Bojkova K, Seifert H (2008) Clonal spread of carbapenem-resistant OXA-23-positive Acinetobacter baumannii in a Bulgarian university hospital. Clin Microbiol Infect 14(7):723–727. https://doi.org/10.1111/j.1469-0691.2008.02018.x

Suzuki M, Nishio H, Asagoe K et al (2015) Genome sequence of a carbapenem resistant strain of Ralstonia mannitolilytica. Genome Announc 3:e00405–e00415

Tan Q, Ogawa AM, Painter RE, Park YW, Young K, DiNinno F, P. (2010) 4,7-Dichloro benzothien-2yl sulfonylaminomethyl boronic acid; first boronic acid derived β-lactamase inhibitor with class A, C and D activity. Bioorg Med Chem Lett 20:2622–2624

Tan Q, Ogawa AM, Raghoobar SL, Wisniewski D et al (2011) Thiophenyl oxime derived phosphonates as nano-molar class β-lactamase inhibitors reducing the MIC of imipenem against Pseudomonas aeruginosa and Acinetobacter baumannii. Bioorg Med Chem Lett 20:2622–2624

Taşbent EF, Özdemir M (2015) The presence of OXA type carbapenemases in Pseudomonas strains: first report from Turkey. Mikrobiyol Bul 49(1):26–34. https://doi.org/10.5578/mb.856

Tian J, Zhang G, Ju Y, Tang N, Li J, Jia R, Feng J (2018) Five novel carbapenem-hydrolysing OXA-type β-lactamase groups are intrinsic in Acinetobacter spp. J Antimicrob Chemother 73: 3279–3284. https://doi.org/10.1093/jac/dky359

Toth M, Antunes NT, Stewart NK, Frase H, Bhattacharya M, Smith CA, Vakulenko SB (2016) Class D β-lactamases do exist in Gram-positive bacteria. Nat Chem Biol 12(1):9–14. https://doi.org/10.1038/nchembio.1950

Valenzuela JK, Thomas L, Partridge SR, van der Reijden T, Dijkshoorn L, Iredell J (2007) Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant Acinetobacter baumannii. J Clin Microbiol 45(2):453–460. https://doi.org/10.1128/jcm.01971-06

Voha C, Docquier JD, Rossolini GM, Fosse T (2006) Genetic and biochemical characterization of FUS-1 (OXA-85), a narrow-spectrum class D β-lactamase from Fusobacterium nucleatum subsp. polymorphum. Antimicrob Agents Chemother 50(8):2673–2679. https://doi.org/10.1128/aac.00058-06

Walsh TR, Hall L, MacGowan AP, Bennett PM (1995) Sequence analysis of two chromosomally mediated inducible β-lactamases from Aeromonas sobria, strain 163a, one a class D penicillinase, the other an AmpC cephalosporinase. J Antimicrob Chemother 36(1):41–52. https://doi.org/10.1093/jac/36.1.41

Walther-Rasmussen J, Høiby N (2006) OXA-type carbapenemases. J Antimicrob Chemother 57(3): 373–383. https://doi.org/10.1093/jac/dki482

Yoon EJ, Jeong SH (2020) Class D β-lactamases. J Antimicrob Chemother 76(4):836–864. https://doi.org/10.1093/jac/dkaa513

Zong Z (2012) Discovery of bla(OXA-199), a chromosome-based bla(OXA-48)-like variant, in Shewanella xiamenensis. PLoS One 7(10):e48280. https://doi.org/10.1371/journal.pone.0048280

Part II

Genetic Basis of Resistance in Gram-Negative Bacteria

Mobile Genetic Elements

8

Anuradha Singh, Mohammad Shahid, Gourav Pratap Singh, and Haris M. Khan

Abstract

Irresponsible use of antimicrobial agents to treat bacterial infections led to development of multidrug resistance in bacteria. The presence of MDR Gram-negative bacilli among hospitalized patients brought the attention of microbiologists and clinicians to understand why this change occurred. It is now documented that capture, accumulation, and dissemination of resistance genes can occur due to mobile genetic elements (MGE). These MGEs are genetic elements that have ability to move from chromosome to a plasmid, or between plasmids within the species, or from one species to another through any mode of genetic transfer. These mobile genetic elements include plasmids, transposons, insertion sequences, gene cassettes, and integrons. Brief description of various types of mobile genetic elements is given in this chapter.

Keywords

Plasmids · Transposons · Integrons · Gene cassettes · IS*Ecp1* · IS*26* · ISCR

A. Singh (✉) · H. M. Khan
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid
Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: mohammeds@agu.edu.bh

G. P. Singh
Holy Family Hospital, New Delhi, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_8

8.1 Introduction

Constant and more often irresponsible use of antimicrobial agents to treat bacterial infections has led to the emergence of multidrug resistant (MDR) strains. In recent years, the trend of high morbidity and mortality among hospitalized patients, associated with MDR Gram-negative bacilli has increasingly gained attention worldwide. The capture, accumulation, and dissemination of resistance genes can be due to the actions of mobile genetic elements (MGE). The term mobile genetic elements is used for the genetic elements that have intracellular mobility from chromosome to a plasmid or between plasmids or can be transferred from one species or cell to another. MGEs including plasmids, transposons, insertion sequences, gene cassettes, and integrons may play an important role in evolution. MGEs like insertion sequences (IS) and transposons (Tn) comprise those segments of DNA which are able to transfer themselves and associate resistance genes to new locations in the same or different DNA molecules within a single cell. Integrons comprise an important part of these elements and have been found in plasmids and/or transposons that enhance the spread of resistance genes. Integrons use site-specific recombination to move resistance genes between defined sites. Multiple copies of different types of mobile genetic elements are frequently present in different locations in a genome (Partridge et al. 2018). In evolutionary context, MGEs can transport virulence factors and antibiotic resistance genes with neighboring bacteria. Class 1 integrons are most frequently isolated from MDR pathogens, and the ongoing use of antibiotics has increased their numbers in recent years (Wu et al. 2012). Intercellular genetic exchange includes conjugation, transduction, and transformation. In this chapter we will briefly discuss about various mobile genetic elements associated with resistance in bacteria.

8.2 Genetic Mechanisms of Resistance

Bacteria may also develop resistance by acquiring new genetic material from other organisms which are already resistant. This has been called as horizontal evolution, and it may take place between the members of same species or among the members of different species or genera. Genetic inheritance in bacteria may occur in two manners: (1) Mutations altering the pre-existing DNA—these alterations could be base changes, DNA deletions or inversions (Avinson and Bennett 2005) and by (2) Acquisition of new genetic material such as expansion of the genome in the cell by capture of new genes. In this phenomenon of acquisition, gene transfer is from any outside source, like other bacteria. These mechanisms of genetic exchange include transduction, transformation, and conjugation between same or different bacterial species (McManus 1997).

Conjugation The process comprises DNA transfers from a donor to a recipient by direct cell-to-cell contact. It so happens by allowing the passage of more than one functional gene at a time rendering the development of multiple resistances within a

single step. Many different organisms act as recipients, allowing reception of DNA (resistant genes) freely from different sources. This is evident in the instances like resistance being transferred from commensals in the gut to a pathogen existing in the same environment. Conjugation is a highly efficient and important process for transferring and acquisition of resistant genes by most of the pathogens.

Transduction Transduction is a process in which DNA is transferred from a donor to a recipient with the help of a host/phage. However, it is still unclear, whether this process is solely responsible for clinically observed resistance to antibiotics. Since transduction is highly dependent on bacterial phages, so it may occur only in specific bacterial species. A restricted amount of DNA can be packed into the head of a phage to transfer. Therefore, transduction is usually not responsible for development of multiple drug resistance.

Transformation The process of transformation involves the passage of DNA to a recipient via a specific medium. This process of transfer of genetic material is mostly observed in vitro by using molecular techniques in the molecular biology laboratory.

8.3 Mobile Genetic Elements

A variety of immoral gene transfer systems, such as bacterial conjugative plasmids, transposable elements, and integron systems can move genes from one DNA system to another and from one bacterial cell to another, not necessarily one related to the gene donor greatly aid in acquisition of bacterial genes needed to elaborate various mechanisms. Mobile genetic elements include bacterial plasmids, integrons including gene cassettes, transposons, and insertion sequences promoting gene mobilization. Plasmids and conjugative transposons with their ability of replication can transfer from one cell to another, while transposons, gene cassettes, and ISCR-mediated gene transfer do not necessarily need replication, rather they require some form of recombination which may or may not include replication (Bennett 2005).

8.4 Bacterial Plasmids

The elements that use the mechanism of horizontal gene transfer, move many bacterial genes from one bacterial cell to another are called as bacterial plasmids or conjugative plasmids. These plasmids can promote their own transfer and transfer of other plasmids as well from one bacterial cell to another. Most of the plasmids are circular, double-stranded DNA molecule with the ability of autonomous replication. Plasmids have been assigned to a special biological category of extra-chromosomal, accessory DNA elements (Reanney 1976; Campbell 1981). Plasmids have been defined as the genetic elements which do not carry genes essential for growth of their host under non-stressed conditions. These special abilities of the plasmids make

them an important part of scientific research as they provide an excellent model of DNA replication. In size, the plasmids may range from 2 to 3 kb (with just 2 or 3 genes) to elements as large as 10% or more relative to the host chromosome to accommodate even 400 or more genes. Resistance plasmids are known to carry one or more resistance genes. Plasmid-encoded antibiotic resistance comprises many classes of antibiotics, like cephalosporins, fluoroquinolones, and aminoglycosides. Most often, resistance plasmids are conjugative as they have the ability to encode the functions necessary to promote cell-to-cell DNA transfer and chiefly their own transfer. Plasmids are usually classified in Incompatibility (Inc) groups, with incompatibility being defined as the inability of two plasmids to be propagated stably with in the same cell line. "Inc" is the manifestation of relatedness, as these share equal partitioning elements or common replication controls (Couturier et al. 1988). Antimicrobial resistance on plasmids originates from a complex multi-factorial process supported by panoply of mobile genetic elements which can transfer resistance determinants by assemblage of modular components, by homologous recombination, transposition, and improper recombination events (Bennett 2004). Multiple genes present on a plasmid, conferring resistance to different classes of antibiotics may provide a selective advantage to the bacterial host when different antimicrobials are simultaneously administered. Once selected by the bacterial host, these determinants can evolve further and then transferred to other bacterial populations, rendering them the ability to penetrate into niches (Carattoli 2008). A variety of insertion sequences (IS) are involved in the assembly of a Resistance Island. Such as IS26 can mobilize sulII-strA-atrB resistance determinants from the IncQ RF1010 broad-host range plasmid and acquired on the pHCM1 scaffold. Transposition-recombination events may even mobilize other resistance determinants, like chloramphenicol (cat1) and β-lactams ($bla_{\text{TEM-1}}$) genes, and the *mer operon* (mercuric ions) inserted into the resistance island of the pHCM1 plasmid (Miriagou et al. 2006; Wain et al. 2003). Bacteria can possess plasmids that encode for more than one β-lactamase in addition to their expression of chromosomal enzyme. Due to the presence of plasmids in bacteria and the immoral promiscuous exchange of genetic material between unrelated bacteria, these antibiotic resistance genes have spread widely (Lee et al. 2001). First plasmid mediated β-lactamases were recognized in early 1960s, in Gram-negative bacteria (Livermore 1993). Even the CTX-M encoding genes are commonly located on plasmids which vary in size from 7 kb to 200 kb (Bonnet 2004; Novais et al. 2007; Coque et al. 2008).

8.5 Transposons

Resistance transposons are also called as jumping gene systems with incorporation of a resistance gene within their element. These elements are able to move both intra- and inter-molecularly. They can jump from one site to another within the same DNA molecule or from one DNA molecule to another such as from one plasmid to a chromosome or from a bacterial plasmid to another and vice versa. Transposons belong to the set of mobile elements designated as transposable elements. This

designation covers small cryptic elements like transposons, transposing bacteriophages, and insertion sequences (IS elements) (Bennett 2004). A transposon differs from an IS element by the ability to encode at least one function that changes the phenotype of the cell, for example, a resistance transposon conferring resistance to a particular antibiotic. Transposons are constructed from a pair of IS elements and a central DNA sequence that is not inherently able to transpose; however, the expression of which alters the cell phenotypically (Bennett 2008). Some well-known transposons areTn*3*, Tn*5*, Tn*10*, and Tn*21*. Tn*5* encodes resistance to aminoglycosides (Kanamycin and neomycin). Tn*10* encodes resistance to tetracycline, while Tn*3* encodes resistance to some β-lactams including ampicillin, and Tn*21* is able to encode resistance to streptomycin, spectinomycin, and sulfonamides as well as mercuric ions are examples of complex transposons, found on the plasmids in the members of *Enterobacteriaceae*.

8.6 Insertion Sequences

Rapid dissemination of β-lactamases involves strain or plasmid epidemics, but these also involve some important mobile elements like insertion sequences. Initially recognized during the studies of model genetic systems, bacterial insertion sequences showed the capabilities to generate mutations due to their translocations. Scholars' interest in antibiotic resistance and transmissible plasmids subsequently revealed the important role for these mobile elements in promotion of gene acquisition and in dissemination of resistance genes (Mahillon and Chandler 1998). It was recently reviewed by Partridge et al. (2018) that insertion sequences are small mobile elements that carry more than one transposes (tnp) gene. These are divided into groups based partially on active site motifs of Tnp and designated according to amino acids of active site, most commonly DDE (Asp, Asp and Glu) but also DEDD and HUH (two His residues separated by large hydrophobic amino acid) (Hickman and Dyda 2015) which is based on whether transposition is a conservative, cut-and-paste mechanism (IS excised from donor and inserted into the recipient) or by replicative mechanism. Replicative transposition includes copy-and-paste mechanism in which IS is replicated to join the donor and recipient in a co-integrate that is resolved to give donor and recipient with the IS (Hallet and Sherratt 1997) or a copy-out-paste-in mechanism, in this IS is replicated into a double-stranded circular intermediate that then integrates into the recipient (Chandler et al. 2015). These insertion sequences comprise the IS*APl1*, the IS*CR1* element (previously called ORF513), and IS*Ecp1*-like insertion sequences (Rossolini et al. 2008; Toleman et al. 2006). IS26 is 820 bp long insertion sequence that characteristically generates 8bp target duplication upon transposition (Mollet et al. 1983). IS*Ecp1*-like insertion sequence captures and mobilizes a number of unrelated antibiotic resistance genes including $bla_{\text{CTX-M}}$ groups (Rossolini et al. 2008; Shahid et al. 2011). The most common type of insertion sequence, DDE is bound by 14-bp terminal inverted repeats, IR_L at the left side and IR_R at the right side with respect to the direction of transcription of the transposes gene. Binding of the IR by the Tnp protein is involved

in transposition, during this process as a result of repair of staggered cuts in different DNA strands, many IS creating short-flanking direct repeats on insertion (DR; typically ~3 to 14 bp, but the length is characteristic for each IS). These are also referred to as target site duplications (TSD), while most of the IS do not appear to target specific sequence motifs. While some IS types may not have IR or create TSD. Because frequent transposition may be deleterious, expression of active transposase may be controlled by a programmed frameshift to create a complete Tnp protein (Chandler and Fayet 1993). The frameshift typically occurs within a "slippery codon" region, e.g., AAAAAAA in IS*Aba1* (Mugnier et al. 2009).

8.7 IS*26* and Composite Transposon

IS*6* family elements IS*26* (also known as IS*6*, IS*15*Δ, IS*46*, IS*140*, IS*160*, IS*176*, and IS*1936*) (Harmer et al. 2014) have played an essential role in the dissemination of resistance determinants in Gram-negative bacteria. These IS encode a single transposase, and the terminal IR of IS*26* and IS*257* both contain a -35 consensus (TTGCAA) that can create a hybrid promoter if accidentally positioned (with an ~17-bp spacer) near a -10 sequence upstream of the gene (Vandecraen et al. 2017). Movement of these IS was originally demonstrated to occur by replicative transposition (Mollet et al. 1985; Needham et al. 1995; Firth and Skurray 2006). This results in cointegration of the donor and recipient molecules with a directly repeated copy of the IS at each junction, creating a "composite transposon"-like structure flanked by characteristic 8-bp TSD.

8.8 IS*Ecp1* and Related Elements

IS*Ecp1* (IS*1380* family; encodes a DDE-type transposase), first identified in *E. coli*, has 14 bp inverted repeat (IR) and creates 5-bp TSD on transposition. IS*Ecp1* appears to be able to use IRL (inverted repeat left) in combination with a sequence beyond its IRR (inverted repeat right) end to move an adjacent region, creating 5-bp (or occasionally 6-bp) TSD flanking the whole "transposition unit" (TU) (Boyd et al. 2004). Upstream insertion of IS*Ecp1* of a chromosomal $bla_{\text{CTX-M-2}}$ gene in *Kluyvera* and consequent movement to a plasmid have been demonstrated (Lartigue et al. 2006), but the exact mechanism and any important characteristics of the sequences that can be used as alternatives to IRR have not been determined. While IS*Ecp1* generally makes use of IRL in conjunction with alternative sequences resembling these IR to mobilize adjacent regions, to generate 5-bp duplications of flanking sequence on transposition (Lartigue et al. 2006; Poirel et al. 2003, 2005; Wachino et al. 2006). As illustrated by transposition of "hybrid" units that include adjacent vector sequence from cloned IS*Ecp1*-resistance gene combinations, IS*Ecp1* is able to mobilize the same adjacent gene as part of different-size "transposition units" (Poirel et al. 2005; Wachino et al. 2006).

8.9 IS*CR1* and Related Elements with Antibiotic Resistance Genes

An element, ORF513, has increasingly been associated with class 1 integrons, this element shows IS91-like characteristics, and it can mobilize adjacent DNA sequences via rolling circle replication process. Hence this element was aptly named as "insertion sequence CRs" (ISCRs), which is a reflection of their name appropriate structure–function properties (Toleman et al. 2006). The first CR element (IS*CR1*) was discovered in the early 1990s. It was reported as a sequence of DNA with size of 2154 bp, incorporating ORF513, which is inserted beside the *sul1* genes of class 1 integrons; In*6* and In*7* (Hall and Stokes 1993). To distinguish it from the 5′ and 3′ conserved sequences (CS) of class 1 integrons, this sequence was termed as CR (common region). ORF513 (also known as IS*CR1*) has been found related to a number of different antibiotic resistance genes, with most of them being closely associated with class 1 integrons and called as complex class 1 integron (Toleman et al. 2006). The sequence is an example of a CR (common region) element, which is a group of putative "mobile" genetic elements, found in "*Salmonella* genomic islands" (SGIs) and on the integrative conjugative element SXT of *Vibrio cholerae* (Beaber et al. 2002). IS*CR1* belongs to a family of unusual insertion sequences, typified by IS*91* (Garcillan-Barcia et al. 2002) and is closely associated with many antibiotic resistance genes. IS*CR1* is associated with genes encoding resistance to chloramphenicol (catAII), trimethoprim (dfrA10, dfrA23, dfrA3b, dfrA19) and aminoglycosides (armA), and also with class A ($bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-20}}$, $bla_{\text{PER-3}}$, $bla_{\text{VEB-3}}$) and class C ($bla_{\text{DHA-1}}$, $bla_{\text{CMY-1}}$, $bla_{\text{CMY-8}}$, $bla_{\text{CMY-9}}$, $bla_{\text{CMY-10}}$, $bla_{\text{MOX-1}}$) β-lactamases. The recently discovered gene, qnr, which confers resistance to quinolones and reduced susceptibility to fluoroquinolones, is also closely linked to IS*CR1* (Mammeri et al. 2005).

8.10 Integrons

Antibiotic resistance genes have frequently been found to be encoded by the determinants carried on mobile genetic elements, such as integrons, plasmids, and transposons which then help in the horizontal transfer of antibiotic resistance genes (Ploy et al. 2000). Integrons are the mobile elements defined by their ability to participate in a powerful site-specific recombination system to capture, accumulate, excise, and organize gene cassettes and then convert them into functional genes by ensuring their correct expression and thus making it the natural expression vectors of these genes (Hall and Collis 1995; Bennet 1999; Carattoli 2001; Mazel 2006). Integrons play a dominant role in spreading antibiotic resistance genes as found in Gram-negative bacteria as a part of the gene cassette inserted into an integron (Fluit and Schmitz 1999; Yu et al. 2004). Five different classes of mobile integrons have been identified on the basis of the sequences which encode integrases (40–58% identity), while only first three classes class 1, class 2, and class 3 are involved in the spread of multi-resistance phenotypes (Mazel 2006), class 4 is a distinctive class

found in *Vibrio cholera* as it is not associated with antibiotic resistance (Mazel et al. 1988). Being detected in 22–59% of Gram-negative isolates, Class 1 integrons are the most widespread and clinically more important (Labbate et al. 2009), these have also been reported in environmental isolates of Gram-negative bacilli (Jones et al. 1997; Fluit and Schmitz 1999). Class 1 type integrons are formed of two conserved segments flanking each other; these have variable length, within which antibiotic resistance gene cassettes are found (Reanney 1976; Fluit and Schmitz 1999). The 5′ conserved segment (5′CS) of class 1 integrons encodes a DNA integrase (IntI1) which mobilizes and inserts gene cassettes through a site-specific recombination mechanism at a specific site (*att1*) adjacent to the *Int1* gene (Hall and Stokes 1993). This 5′ CS region of integron acts as a receptor for gene cassettes, it also contains promoters P_{int} and P_c (P_1) making it possible to transcribe both the integrase and gene cassettes and also to express most of the genes carried on cassettes (Carattoli 2001; Mazel 2006). Three versions of P_1 are known to exist, with different combinations of 210 and 235 sequences in comparison to consensus sequences. These are TTGACAN17TAAACT (a strong promoter), TGGACAN17TAAGCT (a weak promoter), and TGGACAN17TAAACT (a hybrid promoter) (Stokes and Hall 1989; Levesque et al. 1994). Such sequence changes raise the suspicion of a crude mechanism of control of gene expression. In addition to this, the insertion of three guanosine molecules, 119 bases downstream of the promoter P_1 creates a downstream secondary weak promoter, P_2, resulting in a second initiation point of transcription, thereby increasing the expression of inserted gene cassettes (Levesque et al. 1994; Collis and Hall 1992a, 1992b). The 3′ conserved segment (3′CS) of class 1 integrons comprises a truncated antiseptic resistance gene (*qacEΔ1*), a sulfonamide resistance gene (*sul1*), and an open reading frame (*orf5*) of unknown function (Reanney 1976).

8.11 Gene Cassettes and Their Mobility

Gene cassettes, mostly encoding for antibiotic resistance, consist of one coding sequence; a 59-base element located at the 3′ end of this sequence. These may also contain a variable number of non-translated nucleotides. Recently a number of gene cassettes have been reported (Fluit and Schmitz 1999; Partridge et al. 2009), most of these have been found to encode proteins, involved in the resistance to antibiotics. Gene cassettes encoding resistance against antibiotics may cover a wide range of antibiotics and antibiotic classes (Partridge et al. 2009). Gene cassettes mobilization is mediated by the *intI1* gene encoded IntI1, which belongs to the Integrase family. The integrase excises the gene cassettes as covalently closed supercoiled circular molecules (Collis and Hall 1992a), even these circular molecules can also be made to integrate. Deletions, duplications, and rearrangements of gene cassettes in integrons have been observed (Collis and Hall 1992b). The formation of co-integrates between plasmids has been also found to contribute gene cassette exchange (Martinez and de la Cruz 1990). Besides the integrase, the *attI* and 59-base elements are also involved in gene cassette mobility. Gene cassettes are promoter-less genes which contain an

attC recombination site. The *attC*, also known as 59-base elements, is considerably variable in length (Fluit and Schmitz 1999). As reviewed by Partridge et al. (2009), compilation of the *attC* sites has inverted repeats with two 7bp core regions, having the consensus GTTRRRY/RYYYAAC for right-hand/left-hand ends (Hall et al. 1991). Analysis of right-hand consensus sequences which were observed initially indicated GTTAGGC, GTTAGCC, and GTTAGAC as dominant sequences. As demonstrated, in these, "A" is the most common nucleotide present at fourth position but recently G and C have also been seen at this position in one example (*gcu13*, GTTCTGT); therefore, the final nucleotide of right hand was A/G, rather than T/C in a number of *attC* sites but few were found mismatched between 1R and 1L. GTTA and GTTG sequences have been found to play a dominant role in the binding of the four integrase molecules to the *attI1* site; however, it is yet to be ascertained whether all of the four are necessary for recombination (Fluit and Schmitz 1999). One study from China reported that the gene cassettes included those encoding resistance to trimethoprim (dfrA1, dfrA5, dfrA12, dfrA15, dfrA16, dfrA17, and dfrA27), aminoglycosides [aadA, aadA1, aadA2, aadA5, aadA12, aadA13, aadA22, aadB, aac(6′)-II, aac(6′)-Iid, aac(6′)-Ib, aacA4, aacC, aacC1, aacC4 and ant(3″)-Ih], the β-lactamase (bla_{PSE-1}, bla_{OXA-4}, bla_{OXA-10}, bla_{OXA-30}, bla_{IMP-9} and bla_{IMP-25}), chloramphenicol (cmlA1, cmlA6, cmlA8, catB3, and catB8), quinolones (qnrVC-like), and rifampicin (arr-3). Detection of three gene cassettes (ΔMFS-1, HAD-like, and qnrVC-like) in integrons indicated that integrons can efficiently capture and integrate genes. The qnrVC-like gene was included in the catB3-qnrVC-like-aacA4 array that showed 98% identity with the functional qnrVC genes, which differed by 14 and 15 nucleotides compared with qnrVC1 and qnrVC3, respectively. The predicted protein sequence differs from the qnrVC3 sequence only at position 71 (asparagine → aspartic acid). The putative promoter (Pc: -35 TTGACA and -10 TAGTCT) was found in the qnrVC-like cassette with one mutation (C-G) in the -35 motif compared with the functional qnrVC gene promoter (Pc: -35 TTCACA and -10 TAGTCT) (Wu et al. 2012).

Acknowledgements Dr. Anuradha Singh is grateful to the Department of Microbiology, JN Medical College and Hospital, AMU, Aligarh, and also to Aligarh Muslim University for providing necessary facilities during her PhD work.

Disclosure Contents of this chapter have been extracted from the PhD thesis of the primary author of this chapter (Dr. Anuradha Singh) under the supervision of Prof. Mohammad Shahid who is also the co-author in this chapter. The thesis was not published in any form however, the soft copy of the thesis was deposited in the repository of the Maulana Azad Library of the Aligarh Muslim University, the institution from where Dr. Anuradha Singh obtained her PhD. The library and the institution have no objection in publishing the part of the thesis as a book chapter. The soft copy of the thesis is available at http://ir.amu.ac.in/9736/1/T%208440.pdf.

References

Beaber JW, Hachhut B, Waldor MK (2002) Genomic and functional analysis of SXT, an integrating antibiotic resistance gene transfer element derived from Vibrio cholarae. J Bacteriol 184:4259–4269

Bennet PM (1999) Integrons and gene cassettes: a genetic construction kit for bacteria. J Antimicrob Chemother 43:1–4

Bennett PM (2004) Transposable elements. In: Schaechter M (ed) The desk encyclopedia of microbiology. Elsevier, San Diego, pp 1025–1041

Bennett PM (2005) Genome plasticity. In: Woodford N, Johnson A (eds) Methods in molecular biology, vol 266, genomics, proteomics and clinical bacteriology. Humana Press Inc, Totowa, pp 71–113

Bennett PM (2008) Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 153:S347–S357

Bonnet R (2004) Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14

Boyd DA, Tyler S, Christianson S et al (2004) Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum β-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 48:3758–3764

Campbell A (1981) Evolutionary significance of accessory DNA elements in bacteria. Annu Rev Microbiol 35:55–83

Carattoli A (2001) Importance of integrons in the diffusion of resistance. Vet Res 32:243–259

Carattoli A (2008) Evolution of plasmids and evolution of virulence and antibiotic resistance plasmids. In: Baquero F, Nombela G, Cassel H, Gutierrez J (eds) Evolutionary biology of bacterial and fungal pathogens. ASM Press, Washington, DC, pp 155–165

Chandler M, Fayet O (1993) Translational frameshifting in the control of transposition in bacteria. Mol Microbiol 7:497–503. https://doi.org/10.1111/j.1365-2958.1993.tb01140.x

Chandler M, Fayet O, Rousseau P (2015) Copy-out-paste-in transposition of IS911: a major transposition pathway. Microbiol Spectr 3:31. https://doi.org/10.1128/microbiolspec.MDNA3-0031-2014

Collis CM, Hall RM (1992a) Gene cassettes from the insert region of integrons are excised as covalently closed circles. Mol Microbiol 6:2875–2885

Collis CM, Hall RM (1992b) Site-specific deletion and rearrangement of integron insert genes catalyzed by the integron DNA integrase. J Bacterial 174:1574–1585

Coque TM, Novais A, Carattoli A et al (2008) Dissemination of clonally related *Escherichia coli* strains expressing Extended-spectrum β-Lactamase CTX-M-15. Emerg Infect Dis 14:195–200

Couturier M, Bex F, Bergquist PL et al (1988) Identification and classification of bacterial plasmids. Microbiol Rev 52:375–395

Firth N, Skurray RA (2006) The Staphylococcus—genetics: accessory elements and genetic exchange. In: Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Rood JI (eds) Gram-positive pathogens, 2nd edn. ASM Press, Washington, DC, pp 413–426

Fluit AC, Schmitz FJ (1999) Class 1 integrons, gene cassettes, mobility and epidemiology. Eur J Clin Microbiol Infect Dis 18:761–770

Garcillan-Barcia MP, Bernales I, Mendiola MV et al (2002) IS*91* rolling-circle transposition. In: Craig NL, Craigie R, Gellert M, Lambowitz AM (eds) Mobile DNA II. ASM Press, Washington, D.C, pp 891–904

Hall RM, Collis CM (1995) Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol Microbiol 15:593–600

Hall R, Stokes H (1993) Integrons: novel DNA elements which capture genes by site-specific recombination. Genetica 90:115–132

Hall RM, Brookes DE, Stokes HW (1991) Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination cross-over point. Mol Microbiol 5: 1941–1959

Hallet B, Sherratt DJ (1997) Transposition and site-specific recombination: adapting DNA cut-and-paste mechanisms to a variety of genetic rearrangements. FEMS Microbiol Rev 21:157–178. https://doi.org/10.1111/j.1574-6976.1997.tb00349.x

Harmer CJ, Moran RA, Hall RM (2014) Movement of IS*26*-associatedantibiotic resistance genes occurs via a translocatable unit that includes a single IS*26* and preferentially inserts adjacent to another IS*26*. MBio 5:01801. https://doi.org/10.1128/mBio.01801-14

Hickman AB, Dyda F (2015) Mechanisms of DNA transposition. Microbiol Spectr 3:34. https://doi.org/10.1128/microbiolspec.MDNA3-0034-2014

Jones ME, Peters E, Weersink A-M et al (1997) Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. Lancet 349:1742–1743

Labbate M, Case RJ, Stokes HW (2009) The integron/gene cassette system: an active player in bacterial adaptation. Methods Mol Biol 532:103–125

Lartigue MF, Poirel L, Aubert D et al (2006) In vitro analysis of ISEcp1B-mediated mobilization of naturally occurring β-lactamase gene bla_{CTX-M} of *Kluyveraascorbata*. *Antimicrob Agents Chemother* 50:1282–1286

Lee NLS, Yuen KY, Kumana CR (2001) β-lactam antibiotic and β-lactamase inhibitor combinations. J Am Med Assoc 285(4):386–388

Levesque C, Brassard S, Lapointe J et al (1994) Diversity and relative strength of tandem promoters for the antibiotic-resistance genes of several integrons. Gene 142:49–54

Livermore DM (1993) Determinants of the activity of the activity of β-lactamase Inhibitor combinations. J Antimicrob Chemother 31(Suppl A):9–21

Mahillon J, Chandler M (1998) Insertion sequences. Microbiol Mol Biol Rev 62:725–774

Mammeri H, Van de Loo M, Poirel L et al (2005) Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob Agents Chemother 49:71–76

Martinez E, de la Cruz F (1990) Genetic elements involved in Tn*21* site-specific integration, a novel mechanism for the dissemination of antibiotic resistance genes. EMBO J 9:1275–1281

Mazel D (2006) Integrons: agents of bacterial evolution. Nat Rev Microbiol 4:608–620

Mazel D, Dychinco B, Webb VA et al (1988) A distinctive class of integron in the *Vibrio cholerae* genome. Science 280:605–608

McManus MC (1997) Mechanisms of bacterial resistance to antimicrobial agents. Am J Health Syst Pharm 54:1420–1433

Miriagou V, Carattoli A, Fanning S (2006) Antimicrobial resistance islands: resistance gene clusters in *Salmonella* chromosome and plasmids. Microbes Infect 8:1923–1930

Mollet B, Iida S, Shepherd J et al (1983) Nucleotide sequence of IS26, a new prokaryotic mobile genetic element. Nucleic Acids Res 11:6319–6330

Mollet B, Clerget M, Meyer J et al (1985) Organization of the Tn*6*- related kanamycin resistance transposon Tn*2680* carrying two copies of IS*26* and an IS*903* variant, IS*903B*. J Bacteriol 163: 55–60

Mugnier PD, Poirel L, Nordmann P (2009) Functional analysis of insertion sequence IS*Aba1*, responsible for genomic plasticity of *Acinetobacter baumannii*. J Bacteriol 191:2414–2418. https://doi.org/10.1128/JB.01258-08

Needham C, Noble WC, Dyke KGH (1995) The staphylococcal insertion sequence IS*257* is active. Plasmid 34:198–205. https://doi.org/10.1006/plas.1995.0005

Novais A, Canton R, Moreira R et al (2007) Emergence and dissemination of *Enterobacteriaceae* isolates producing CTX-M-1-like enzymes in Spain are associated with IncF II (CTX-M-15) and broad-host-range (CTX-M-1,-3, and -32) plasmids. Antimicrob Agents Chemother 51:796–799

Partridge SR, Tsafnat G, Coiera E et al (2009) Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev 33:757–784

Partridge SR, Kwong SM, Firth N et al (2018) Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31:e00088–e00017. https://doi.org/10.1128/CMR.00088-17

Ploy MC, Lambert T, Couty JP et al (2000) Integrons: an antibiotic resistance gene capture and expression system. Clin Chem Lab Med 38:483–487

Poirel L, Decousser JW, Nordmann P (2003) Insertion sequence IS*Ecp1B* is involved in expression and mobilization of a bla_{CTX-M} β-lactamase gene. Antimicrob Agents Chemother 47:2938–2945

Poirel L, Lartigue MF, Decausser JW et al (2005) IS*Ecp1*B-mediated transposition of bla_{CTX-M} in *Escherichia coli*. Antimicrob Agents Chemother 49:447–450

Reanney D (1976) Extrachromosomal elements as possible agents of adaptation and development. Bacteriol Rev 40:552–590

Rossolini GM, D'Andrea MM, Mugnaioli C (2008) The spread of CTX-M-type extended-spectrum β-lactamases. Clin Microbiol Infect 14(Suppl. 1):33–41

Shahid M, Singh A, Sobia F et al (2011) An overview of CTX-M β-lactamases. Rev Med Microbiol 22:28–40

Stokes HW, Hall RM (1989) A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1669–1683

Toleman MA, Bennett PM, Walsh TR (2006) IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev 70:296–316

Vandecraen J, Chandler M, Aertsen A et al (2017) The impact of insertion sequences on bacterial genome plasticity and adaptability. Crit Rev Microbiol 43:709–730. https://doi.org/10.1080/1040841X.2017.1303661

Wachino J, Yamane K, Kimura K et al (2006) Mode of transposition and expression of 16S rRNA methyltransferase gene *rmtC* accompanied by IS*Ecp1*. Antimicrob Agents Chemother 57:154–155

Wain J, Diem Nga LT, Kidgell C et al (2003) Molecular analysis of incHI1 antimicrobial resistance plasmids from *Salmonella serovar Typhi* strains associated with typhoid fever. Antimicrob Agents Chemother 47:2732–2739

Wu K, Wang F, Suna J et al (2012) Class 1 integron gene cassettes in multidrug-resistant Gram-negative bacteria in southern China. Int J Antimicrob Agents 40:264–267

Yu HS, Lee JC, Kang HY et al (2004) Prevalence of *dfr* genes associated with integrons and dissemination of *dfrA17* among urinary isolates of *Escherichia coli* in Korea. J Antimicrob Chemother 53:445–450

Plasmids Associated with Beta-Lactamases in Bacteria

9

Hiba Sami, Mohammad Shahid, and Haris M. Khan

Abstract

Different resistance components are capable of resistance in Family Enterobacteriaceae, among which plasmid-encoded extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase are the most significant. Extended-spectrum β-lactamases (ESBLs) are a differing, complex, and quickly advancing bunch of plasmid-mediated proteins that are displaying a major helpful challenge within the care of hospitalized and community-based patients nowadays. ESBL-related infections range from uncomplicated infections of the urinary tract to life-threatening sepsis. Enterobacteriaceae have been identified as a major cause of hospital-acquired infections since the 1980s, particularly *Klebsiella* spp. harboring enzyme ESBLs such as SHV and TEM forms. Resistance to unrelated antibiotics is often co-transferred by plasmids encoding extended-spectrum beta-lactamases; therefore, plasmid profiling is a valuable diagnostic method as well as an effective tool for epidemiological typing.

Keywords

Plasmids · ESBL · Hospital-acquired infections

H. Sami (✉) · H. M. Khan
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid
Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: mohammeds@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_9

9.1 Introduction

A plasmid could be a small extrachromosomal DNA entity ranging in size from 1 kbp to 200 kbp that is physically isolated from chromosomal DNA and can replicate independently. The number of indistinguishable plasmids in a single cell can extend from one to thousands in a few cases. Naturally, plasmids bear qualities that help the organism's survival and give a specific advantage, such as antimicrobial resistance. Not at all like chromosomes, which are expansive and contain all of the hereditary material required for ordinary life, plasmids are as a rule small and contain extra genes that will be valuable in specific circumstances or conditions. Later research revealed that plasmids were self-replicating DNA molecules capable of cell-to-cell self-transmission and of mobilizing chromosome segments via a mechanism known as high-frequency recombination (Hfr) (Adelberg and Pittard 1965). Nowadays man-made plasmids are being used as vectors in molecular cloning. Plasmids can be inserted into a cell through transformation in the laboratory (Fig. 9.1).

9.2 Plasmid's Significance in Antibiotic Resistance

The development and spread of antibiotic-resistant bacteria have become a major concern in hospital infection control in the last decade (Rajaee Behbahani et al. 2019). Knowledge of the resistance mechanism is essential to plan stewardship programs to curtail the spread of these resistant pathogens. Antibiotic resistance

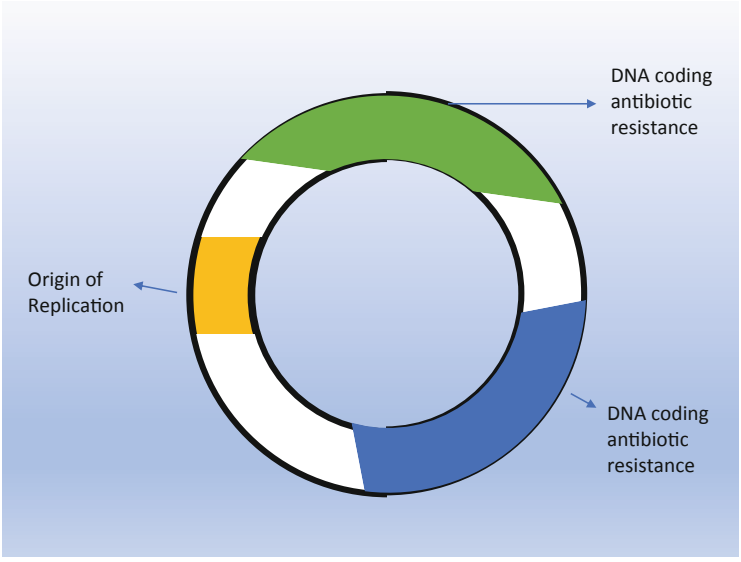


Fig. 9.1 An illustration of plasmid

genes are found on the genetic components of bacteria, such as chromosomes and plasmids. Resistance transmission is increased by genes on plasmids frequently observed in nosocomial infections because of the high transferability of these genetic materials (Lerminiaux and Cameron 2019). In Gram-negative bacteria, plasmid-defined β-lactamase activity is usually the source of high-level resistance to broad-spectrum β-lactam antibiotics (Foster 1983). In hospital and community-acquired infections, *E. coli* and *Klebsiella pneumoniae* pose the greatest threat of plasmid-mediated drug resistance (Schultsz and Geerlings 2012).

9.3 Mechanism of Drug Resistance in Plasmids

Plasmids use four classic resistance mechanisms:

(1) Drug inactivation, (2) decreased cell permeability, (3) bypass, and (4) repositioning of the target (Davies and Smith 1978) as shown in Fig. 9.2.

Some authors have given plasmid-specific Gram-negative bacteria enzymes names based on the bacterial host from which they were isolated or the β-lactam drug that they better hydrolyze.

9.4 Why to Study Plasmids?

Plasmids are the ideal vectors for the spread of antibiotic resistance because of their ability to acquire new genes via transposons or insertion sequences, which are mobile genetic elements, in addition to their ability to propagate in a variety of hosts. In the 1980s, Cephalosporins, carbapenems, and monobactams, which have a higher β-lactamase stability, were introduced. Small RNAs (sRNA) resistance first appeared in organisms including *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, which were able to

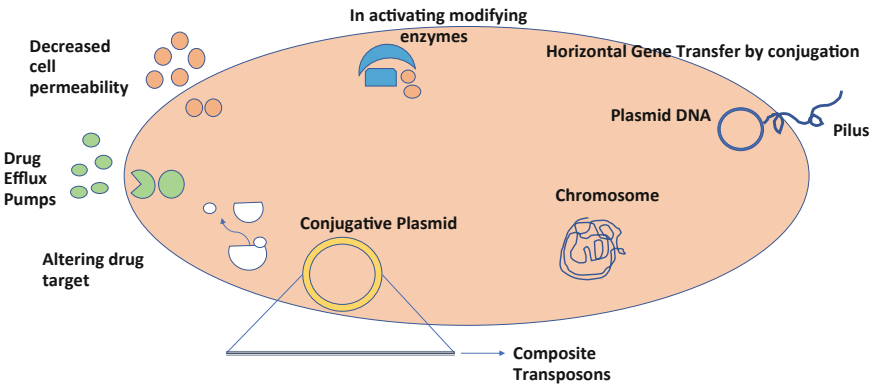


Fig. 9.2 Mechanisms to counteract effect of antibiotics

overproduce their chromosomal AmpC β-lactamase (also known as class C or group 1) via mutation, resulting in resistance to both oxyimino- and 7-methoxy-cephalosporins as well as monobactams (Sanders 1987). Resistance was later discovered in bacteria lacking an inducible AmpC enzyme, such as *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp., and *Proteus mirabilis*, and it was discovered that this resistance was mediated by plasmids encoding extended-spectrum β-lactamases (ESBLs), which are enzymes that developed from mutations in TEM or SHV β-lactamases with less hydrolytic capacity (Philippon et al. 2002). As a result, identifying plasmid features and behavior in various bacterial hosts provides critical information about AMR transmission.

9.5 Plasmids Linked to β-lactamases in Gram-Negative Bacteria

In Gram-negative bacteria, plasmid-controlled β-lactamase activity is frequently the source of high-level resistance to broad-spectrum β-lactamase antibiotics. Table 9.1 summarizes some common β-Lactamases transferred through plasmids.

9.5.1 TEM ESBLs

In Gram-negative bacteria, the TEM enzyme was the first R-plasmid-linked β-lactamase to be discovered. The RTEM plasmid inspired the enzyme's name. TEM-1 and TEM-2 are two varieties that can be identified by isoelectric focusing (Foster 1983). Their ubiquitous occurrence is due to transposons carrying the TEM β-lactamase gene.

Table 9.1 Common β-Lactamases transferred through plasmids

Plasmid-mediated β-lactamases	Commonly found in species
TEM	*Klebsiella pneumoniae*, *Escherichia coli*
SHV-1	*Klebsiella pneumoniae*
AmpC-type	*Klebsiella pneumoniae*
CTX-M	*Klebsiella pneumoniae*, *Escherichia coli*
Plasmid-encoded MBL (IMP-1)	*Pseudomonas aeruginosa*
OXA-1	*Klebsiella pneumoniae*
OXA-48 carbapenemase	*Klebsiella pneumoniae*
PSE-1	*Pseudomonas aeruginosa*
PSE-4	*Pseudomonas aeruginosa*
CEP-1	*Proteus mirabilis*
MCR-1	*Escherichia coli*
HMS-1	*Proteus mirabilis*
ROB	*Haemophilus influenzae type b*
VIM, IMP, NDM	*Klebsiella pneumoniae*, *Escherichia coli*

9.5.2 SHV ESBLs

Klebsiella species have developed resistance to a wide spectrum of antibiotics in recent years. The penicillinase enzyme SHV-1 has been found to confer resistance to penicillins, particularly ampicillin and carbenicillin, in *Klebsiella. K. pneumoniae* carbapenemases are a unique carbapenem resistance mechanism established by *K. pneumoniae* (KPCs). Antibiotic tolerance is conferred by a plasmid-encoded KPC that can hydrolyze cephalosporins, monobactams, and even carbapenems. It has been reported that these resistance plasmids have spread to other Gram-negative bacteria such as *Enterobacter*, *K. oxytoca*, *E. coli*, *Serratia marcescens*, *and Pseudomonas species*. In Enterobacteriaceae, a mutant bla_{SHV-27} (Corkill et al. 2001) has been discovered on various plasmids and has been connected to a wide range of antibiotic resistance genes (bla_{DHA1}, bla_{TEM-1}, bla_{TEM1b}, bla_{CMY-2}, bla_{IMP}, $bla_{CTX-M-14}$, $bla_{CTX-M-15}$, bla_{SHV-12}, bla_{SHV} (Liakopoulos et al. 2016; Muratani et al. 2006).

9.5.3 Pseudomonas-Specific Enzymes (PSE)

Carbenicillin is hydrolyzed at a faster rate than penicillin by certain plasmid-specific β-lactamases. They are named pseudomonas-specific enzymes (PSE) because they were found in *P. aeruginosa.* The PSE-1/CARB-2 enzyme was first identified as a determinant of *P. aeruginosa* IncP2 plasmids with a confined host range; however, it has recently been found in intestinal bacteria and connected to transposons. The CARB-4/PSE-3 gene is controlled by a single plasmid in *P. aeruginosa* (Foster 1983).

9.5.4 CEP-1

There have been reports of β-lactamases with plasmid-specificity that primarily hydrolyze cephalosporins. The CEP-1 determinant was passed from *Proteus mirabilis* to *Escherichia coli*, where it was identified as a β-lactamase with properties that were similar to the chromosomal Amp C enzyme in *E. coli* (Philippon et al. 2002).

9.5.5 Amp C β-lactamases Mediated by Plasmids (pAmpC)

Plasmids that transmit class C β-lactamases appeared as a result of the therapeutic use of 7-methoxy-cephalosporins (cefoxitin and cefotetan) and the advent of β-lactamase inhibitor combinations (amoxicillin or ticarcillin plus clavulanate, sulbactam plus ampicillin, and tazobactam plus piperacillin) (Medeiros 1997). While the chromosomal mediated AmpC is mainly present in members of the Enterobacteriaceae family's group II (like *Enterobacter* spp., *Serratia* spp., *Citrobacter freundii*, *Providentia* spp., *Morganella morganii*, and *Hafnia alvei*)

making them resistant to third generation cephalosporins (Rensing et al. 2019). The pAmpC (plasmid-mediated AmpC) plasmids were first found in Enterobacteriaceae that either have reduced expression of chromosomally encoded AmpC or do not have it at all (such as *Klebsiella* spp., *Escherichia coli*, *Proteus* spp., *Salmonella* spp., and *Shigella* spp.) (Jacoby 2009). pAmpC can also provide carbapenem resistance in a strain with reduced outer membrane permeability, as shown by the results of an epidemic in New York, where a large number of *K. pneumoniae* isolates were collected and studied (Bradford et al. 1997). TEM-1, TEM-2, or even an ESBL, such as SHV-5, are produced by many strains having plasmid-determined AmpC enzymes (Philippon et al. 2002).

9.5.6 CTXM Enzymes

Class A extended-spectrum β-lactamases (ESBLs) are a subset of CTX-M-type enzymes that are rapidly proliferating in Enterobacteriaceae. Around 50 different alleles have been described, and six distinct sub-lineages have been established. One such paper reports the isolation of the CTX-M-encoding genes from *Kluyvera* species and demonstrates that these genes can be distributed through pathogenic enterobacteria (Jiang et al. 2017). CTX-M elements can be distributed through a variety of genetic mechanisms. A newly discovered ISEcp1 insertion sequence is a transposable element that can mediate the movement of flanking DNA segments through a one-ended transposition mechanism (CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-25). Inter-replicon transmission possibly included the assistance of ISEcp1 and the CR1-associated recombination mechanism in the capturing of $bla_{CTX\text{-}M}$ genes by conjugative plasmids, which were then distributed between the replicons by way of Tn402-like backbone interactions. The importance of plasmids, which are mostly self-conjugative and contain additional resistance markers, in the horizontal transfer of $bla_{CTX\text{-}M}$ genes is well-known (Bonnet 2004).

9.5.7 Carbapenemases

Plasmids are increasingly essential for the successful transmission of carbapenemases, particularly the VIM, IMP, and NDM metallo-β-lactamases (MBL), the serine-carbapenemase KPC, and carbapenem-hydrolyzing class D OXA β-lactamases (CHDLs). The $bla_{NDM\text{-}1}$ gene is present primarily on plasmids, but various plasmid varieties, including IncL/M, IncA/C, IncF, IncHI1, and distinct IncN and IncHI1 plasmid variants, were mainly responsible for the distribution of the $bla_{NDM\text{-}1}$ gene in nonclonally associated enterobacterial isolates (Carattoli 2013). bla_{NDM} is present on bacterial chromosomes, but most carriage occurs on plasmids, which aid its dissemination. bla_{NDM} has been found on plasmids carrying a variety of replicon types (Wu et al. 2019).

9.5.8 Plasmid Typing

Datta and Hedges developed the first plasmid typing scheme in 1971 (Datta and Hedges 1971). By classifying plasmids into various kinds based on their phylogenetic relatedness, the epidemiology of antibiotic resistance mediated by plasmids has been better understood. Presently the common typing methods use either replication fork loci (replicon typing) or plasmid mobility (MOB typing). For plasmid typing, traditional PCR-based approaches are still commonly employed (Orlek et al. 2017). Table 9.2 shows a list of some common plasmids.

9.6 Replicon Typing Scheme

9.6.1 Incompatibility (Inc) Grouping

Inc typing is a method of classifying plasmids based on their ability to live in a stable manner in the same bacterial strain as other plasmids, which is determined by their replication machinery. When coresident plasmids have the same replication machinery, they are incompatible. Because the Inc group is determined by the plasmid replicon type, the terms Inc and Rep type are interchangeable when describing plasmid kinds (Johnson and Nolan 2009). Plasmids from the Enterobacteriaceae family have been assigned to the Inc group, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Datta and Hedges 1971; Taylor et al. 2004). The approach has been useful in tracing the transmission of plasmids that confer antimicrobial resistance, as well as in tracking the evolution and dissemination of new plasmids (Carattoli et al. 2005).

9.6.2 PCR-Based Replicon Typing (PBRT)

The presence of several plasmids inside a single cell will make plasmid recognition challenging, rendering it difficult to determine a bacterial strain's total plasmid content. To overcome this PBRT has been widely used. This technique has been estimated to have screened thousands of strains, permitting the detection of supposed "epidemic" plasmids that dispersed in Enterobacteriaceae beyond the limits of organisms and at far-flung topographical locations (Carattoli 2013). The PBRT-defined replicon content has been utilized to investigate outbreak clones microbiologically and track the transmission of certain resistant determinants in groups of epidemiologically related but genetically unrelated bacterial isolates (Carattoli 2013). PBRT now detects 28 replicons in plasmids from the well-studied Enterobacteriaceae family (dependent on many genetic loci, including replication regulatory sequences and rep genes) (Orlek et al. 2017). In a recent study, in silico research revealed 22 replicons, and 5 more were discovered and cloned from previously unidentified *A. baumannii* resistance plasmids containing the carbapenem-hydrolyzing oxacillinase OXA-58 (Bertini et al. 2010).

Table 9.2 Summary of plasmids associated with β-lactamases

Plasmid	Characteristics	Host range and transferability
IncF plasmids	Conjugative plasmids with low copy number that vary in size from 45 to 200 kb (classified as MOB_F according to relaxase typing) (Garcillán-Barcia et al. 2011). The most frequently mentioned resistance genes on IncF plasmids are ESBL genes, carbapenemase genes, aminoglycoside-modifying enzyme genes, and plasmid-mediated quinolone resistance (PMQR) genes (Rozwandowicz et al. 2018)	Enterobacteriaceae; conjugative
IncI plasmids	IncI, also known as MOB_P, is a family of low copy number, plasmids that vary in size from 50 to 250 kb (Garcillán-Barcia et al. 2011). $bla_{CTX-M-1}$ is the most frequently found gene on IncI plasmid ST7 and 3 and it has been related to *E. coli* strains ST10, 58, 117, and 131 (Leverstein-van Hall et al. 2011; Wang et al. 2014). IncI2 plasmids were recently discovered to be linked to the *mcr-1* colistin resistance gene and its counterparts *mcr-1.3* and *mcr-1.5* (Yang et al. 2017; Tijet et al. 2017)	Limited; conjugative
I-plasmid complex	I-Plasmid complex comprises IncK, IncB/O, and IncZ plasmids. The resemblance of the IncK, IncB/O, and IncZ RNAI sequences, which are targets in the PBRT system, makes their typing problematic. In Europe, IncK plasmids are mostly linked to the dissemination of the bla_{CMY-2} as well as $bla_{CTX-M-14}$ genes (Rozwandowicz et al. 2018)	Limited; conjugative
Inc A/C plasmids	IncA/C is a family of low copy number plasmids that range in size from 40 to 230 kb; however, smaller conjugative variations of 18–25 kb have also been found (Lee et al. 2014). Classified as MOB_H in relaxase typing (Garcillán-Barcia et al. 2011). IncA/C plasmids replicate not only in Enterobacteriaceae but also in *Pseudomonas* and other bacteria like *Photobacterium damselae*. These plasmids include resistance determinants to aminoglycosides,	Limited; conjugative

(continued)

Table 9.2 (continued)

Plasmid	Characteristics	Host range and transferability
	sulfonamides, trimethoprim, and chloramphenicol, as well as restriction enzymes, antirestriction DNA methylases, and partitioning mechanisms, which help in their persistence and durability (Johnson and Lang 2012)	
IncH	IncH is a family of low copy number, plasmids that range in size from 75 to 400 kb. Classified as MOB_H in relaxase typing (Garcillán-Barcia et al. 2011). IncHI1 plasmids, which were historically only present in *Salmonella* isolates and have a transition temperature of 22–30 °C, have now been discovered in other bacteria, raised the possibility that they could help spread the bla_{NDM-1} gene among bacteria in environment (Dolejska et al. 2013)	Enterobacteriaceae, many Gram-negative species as *Aeromonas* spp., *Vibrio* spp., etc; conjugative
IncP plasmids	IncP (MOB_P) is a group of broad host range, low copy number plasmids, 70–275 kb in size. These plasmids are designated as IncP in Enterobacteriaceae and IncP-1 in *Pseudomonas* spp. The IncP plasmid has recently been related to the colistin resistance gene *mcr-1* and its variant *mcr-1.6*. The resistance genes dfrA1, tet(A), and sul1 were all found on this plasmid (Malhotra-Kumar et al. 2016; Lu et al. 2017)	Broad; conjugative
IncL/M plasmids	IncL/M, often known as MOB_P due to its relaxase type, is a collection of broad host range plasmids with sizes ranging from 50 to 80 kb and low copy numbers (Garcillán-Barcia et al. 2011). The ArmA 16S RNA methylases were carried on pCTXM-3, a self-transmissible plasmid of the IncL/M family that propagated the $bla_{CTX-M-3}$ gene in Europe and China (Gołębiewski et al. 2007). pCTX-M-3 is closely linked to the IncL/M plasmids that have been linked to NDM-1 and ArmA. Globally, bla_{OXA-48} has been linked to a 60 kb IncL plasmid, formerly known as IncL/M, while this gene has also been found on IncF and IncP plasmids.	Broad; conjugative

(continued)

Table 9.2 (continued)

Plasmid	Characteristics	Host range and transferability
	Nosocomial infections are believed to be caused by *K. pneumoniae* bearing these IncL plasmids with bla_{OXA-48} (Potron et al. 2013)	
IncN plasmids	IncN, also known as MOB_F in relaxase typing, is a family of broad-host range plasmids with size ranging from 30 and 70 kb whose copy number is regulated by iterons (Garcillán-Barcia et al. 2011). Unlike NDM-1, which is transferred by a broad range of plasmids, MBL VIM-1 is spread by a limited number of plasmids. The bla_{VIM-1} gene was found in abundance on various IncN family variants in Italy and Greece. VIM-1-IncN plasmids spread in unlinked *Klebsiella* spp. bacteria, staying in different hospitals for lengthy periods of time and gaining PMQR genes and added resistance determinants (Carattoli 2013)	Broad; conjugative
Colicinogenic plasmids	Colicins, which are members of the bacteriocin family, are proteins formed by some *E. coli* strains that are fatal to linked *E. coli* bacteria (Cascales et al. 2007). Colicins are primarily encoded by genes found on plasmids. The ColE1 plasmid is the source of the majority of qnr-carrying plasmids conferring quinolone resistance. They are most commonly found in strains of *S. enterica* recovered from human samples	Limited; mobilizable
IncX	IncX is a set of narrow-host range plasmids classified as MOBP by relaxase typing. There are six known subtypes of IncX plasmids (X1–X6), with sizes from 30 to 50 kb range (Garcillán-Barcia et al. 2011). *Salmonella* strains obtained prior to the widespread use of antibiotics included IncX plasmids (Jones and Stanley 1992). IncX plasmids are now mostly obtained from both human and animal sources of *Salmonella* and *E. coli* (Norman et al. 2008). These plasmids largely encode antimicrobial resistance determining factor for extended-spectrum	Limited

(continued)

Table 9.2 (continued)

Plasmid	Characteristics	Host range and transferability
	b-lactams and quinolones. IncX plasmids can also carry resistance genes for tetracycline and trimethoprim. Carbapenemase genes (mostly bla_{KPC} and bla_{NDM}) have been found on IncX plasmids (Pál et al. 2017) (Venditti et al. 2017). The colistin resistance genes mcr-1 and mcr-2 have recently been linked to an IncX4 plasmid (Xavier et al. 2016)	

9.6.3 Southern Blot Hybridization with Replicon Probes

Plasmids can be also classified based on hybridization using replicons as probes but this method is now obsolete. When closely similar replicons are present, probe hybridization loses specificity, and it is no longer extensively employed unless in the context of PCR-based replicon typing (PBRT), a technique that uses PCR amplicons as probes to sort plasmids separated on a gel (Orlek et al. 2017).

9.7 MOB Typing Scheme

The relaxase (relaxases are required for the conjugation of plasmids) genes expressed by transmissible plasmids are detected by degenerate primer MOB typing (DPMT) using PCR. Since the genes for relaxase (also known as Mob proteins) are more conserved than those for repA, this technique has the ability to detect all proteobacterial transmissible plasmids and classify them into one of five phylogenetic relaxase MOB groups (Carattoli et al. 2005). MOB typing is more sensitive but has a low resolution, and it cannot be used on plasmids that do not contain the relaxase gene. pMLST techniques for plasmid subtyping have been developed for enterobacteria plasmid families.

9.8 Plasmid Multilocus Sequence Typing (pMLST)

For pMLST review, a weekly modified database was developed from www.pubmlst.org and incorporated into a web program called in silico pMLST. PlasmidFinder and pMLST are web-based applications that allow users to search WGS data from a variety of genome sequencers and extract plasmid data for clinical and epidemiological research (Carattoli et al. 2014).

9.9 Restriction Fragment Length Polymorphism (RFLP) Typing of Plasmids

Since the early 1970s, plasmid profile typing (size and number) and plasmid fingerprinting (processed plasmid DNA) are commonly used to examine the links between epidemiologically associated Gram-positive microbe strains and to monitor the transfer of genes of public health significance like antibiotic resistance, Bacteriocin production, etc. The use of restriction endonuclease analysis can substantially improve the investigator's capacity to distinguish strains that only have one plasmid. At what is known as a restriction site, a restriction enzyme chops DNA segments within a specified nucleotide sequence. These recognition sequences are usually four, six, eight, ten, or twelve nucleotides long and palindromic (that is, they all have the same nucleotide sequence in the 5′–3′ direction). Plasmid fingerprinting is frequently the only distinguishing feature for strains engaged in outbreaks (Tenover 1985).

9.10 Conclusion

The evidence clearly shows that some plasmid families are more widely diffused and scattered among bacteria in nature, while others show distinct differences in the distributions in pathogens and commensal bacteria. Epidemic plasmids linked to the propagation of resistance through β-lactamases like CTX-M-1, CTX-M-15, NDM-1, OXA-48, and VIM-1 have been found in genetically diverse strains discovered over vast distances with no apparent epidemiological linkages. Plasmids are the most difficult threat to antimicrobial resistance spread because they contribute to the evolution and emergence of evolving and relevant resistance traits, are highly suited to the host, and are hard to cure or prevent with existing therapies.

References

Adelberg EA, Pittard J (1965) Chromosome transfer in bacterial conjugation. Bacteriol Rev 29: 161–172

Bertini A, Poirel L, Mugnier PD, Villa L, Nordmann P, Carattoli A (2010) Characterization and PCR-based replicon typing of resistance plasmids in Acinetobacter baumannii. Antimicrob Agents Chemother 54:4168–4177. https://doi.org/10.1128/AAC.00542-10

Bonnet R (2004) Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14. https://doi.org/10.1128/AAC.48.1.1-14.2004

Bradford PA, Urban C, Mariano N, Projan SJ, Rahal JJ, Bush K (1997) Imipenem resistance in Klebsiella pneumoniae is associated with the combination of ACT-1, a plasmid-mediated AmpC beta-lactamase, and the foss of an outer membrane protein. Antimicrob Agents Chemother 41:563–569. https://doi.org/10.1128/AAC.41.3.563

Carattoli A (2013) Plasmids and the spread of resistance. IJMM 303:298–304. https://doi.org/10.1016/j.ijmm.2013.02.001

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018

Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H (2014) In silico detection and typing of plasmids using plasmid finder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14

Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, Riley M, Slatin S, Cavard D (2007) Colicin biology. MMBR 71:158–229. https://doi.org/10.1128/MMBR.00036-06

Corkill JE, Cuevas LE, Gurgel RQ, Greensill J, Hart CA (2001) SHV-27, a novel cefotaxime-hydrolysing β-lactamase, identified in Klebsiella pneumoniae isolates from a Brazilian hospital. J Antimicrob Chemother 47:463–465. https://doi.org/10.1093/jac/47.4.463

Datta N, Hedges RW (1971) Compatibility groups among fi − R factors. Nature 234:222–223. https://doi.org/10.1038/234222a0

Davies J, Smith DI (1978) Plasmid-determined resistance to antimicrobial agents. Annu Rev Microbiol 32:469–518. https://doi.org/10.1146/annurev.mi.32.100178.002345

Dolejska M, Villa L, Poirel L, Nordmann P, Carattoli A (2013) Complete sequencing of an IncHI1 plasmid encoding the carbapenemase NDM-1, the ArmA 16S RNA methylase and a resistance-nodulation-cell division/multidrug efflux pump. J Antimicrob Chemother 68:34–39. https://doi.org/10.1093/jac/dks357

Foster TJ (1983) Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol Rev 47:361–409

Garcillán-Barcia MP, Alvarado A, de la Cruz F (2011) Identification of bacterial plasmids based on mobility and plasmid population biology. FEMS Microbiol Rev 35:936–956. https://doi.org/10.1111/j.1574-6976.2011.00291.x

Gołębiewski M, Kern-Zdanowicz I, Zienkiewicz M, Adamczyk M, Żylińska J, Baraniak A, Gniadkowski M, Bardowski J, Cegłowski P (2007) Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum β-lactamase gene blaCTX-M-3. Antimicrob Agents Chemother 51:3789–3795. https://doi.org/10.1128/AAC.00457-07

Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22:161–182. https://doi.org/10.1128/CMR.00036-08

Jiang X, Liu X, Law COK, Wang Y, Lo WU, Weng X, Chan TF, Ho PL, Lau TCK (2017) The CTX-M-14 plasmid pHK01 encodes novel small RNAs and influences host growth and motility. FEMS Microbiol Ecol 93:90. https://doi.org/10.1093/femsec/fix090

Johnson TJ, Lang KS (2012) IncA/C plasmids. Mob Genet Elem 2:55–58. https://doi.org/10.4161/mge.19626

Johnson TJ, Nolan LK (2009) Plasmid replicon typing. In: Caugant DA (ed) Molecular epidemiology of microorganisms: methods and protocols, methods in molecular biology. Humana Press, Totowa, pp 27–35. https://doi.org/10.1007/978-1-60327-999-4_3

Jones C, Stanley J (1992) Salmonella plasmids of the pre-antibiotic era. J Gen Microbiol 138:189–197. https://doi.org/10.1099/00221287-138-1-189

Lee K-E, Lim S-I, Choi H-W, Lim S-K, Song J-Y, An D-J (2014) Plasmid-mediated AmpC β-lactamase (CMY-2) gene in Salmonella typhimurium isolated from diarrheic pigs in South Korea. BMC Res Notes 7:329. https://doi.org/10.1186/1756-0500-7-329

Lerminiaux NA, Cameron ADS (2019) Horizontal transfer of antibiotic resistance genes in clinical environments. Can J Microbiol 65:34–44. https://doi.org/10.1139/cjm-2018-0275

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJM, Mevius DJ (2011) Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17:873–880. https://doi.org/10.1111/j.1469-0691.2011.03497.x

Liakopoulos A, Mevius D, Ceccarelli D (2016) A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01374

Lu X, Hu Y, Luo M, Zhou H, Wang X, Du Y, Li Z, Xu J, Zhu B, Xu X, Kan B (2017) MCR-1.6, a new MCR variant carried by an IncP plasmid in a colistin-resistant Salmonella enterica serovar typhimurium isolate from a healthy individual. Antimicrob Agents Chemother 61:16. https://doi.org/10.1128/AAC.02632-16

Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Butaye P, Goossens H (2016) Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid. Lancet Infect Dis 16:283–284. https://doi.org/10.1016/S1473-3099(16)00012-8

Medeiros AA (1997) Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. Clin Infect Dis 24(Suppl 1):19–45. https://doi.org/10.1093/clinids/24.supplement_1.s19

Muratani T, Kobayashi T, Matsumoto T (2006) Emergence and prevalence of β-lactamase-producing Klebsiella pneumoniae resistant to cephems in Japan. Int J Antimicrob Agents 27:491–499. https://doi.org/10.1016/j.ijantimicag.2006.03.007

Norman A, Hansen LH, She Q, Sørensen SJ (2008) Nucleotide sequence of pOLA52: a conjugative IncX1 plasmid from Escherichia coli which enables biofilm formation and multidrug efflux. Plasmid 60:59–74. https://doi.org/10.1016/j.plasmid.2008.03.003

Orlek A, Stoesser N, Anjum MF, Doumith M, Ellington MJ, Peto T, Crook D, Woodford N, Walker AS, Phan H, Sheppard AE (2017) Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. Front Microbiol 8:182. https://doi.org/10.3389/fmicb.2017.00182

Pál T, Ghazawi A, Darwish D, Villa L, Carattoli A, Hashmey R, Aldeesi Z, Jamal W, Rotimi V, Al-Jardani A, Al-Abri SS, Sonnevend Á (2017) Characterization of NDM-7 carbapenemase-producing Escherichia coli isolates in the Arabian peninsula. Microb Drug Resist 23:871–878. https://doi.org/10.1089/mdr.2016.0216

Philippon A, Arlet G, Jacoby GA (2002) Plasmid-determined AmpC-type β-lactamases. Antimicrob Agents Chemother 46:1–11. https://doi.org/10.1128/AAC.46.1.1-11.2002

Potron A, Poirel L, Rondinaud E, Nordmann P (2013) Intercontinental spread of OXA-48 beta-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. Euro Surveill. Bull Eur Sur Mal Transm Eur Commun Dis Bull 18:20549. https://doi.org/10.2807/1560-7917.es2013.18.31.20549

Rajaee Behbahani M, Keshavarzi A, Pirbonyeh N, Javanmardi F, Khoob F, Emami A (2019) Plasmid-related β-lactamase genes in Pseudomonas aeruginosa isolates: a molecular study in burn patients. J Med Microbiol 68:1740–1746. https://doi.org/10.1099/jmm.0.001105

Rensing KL, Abdallah HM, Koek A, Elmowalid GA, Vandenbroucke-Grauls CM, Al Naiemi N, van Dijk K (2019) Prevalence of plasmid-mediated AmpC in Enterobacteriaceae isolated from humans and from retail meat in Zagazig, Egypt. Antimicrob Resist Infect Control 8:45. https://doi.org/10.1186/s13756-019-0494-6

Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J (2018) Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121–1137. https://doi.org/10.1093/jac/dkx488

Sanders CC (1987) Chromosomal cephalosporinases responsible for multiple resistance to newer β-lactam antibiotics. Annu Rev Microbiol 41:573–594. https://doi.org/10.1146/annurev.mi.41.100187.003041

Schultsz C, Geerlings S (2012) Plasmid-mediated resistance in Enterobacteriaceae. Drugs 72:1–16. https://doi.org/10.2165/11597960-000000000-00000

Taylor DE, Gibreel A, Lawley TD, Tracz DM (2004) Antibiotic resistance plasmids. In: Plasmid biology. Wiley, Hoboken, pp 473–491. https://doi.org/10.1128/9781555817732.ch23

Tenover FC (1985) Plasmid fingerprinting. A tool for bacterial strain identification and surveillance of nosocomial and community-acquired infections. Clin Lab Med 5:413–436

Tijet N, Faccone D, Rapoport M, Seah C, Pasterán F, Ceriana P, Albornoz E, Corso A, Petroni A, Melano RG (2017) Molecular characteristics of mcr-1-carrying plasmids and new mcr-1 variant

recovered from polyclonal clinical Escherichia coli from Argentina and Canada. PLoS ONE 12: e0180347. https://doi.org/10.1371/journal.pone.0180347

Venditti C, Fortini D, Villa L, Vulcano A, D'Arezzo S, Capone A, Petrosillo N, Nisii C, Carattoli A, Di Caro A (2017) Circulation of blaKPC-3-carrying IncX3 plasmids among Citrobacter freundii isolates in an Italian Hospital. Antimicrob Agents Chemother 61:e00505. https://doi.org/10.1128/AAC.00505-17

Wang J, Stephan R, Power K, Yan Q, Hächler H, Fanning S (2014) Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant Escherichia coli isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans. J Antimicrob Chemother 69:2658–2668. https://doi.org/10.1093/jac/dku206

Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z (2019) NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 32:18. https://doi.org/10.1128/CMR.00115-18

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S (2016) Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Eur Sur Mal 21:27. https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280

Yang Y-Q, Li Y-X, Song T, Yang Y-X, Jiang W, Zhang A-Y, Guo X-Y, Liu B-H, Wang Y-X, Lei C-W, Xiang R, Wang H-N (2017) Colistin resistance gene mcr-1 and its variant in Escherichia coli isolates from chickens in China. Antimicrob Agents Chemother 61:16. https://doi.org/10.1128/AAC.01204-16

Transposons Associated with Antibiotic-Resistant Genes in Gram-Negative Bacteria

10

Hiba Sami, Parvez Anwar Khan, and Anuradha Singh

Abstract

Transposons are a group of mobile genetic elements. Transposons can switch among various places of the genome, hence also called as jumping genes. They are divided into two main groups: retrotransposons (classified) and DNA transposons (classified). While retrotransposons are mainly present in eukaryotes, they can be found in prokaryotes also. Transposons may shift from one plasmid to another or from a chromosome to a plasmid and the other way round, causing genes of antibiotic resistance in bacteria to be transmitted. In Gram-negative bacteria, some examples of composite transposons are Tn5, Tn9, Tn10, Tn903, Tn1525, and Tn2350. Most studied Tn21 bear determinants of OXA (a carbapenem, possessing oxacillinase activity) and PSE (β-lactam gene *Pseudomonas* specific enzyme) PSE (*Pseudomonas* unique enzyme-lactam gene) makes them resistant to aminoglycoside antibiotics.

Keywords

Transposons · Jumping genes · Antibiotic resistance · Gram-negative bacteria

10.1 Introduction

A transposable element, also known as TE or transposon or jumping gene, is a sequence of DNA that can change its location within a genome and thus can cause or reverse a mutation and alter the genetic identity and size of the cell. Antibiotic-resistant bacteria are a significant source of infections associated with health care

H. Sami (✉) · P. A. Khan · A. Singh
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_10

settings. Morbidity, mortality, and health care costs are greatly increased by infections caused by these multi-resistant species. The selection, accumulation, and distribution of resistant genes are mainly because of the behaviour of mobile genes.

10.2 Historical Perspective

Zea mays was the first cereal crop to be found as having transposable elements (TEs). McClintock was working with broken-segmented chromosome in maize plants (McGrayne 2001). She reported that the variable colour patterns on corn kernels were due to the action of some distinct genetic units which can shift from one site to another on different chromosomes of maize. The placement of these genetic units into genes switches the expression of genes on or off, controlling thereby their expression. She identified them as elements of power. This Barbara McClintock discovery earned her a Nobel laureate (McClintock 1950).

Structure of transposon: the basic structure of transposon is shown in Fig. 10.1.

10.3 Classification

TEs are classified into two groups based on their function in recognising and recombining unique sequences:

1. Composite transposons or class I transposons are those holding a variety of resistance genes possessing similar structural and functional characteristics. However, there is just a small amount of DNA homology.
2. Complex transposons or class II transposons possess dissimilar but interconnected families—Tn3, Tn21, and Tn2501.

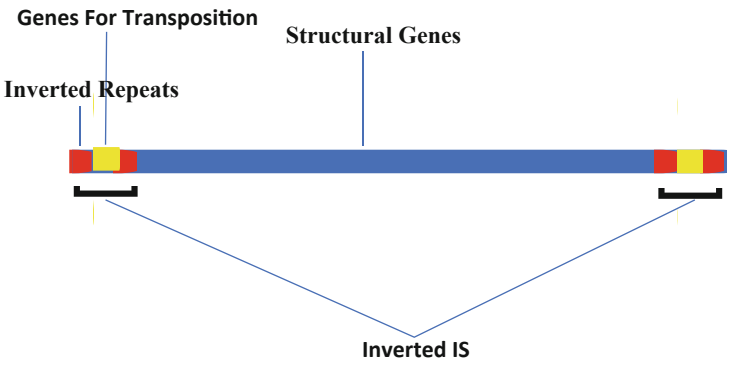


Fig. 10.1 A bacterial DNA transposon

10.4 Autonomous and Non-autonomous

In both class I and class II TEs, transposition may be defined as either "autonomous" or "non-autonomous." Autonomous TEs are capable of travelling on their own, whereas non-autonomous TEs need another TE to be present for movement. This is quite frequent due to lack of reverse transcriptase in class I or transposes in class II TEs. For example, dissociation elements (Ds) are non-autonomous TEs, while activator elements (Ac) are autonomous.

10.5 Mechanism of Action of Transposons

The primary transposition mechanisms have been discovered through the investigation of a limited number of transposons, demonstrating that transposition is mediated by a small number of mechanisms that are applied in various combinations. These transposons are characterised by several inherent properties, including their length and sequence of their ends, their intermediary forms, and the sequences that they target (Curcio and Derbyshire 2003; Siguier et al. 2015).

Their capacity to incorporate into foreign DNA sequences is the fundamental property of transposons. This non-homologous recombination varies from the normal recombination mediated by the host's recA framework catalysed by the transposon-encoded transposase. Conservative transposition and replicative transposition are two strategies of transposon insertion into foreign DNA. In conservative transposition, the transposing factor moves from a donor to a recipient site as a physical object. The donor replicon may be destroyed after the transposition, or the damage caused by the loss of the transposon may be restored, but the exact fate is unknown, in replicative transposition, the transposon is copied while in motion, leaving one copy at the original location and a new copy at a new location.

The events in conservative and replicative transposition can be described using a variety of models (Grindley and Reed 1985). The first step in both modes of transposition is transposon cleavage at both ends and cleavage of the target molecule at 5–9 bp-spaced sites. The transposon is then connected to one of the target's Sticky single stands, forming an intermediate between the donor and recipient replicon. In conservative transposition, the second stage involves filling the phased cuts of the receiver replicon. As a result, the finished product is a replicon, with the transposon inserted between direct repeats of the target sequence. For class I transposons, conservative replication is common. In the second stage, the transposon is replicated in replicative transposition, and an intermediate cointegrate is formed. The cointegrate is resolved in a reaction called resolution, which involves the enzyme resolvase, after the two copies of the transposable element have been recombined at the res site. As a result, the donor replicon and a receptor replicon with the transposed sequence flanked by direct repeats are obtained. For class II transposons, replicative transposition is common. Transposable components may be randomly inserted into different receptor replicon loci; some show a preference for hot spots (for example, A-T rich regions) and just a few inserts in specific areas.

10.6 Transposons and Antibiotic Resistance in Gram-Negative Bacteria

TEs in bacteria typically have an extra gene for roles other than transposition that are usually those involved in antibiotic resistance. Transposons in bacteria can jump back and forth between chromosomal and plasmid DNA, allowing for the permanent addition and transition of genes such as those that code for antibiotic resistance (this method can create multi-antibiotic-resistant bacterial strains). This type of bacterial transposon belongs to the Tn family. Insertion sequences are transposable elements that do not have any extra genes. Different transposons classes and their role in antibiotic resistance is summarised below.

10.7 Retrotransposons (Class I Transposons)

RTNs can be transmitted by reverse transcriptase, a process termed transposition. Retrotransposons are classified as autonomous and non-autonomous. Autonomous RTns have genes (gag and pol), and an open read framework (ORF), which encodes proteins for transposition. Non-autonomous RTns cannot encode these proteins and hence utilise the existing autonomous RTn proteins (Wessler 2006). Long terminal repeat (LTR) and non-LTR retrotransposons are further subtypes of retrotransposons (Han 2010).

10.8 DNA Transposons (Class II Transposons)

Most DNA Tns are transferred through the cut-and-paste mechanism and are usually shorter than RTns (Skipper et al. 2013). DNA sequences comprising inverted repeats (IRs) and a gene for manufacturing Tase enzyme are typically found in the two ends of DNA Tns (Skipper et al. 2013). In the cut-and-paste method, the sequences of IRs are identified by Tase, then Tns are incorporated into the target site (Babakhani and Oloomi 2018). DNA transposons are divided into four different categories in bacteria, namely composite and non-composite transposons, transposable phage Mu, and insertion sequences (IS). Bacterial transposons are further divided into replicative (Tn3 and Mu Phage) and non-replicative transposons (IS10, Tn5, Tn7, and Tn10).

10.9 Insertion Sequence (IS)

Insertion sequences (ISs) are perhaps the minutest and most abundant autonomous transposable elements (TEs) (Siguier et al. 2014). In the insertion site ISs may trigger gene inactivation by direct inclusion and together with a composite Tn can cause antibiotic resistance genes dissemination to different bacteria. For example, IS256, which is part of the Tns of Tn4001, has a role in antibiotic resistance (Babakhani and

Oloomi 2018). *Acinetobacter baumannii* resistance gene expression is ascribed to ISAba1. Among all bacteria, IS26 (which belongs to the IS6 family) is a crucial component in antibiotic resistance (Mugnier et al. 2009). One of the primary functions of the insertion sequence IS26 is to move antibiotic resistance genes creating regions of higher antibiotic resistance that are located next to and edged with copies of IS26 (Harmer et al. 2014). The antibiotic resistance of ISs is mostly linked to their presence in TNs, such as Tn10 and Tn902, which have tetracycline and kanamycin resistance genes, respectively. Tn10 and Tn902 are supported, respectively, by IS10 and IS903 (Mahillon and Chandler 1998). An insertion element ISPa133 has been demonstrated to create *Pseudomonas aeruginosa* PrD protein, which results in carbapenems resistance (Ruiz-Martínez et al. 2011).

10.10 Composite Transposons

A composite transposon is made up of two inverted repetitions from two different transposons that move together as one unit and transport DNA (Clark et al. 2019). These transposons are flanked by insertion sequences. They are carriers of antibiotic resistance genes. Tn5, Tn6, Tn9, Tn10, Tn903, Tn1525, Tn2010, Tn2680, Tn4001, Tn4003, Tn2700, and Tn3411 are all composite types. Tn5 (Kanamycin Resistance), Tn9 (chloramphenicol resistance), Tn10 (tetracycline resistance), and Tn903 being most important in mediating antibiotic resistance especially in *E. coli* (Goryshin et al. 1998).

Tn5
Tn5 was among the first transposons to be discovered to carry antibiotic resistance. IS50R and IS50L are to the right and left of Tn5 and contain neomycin/kanamycin resistance genes, bleomycin (bleR), and streptomycin (Babakhani and Oloomi 2018).

Tn10
Tn10 is edged by IS10R and IS10L, and it also comprises tetA (involved in tetracycline outflow pump), tetR (which controls transcription of tetracycline-responsive genes), tetC (which regulates transcription of tetracycline-responsive genes), and tetD resistance genes (Partridge 2011).

Tn9
Chloramphenicol acetyltransferase (CAT) resistant gene which confers chloramphenicol resistance is carried by Tn9, which is located between IS1, which is the smallest insertion sequence found in bacteria (Alton and Vapnek 1979).

Tn6
The Tn6 molecule carries the kanR gene and has a length of roughly 5 kilobases. It is fringed by IS26 (Mollet et al. 1985).

Tn1
Ampicillin resistance is conferred by the bla_{TEM} genes located on Tn1. Tn1 is located on the plasmids PR1, PR4, R8, and R68. The tnps Tase and Rvase, in addition to bla_{TEM}, are also present (Bailey et al. 2011).

Tn 903
Tn903 transports two IS903. The IS903 is located on the ColE1-like plasmid found in *Salmonella* serotypes. This plasmid has the kanamycin resistant gene (Gray and Fitch 1983).

10.11 Non-composite Transposons

Non-composite Tns are also known as the Tn3 family, as opposed to composite Tns, these lack IS. Even though it lacks the IS parts, it features inverted repeats on both ends. Tn3 is the best example of a non-composite TE.

Unit transposons as originally described are the unit or complex transposons (Tn) of the Tn3 family which besides encoding the functions of transposition are larger than insertion sequences and also bear antibiotic resistance and/or other genes (Partridge 2011). Tn3-like and Tn21-like transposons are two subgroups of the Tn3 transposon family (Grindley 2002). Both forms have a resolution gene, transposase A (tnpA), and a resolution site bound by a 38-bp IR (Partridge 2011). These transposons are transferred through a replicative process involving TnpA's identification of the inverted repeats (IR) and the generation of a cointegrated intermediate consisting of two copies of the transposon separated by the donor and the recipient molecules. The cointegration is resolved by site-specific recombination between the two directly oriented sites, which is accelerated by TnpR, a resolvase gene (Grindley 2002). Differences in both sequence and organisation differentiate the two subgroups. In Tn3-like subgroup members, Res is located between tnpA and tnpR, both of which face in opposite directions (Grindley 2002) as shown if Fig. 10.2. In Tn21-like subgroup transposons, TnpA and tnpR have the same orientation, with res at the beginning of tnpR (Partridge 2011). The resistance gene(s) in Tn3-like and some Tn21-like transposons are found outside of tnpR, but how they were captured is unknown. Transposons Tn21 and similar transposons frequently carry a mercury resistance (mer) operon, these transposons are significant in antibiotic resistance movement (Partridge et al. 2018).

The tetracycline resistance determinant is an excellent example of resistance genes spreading widely via transposons (Kayser and Berger-Bächi 1989). In Gram-negative bacteria, five groups of determinants (tetA-E) which are frequently found on transposons have been identified. The class B determinant can be found in *Enterobacteriaceae* and *Haemophilus* organisms, where it is part of a transposon that is similar to Tnl0, which was first discovered in *Enterobacteriaceae*. Several genetic studies on beta-lactamase-producing *Haemophilus Influenzae* indicate that the antibiotic-resistant transposon Tn3 was introduced from *Enterobacteriaceae* into

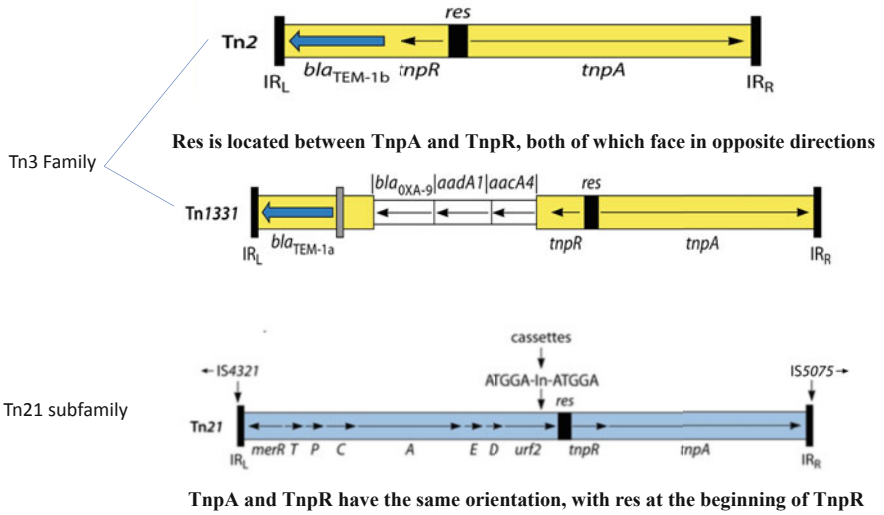


Fig. 10.2 Diagrammatic representation of orientation of Res, TnpA, and TnpR in Tn3 and Tn21 transposons

phenotypically cryptic plasmids found naturally in a few *Haemophilus* strains (Kayser and Berger-Bächi 1989).

Tn3

Tn3 is the ampicillin resistance gene carrier found in both Gram-negative and Gram-positive bacteria. In Gram-negative bacteria, Tn3 acts as a carrier of β-lactamase genes (e.g. bla_{TEM-1}) (Gómez-Lus 1998).

Tn7

Being a non-composite transposon, majority of antibiotic resistance genes are carried by Tn7. Resistance to streptomycin, trimethoprim, as well as spectinomycin is caused by this Tn (Waddell and Craig 1988).

Tn21

Tn21 being significantly investigated carries carbapenemase having oxacillinase activity (OXA) and *Pseudomonas* specific enzyme (PSE) determinants that renders them resistant to aminoglycosides (Sultan et al. 2018).

Tn501 and Tn5053

Found in *P. aeruginosa* and *E. coli*, respectively, they carry Mercury Resistant Genes (Babakhani and Oloomi 2018).

10.12 Transposable Phage Mu

Transposable phage Mu is a member of the *Myoviridae* family and is responsible for the spread of resistant genes among bacteria. Bacteriophage Mu, which infects bacteria, was found in *E. coli* in 1950s. Since the discovery of bacteriophages, the intricacy and their role in gene transmission have been better understood (Harshey 2014). Due to the dual transposition mechanisms used by Mu phage, it is particularly notable as a transposable element that undergoes both lytic and lysogenic (infectious) cycles (Mitkina 2003).

Examples of transposons in gram-negative bacteria[a]

Element	Gene	Resistance transferred
Tn*2003*	bla_{SHV}	β-Lactams
	cfr	Phenicols/lincosamides/oxazolidinones/pleuromutilins/streptogramin A
Tn5	aph(3′)-IIa-ble-aph(6)-Ic	Kanamycin, bleomycin, streptomycin
Tn*903*	*aphA1*	Kanamycin
Tn*1999*	bla_{OXA-48}-like	Carbapenems
Tn*6330*	*mcr-1*	Colistin
	mcr-2	Colistin
Tn*aphA6*	*aphA6*	Kanamycin
Tn*2006*	bla_{OXA-23}	Carbapenems
	$bla_{OXA-237}$	Carbapenems
Tn*125*	bla_{NDM}	Carbapenems

[a]See the Tn registry (http://transposon.lstmed.ac.uk/) for further details

10.13 Conclusions

Bacterial infections are now one of the most common causes of morbidity and death around the world. The rate of resistance growth has increased as a result of excessive and imprudent antibiotic usage, as well as widespread distribution of resistant determinants as part of mobile genetic elements. Over the past years, biological research has discovered key information on how antibiotic resistance genes spread and the process by which they are passed on. Apart from insertion, other forms of DNA rearrangements that can affect the host's resistance phenotype include deletion, inversion, excision, and replicon fusion, which are all promoted by transposable DNA. While antibiotic resistance is provided by transposons due to the presence of an extra gene on a plasmid, transposons may jump from chromosomal DNA to plasmid DNA and vice versa for resistance growth (Wagner 2006). The potential for recombination of genes from different bacterial populations is enormous, and it appears that bacteria do not take long to acquire the genetic

resources they need to thrive in environments that would otherwise stifle their growth. Mutations in the resistance genes located on transposons may result in the evolution of novel markers encoding resistance to new drugs when antibiotics provide the necessary selective pressure, as shown by the production of resistance to third generation cephalosporins (Sultan et al. 2018). In order to combat the threat of antibiotic resistance, it seems that we should have a basic understanding of the mechanisms in order to ensure effective antibiotic use from the environment and to halt the spread of antibiotic-resistant supergerms.

References

Alton NK, Vapnek D (1979) Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. Nature 282:864–869. https://doi.org/10.1038/282864a0

Babakhani S, Oloomi M (2018) Transposons: the agents of antibiotic resistance in bacteria. J Basic Microbiol 58:905–917. https://doi.org/10.1002/jobm.201800204

Bailey JK, Pinyon JL, Anantham S, Hall RM (2011) Distribution of the bla_{TEM} gene and bla_{TEM}-containing transposons in commensal Escherichia coli. J Antimicrob Chemother 66:745–751. https://doi.org/10.1093/jac/dkq529

Clark DP, Pazdernik NJ, McGehee MR (2019) Chapter 25 - mobile DNA. In: Clark DP, Pazdernik NJ, McGehee MR (eds) Molecular biology, 3rd edn. Academic Cell, Amsterdam, pp 793–829. https://doi.org/10.1016/B978-0-12-813288-3.00025-2

Curcio MJ, Derbyshire KM (2003) The outs and ins of transposition: from mu to kangaroo. Nat Rev Mol Cell Biol 4:865–877. https://doi.org/10.1038/nrm1241

Gómez-Lus R (1998) Evolution of bacterial resistance to antibiotics during the last three decades. Int Microbiol 1:279–284

Goryshin IY, Miller JA, Kil YV, Lanzov VA, Reznikoff WS (1998) Tn5/IS50 target recognition. Proc Natl Acad Sci U S A 95:10716–10721. https://doi.org/10.1073/pnas.95.18.10716

Gray GS, Fitch WM (1983) Evolution of antibiotic resistance genes: the DNA sequence of a kanamycin resistance gene from Staphylococcus aureus. Mol Biol Evol 1:57–66. https://doi.org/10.1093/oxfordjournals.molbev.a040298

Grindley NDF (2002) The movement of Tn3-like elements: transposition and cointegrate resolution. In: Mobile DNA II. American Society of Microbiology, Washington, pp 272–302

Grindley NDF, Reed RR (1985) Transpositional recombination in prokaryotes. Annu Rev Biochem 54:863–896. https://doi.org/10.1146/annurev.bi.54.070185.004243

Han JS (2010) Non-long terminal repeat (non-LTR) retrotransposons: mechanisms, recent developments, and unanswered questions. Mob DNA 1:15. https://doi.org/10.1186/1759-8753-1-15

Harmer CJ, Moran RA, Hall RM (2014) Movement of IS26-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS26 and preferentially inserts adjacent to another IS26. MBio 5:e01801. https://doi.org/10.1128/mBio.01801-14

Harshey RM (2014) Transposable phage Mu. Microbiol Spectr 2:7. https://doi.org/10.1128/microbiolspec.MDNA3-0007-2014

Kayser FH, Berger-Bächi B (1989) Transposon transfer of drug resistance. In: Jackson GG, Schlumberger HD, Zeiler HJ (eds) Perspectives in antiinfective therapy. Vieweg+Teubner Verlag, Wiesbaden, pp 109–114. https://doi.org/10.1007/978-3-322-86064-4_15

Mahillon J, Chandler M (1998) Insertion sequences. Microbiol Mol Biol Rev 62:725–774. https://doi.org/10.1128/MMBR.62.3.725-774.1998

McClintock B (1950) The origin and behavior of mutable loci in maize. Proc Natl Acad Sci 36:344–355. https://doi.org/10.1073/pnas.36.6.344

McGrayne SB (2001) Nobel prize women in science: their lives, struggles, and momentous discoveries, 2nd edn. Joseph Henry Press, Washington

Mitkina LN (2003) Transposition as a way of existence: phage Mu. Genetika 39:637–656

Mollet B, Clerget M, Meyer J, Iida S (1985) Organization of the Tn6-related kanamycin resistance transposon Tn2680 carrying two copies of IS26 and an IS903 variant, IS903. Br J Bacteriol 163: 55–60. https://doi.org/10.1128/jb.163.1.55-60.1985

Mugnier PD, Poirel L, Nordmann P (2009) Functional analysis of insertion sequence ISAba1, responsible for genomic plasticity of Acinetobacter baumannii. J Bacteriol 191:2414–2418. https://doi.org/10.1128/JB.01258-08

Partridge SR (2011) Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev 35:820–855. https://doi.org/10.1111/j.1574-6976.2011.00277.x

Partridge SR, Kwong SM, Firth N, Jensen SO (2018) Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31:17. https://doi.org/10.1128/CMR.00088-17

Ruiz-Martínez L, López-Jiménez L, d'Ostuni V, Fuste E, Vinuesa T, Viñas M (2011) A mechanism of carbapenem resistance due to a new insertion element (ISPa133) in Pseudomonas aeruginosa. Int Microbiol 14(1):51–58

Siguier P, Gourbeyre E, Chandler M (2014) Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol Rev 38:865–891. https://doi.org/10.1111/1574-6976.12067

Siguier P, Gourbeyre E, Varani A, Ton-Hoang B, Chandler M (2015) Everyman's guide to bacterial insertion sequences. Microbiol Spectr 3:2014. https://doi.org/10.1128/microbiolspec.MDNA3-0030-2014

Skipper KA, Andersen PR, Sharma N, Mikkelsen JG (2013) DNA transposon-based gene vehicles - scenes from an evolutionary drive. J Biomed Sci 20:92. https://doi.org/10.1186/1423-0127-20-92

Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR (2018) Antibiotics, resistome and resistance mechanisms: a bacterial perspective. Front Microbiol 9:02066. https://doi.org/10.3389/fmicb.2018.02066

Waddell CS, Craig NL (1988) Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Dev 2:137–149. https://doi.org/10.1101/gad.2.2.137

Wagner A (2006) Periodic extinctions of transposable elements in bacterial lineages: evidence from intragenomic variation in multiple genomes. Mol Biol Evol 23:723–733. https://doi.org/10.1093/molbev/msj085

Wessler SR (2006) Transposable elements and the evolution of eukaryotic genomes. Proc Natl Acad Sci U S A 103:17600–17601. https://doi.org/10.1073/pnas.0607612103

Integrons and Insertion Sequences Associated with Beta-Lactamases

11

Sana Jamali

Abstract

As there is a rising concern about multi-drug resistant bacteria striking a threat to life, integrons and insertion sequences (Is) have emerged as important causative factors for wide dissemination of resistance genes among gram-negative pathogens in association with beta-lactamases. These mobile genetic elements are capable of capturing drug-resistance genes and hence play an important role in life-threatening bacterial infections caused by antibiotic resistant bacteria. To effectively combat this major global health crisis, joint effort from across the world is highly needed to put more emphasis on genetic based research work in this field on priority basis, particularly at the level of diagnostics, so that more effective measures can be taken to monitor and control spread of this global problem of antibiotic resistance.

Keywords

Integrons · Insertion sequences · Beta-lactamases · Antibiotic resistance · Gram-negative

11.1 Introduction

Resistance to antibiotics, particularly multi-drug resistance is increasingly posing a great threat to the world. The present scenario on the ineffectiveness of the available pool of antibiotics to pathogenic gram-negative bacteria is quite alarming across different parts of the world. It is high time to introspect the various mechanisms

S. Jamali (✉)
Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_11

utilized by bacteria to make themselves antibiotic resistant, so as to avert the more dire consequences of this major public health problem in the near future.

Variety of gram-negative bacterial species has evolved dramatically to effectively combat antimicrobial chemotherapy by producing beta-lactamases. Diverse mechanisms to capture the genes expressing these enzymes have a significant role in horizontal spread of antibiotic resistance among gram-negative pathogenic micro-organisms. Mobile genetic elements such as integrons and insertion sequences (ISs) have been reported to facilitate the expeditious dissemination of beta-lactamases and thus antimicrobial resistance among bacteria.

11.2 Integrons

Integrons are DNA elements possessing the unique property of capturing gene cassettes and thus capable of acquiring antibiotic resistance genes and disseminating them with the help of mobile genetic elements (Stokes and Hall 1989). They are characterized by a variable region containing the gene cassettes and two conserved regions (5′-CS and 3′-CS) on either side of it. There are three essential components of integron at 5′-CS end, which are (1) the *int* gene which codes for an integrase enzyme, belonging to tyrosine-recombinase family; (2) *attI* site for primary recombination (*attI*); and (3) a promoter (*Pc*), which helps in the expression of the cassette genes. Conversely, 3′-CS conserved part is constituted by (1) a truncated gene *qac*ED1, which encodes resistance to quaternary ammonium compounds, (2) *sul1* gene, expressing sulfonamide resistance, and (3) an *orf*5 site, whose function is still unknown (Galani et al. 2006). Site-specific recombination mechanisms used by integrons play a crucial role in mobilizing resistance genes between defined sites. Multi-resistance in bacteria could be due to the insertion of multiple cassettes into the same integron (Partridge et al. 2009).

11.3 Classification of Integrons

Sequence analysis of *IntI*, led to the classification of integrons into different classes, termed as *IntI1*, *IntI2*, *IntI3*, etc., with cognate *attI1*, *attI2*, and *attI3* sites (Escudero et al. 2015; Hall 2012; Partridge et al. 2009).

Most of the integron classes, which are at least 90 in numbers, have chromosomal existence and approximately 10% of bacterial genomes carrying these elements have been sequenced (Barlow et al. 2004; Mazel 2006).

Class 1 (*intI1*) and class 2 (*intI2*) integrons have been reported to be associated with majority of the cases of antibiotic resistances across the world (Fluit and Schmitz 2004; Kaushik et al. 2018; Machado et al. 2005; Odetoyin et al. 2017; Saenz et al. 2004).

11.4 Class 1 Integrons

The promoter component, i.e., *Pc* in class 1 integrons is incorporated in the *int1* gene, and *Pc* strength and *IntI1* activity become inversely proportional to each other due to any minor variations in sequence (Jove et al. 2010). Some portion of the *tni* region has been substituted by the (3′-CS) in the more commonly found "clinical" or "*sul1*-type" class 1 integrons.

The term "class 1 In/Tn" has been coined to include structures having *intI1/attI1/* Pc and either a full or truncated *tni* region (Partridge 2011). 5′-conserved segment (5′-CS) of class 1 In/Tn includes the region extending from IRi to the end of the *attI1*. The 25-bp IR termed as IRi is found at the integrase end whereas IRt has been described at the *tni* end. Though, in few classes 1 In/Tn, *tni* transposition functions are absent, supporting evidences are there that they can be shifted, presumably with the help of compatible Tni proteins present in the same cell (Petrovski and Stanisich 2010).

As class 1 integrons have evolved themselves to acquire and express numerous kinds of resistance genes, they are causing major crisis in the treatment of life-threatening infections by drug-resistant bacteria. Apart from this, their location on mobile genetic elements as plasmids and transposons facilitates the rapid dissemination of transferable antibiotic resistance (Davies 2007; Rowe-Magnus et al. 2002). They are found extensively in gram-negative isolates. A high percentage of gram-negative pathogens (40–70%) isolated from clinical specimens have been reported to harbor class 1 integrons (Essen-Zandbergen et al. 2007; Martinez-Freijo et al. 1998).

11.5 Class 2 and Other Integron Classes

In contrast to class 1 integrons, there is paucity of information related to distribution of class 2 Integrons. Conversely, Tn7 transposons have been studied extensively, which are closely linked with class 2 integrons (Peters and Craig 2001; Waddell and Craig 1998; Wolkow et al. 1996). This class of integrons possesses a limited variety of gene cassettes because of an internally situated stop codon, which makes IntI2 gene nonfunctional (Ramírez et al. 2010).

Class 3 integrons mostly carrying cassettes encoding beta-lactamases have got connection with Tn*402*-like transposons and are more or less similar to class 1 integrons (Collis et al. 2002).

Class 4 integron was formerly referred to the one discovered in *V. Cholerae*. Though they contain vast array of cassettes, resistance gene containing cassettes constitute a minor part of "sedentary chromosomal integrons" (SCI; formerly called CI) (Escudero et al. 2015). Occurrence of class 4 and 5, which are "Mobile" Integron types, is rare (Escudero et al. 2015).

11.6 Integron Association with Beta-lactam Resistance

Recently Bohm et al. from Sweden reported about a novel class 1 integron regulated class C beta-lactamase. During their research work, they recognized two integron-derived cephalosporinase bla_{ampC} genes, $bla_{IDC-1,}$ and $bla_{IDC-2,}$ having amino acid sequence similarity under 85%, when compared with already annotated AmpC sequence beforehand (Böhm et al. 2020a). In addition to this, they also identified an integron-derived gene cassette which codes for a protein with a garosamine moiety in high-level aminoglycoside resistant *E. coli* strains. The gene is known as garosamine-specific aminoglycoside resistance (gar) against its specificity (Böhm et al. 2020b).

Previous studies have also well documented the more common association of class 1 integron with $bla_{CTX-M-15}$ gene expressing cefotaximases, as compared to class 2 integron (Zhao and Hu 2013; Kaushik et al. 2018).

Similarly, occurrence of class 1 (intI1) and class 2 (intI2) integrons was found to be 91.7% and 5.5%, respectively, among ESBL producing *E. coli* isolates (Pérez-Etayo et al. 2018).

Furthermore, a study carried out on drug-resistant clinical strains of *K. pneumoniae* in Tehran, Iran demonstrated a high level of class I integrons (8%) and bla_{TEM} (38%), bla_{VIM} (33%), beta-lactamases, suggesting that genes constituting class I integrons might have a significant contribution in the expression of β-lactamase-encoding genes among these clinical isolates resistant to β-lactam group of antibiotics. However, these class 1 integron rich isolates were found to be devoid of class II and III integrons (Sedighi et al. 2017).

A similar study carried out in Iran demonstrated a higher prevalence of class 1 integron among ESBL positive isolates of *E. coli* suggesting the importance of integron-mediated resistance profile in these gram-negative strains (Mehdipour Moghaddam et al. 2015).

Though, there are few reports about the distribution of integrons belonging to class 2, study by Ramirez et al. from Argentina and Uruguay showed its high prevalence (36.61%) among multi-drug resistant isolates of *A. baumannii* (Ramírez et al. 2012).

Few years back, a gene cassette in class 2 integron expressing β-lactamases was reported. Carbenicillin-resistant β-lactamase gene bla_{CARB-4} was demonstrated, and variable region constituted novel class 2 integron arrays among A. *baumannii* isolates. Though class 1 integrons are widely disturbed among gram-negative pathogens, this study reported class 2 integrons in abundance among clinical isolates of *Acinetobacter baumannii* and *Enterobacter cloacae* (Ramírez et al. 2010).

Mavroidi et al. from Greece characterized an extended-spectrum β-lactamase, IBC-2, among isolated pathogenic strains of *Pseudomonas aeruginosa*. It was found that class 1 integron harbors bla_{IBC-2} as a sole gene cassette, within its variable region, most probably located in the chromosome (Mavroidi et al. 2001).

There are reports about the occurrence of class 3 integrons in few pathogens such as *Acinetobacter spp.*, *Alcaligenes*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Salmonella spp*, *and*

Serratia marcescens. They mostly confer resistance by expressing IMP-1 metallo-beta-lactamases. Furthermore, they have also been detected in *E. coli* in association with bla_{GES-1} (Arakawa et al. 1995; Ploy et al. 2003; Rowe-Magnus et al. 1999, 2001). Even, isolates highly resistant to ceftazidime and sulbactam-cefoperazone have been reported to harbor class 3 integrons (Rowe-Magnus et al. 2001).

Like class 3 integrons, class 4 have been limited to a few micro-organisms, namely *Vibrionaceae*, *Shewanella*, *Xanthomonas*, *Pseudomonad*, and *other proteobacteria* (Rowe-Magnus and Mazel 2001; Poirel et al. 2010; Clark et al. 2000). Gene cassettes in connection with class 4 integrons have been reported to impart resistance against chloramphenicol and fosfomycin (Fluit and Schmitz 2004).

There are reports of MBL association with integrons too. Coexistence of MBL-encoding genes among all the 10.3% carbapenem resistant strains of *P. aeruginosa* mediated by class I integron was reported in an Indian study (Mohanam and Menon 2017).

Efforts of Sobia et al. from Aligarh, India also paved the way to the demonstration of integron carrying plasmids in association with β-lactamase genes. Thus, isolates harboring integron were found to be highly resistant to β-lactam antibiotics of various classes (Sobia et al. 2016).

In another similar study on VEB-1 ESBL producing clinical isolates of *Pseudomonas aeruginosa* ($n = 14$) from India, which were only polymyxin B sensitive, class 1 integron harboring VEB-1 within variable regions was detected (Maurya et al. 2014).

11.7 Insertion Sequences (Is)

Insertion sequences (IS) are undoubtedly the smallest transposable or mobile genetic elements (MGEs) having size under 2.5 kb and their main classification system into different families is based on enzymes involved in catalyzing their movement, i.e., transposases (Mahillon and Chandler 1998; Zhao and Hu 2013). Usually, they are non-expressive except those related to their mobility. Two main factors in *cis* facilitating their mobility are recombinant-active DNA sequences describing the terminals of the elements, and Tpase enzyme, which has got the role of recognition followed by processing of these ends. Many IS elements have also got the unique property to turn dormant adjoining genes into active expressive forms (Mahillon and Chandler 1998).

They were first reported in the year 1989 (Berg and Howe 1989) and since then several continuous research works in this area led to the discovery of over 500 distinct IS (Siguier et al. 2006).

Studies related to bacterial drug resistance and transmissible plasmids project a very prime role of these mobile genetic elements in acquisition and transmission of genes encoding antibiotic resistance. Strikingly, it was noticed that within plasmid genomes, diverse form of elements often congregates together to form "islands," which aids in the integration and excision of plasmid (Bukhari et al. 1997).

Genetically, the most common combination involved in antimicrobial resistance to beta-lactam group of antibiotics is IS26, ISEcp1, ISCR1, and IS903, with class 1 integrons (Arduino et al. 2002; Cheng et al. 2016; Cullik et al. 2010; Diestra et al. 2008; Eckert et al. 2006).

11.8 Insertion Sequence Families

Though there are a number of software tools for searching sequence databases, IS finder defines IS families using an initial manual BLAST (Basic Local Alignment Search Tool) survey, thereafter reiterative BLAST analyses with the initial transposase enzyme sequence of representative elements used as a query during a BLASTP (Altschul et al. 1990) exploration of genomes constituting micro-organisms.

In spite of limitations, Mahllon and Chandler characterized a collection of 443 members, into 17 families (IS1, IS3, IS5, IS6, etc.) based on the four criteria: first, on the basis of resemblance in the arrangement of ORFs (open reading frames), i.e., genetic organization; second, significant similarities in their common domains or motifs, i.e., Tpases; third criteria was based on similarities of their terminal IRs and the last criteria regarding nucleotide sequence fate of their target sites, i.e., production of a target duplication of determined length directly, was taken into account. Out of 500 ISs in the database, 54 are unclassified. Out of these, 33 remained unclassified due to complete absence of knowledge about nucleotide sequence of these elements or having restricted information about the sequence, making them unfit to allot any distinct family or elements having complete information about their nucleotide sequence but exhibiting no significant association with more than one other element (out of 21 ISs, 5 are isoforms) (Mahillon and Chandler 1998).

Of all the ISs, IS*1* was the first insertion sequences which was isolated and characterized in bacteria (Fiandt et al. 1972; Hirsch et al. 1972).

11.9 Insertion Sequence Association with Beta-lactam Resistance

Remarkably, in a study carried out on *E. coli* isolates producing extended-spectrum beta-lactamases, prevalence of insertion sequences IS26, ISEcp1, IS903, and ISCR1 was analyzed to be 100%, 72.3%, 91.6%, and 25%, respectively (Pérez-Etayo et al. 2018).

Montana et al. during their study on extensively drug-resistant (XDR) *Acinetobacter* spp. isolates recovered from various clinical specimens observed a high prevalence of IS*26* (93%) and IS*CR2* (66%) among these nosocomial pathogens suggesting a possible association of IS*26* and IS*CR2* with genes conferring drug resistance in *Acinetobacter* spp. (Montaña et al. 2017).

Research work by Poirel et al. exhibited that the IS*Ecp1B* factor played an important role in the dispersal of cefotaximases-type β-lactamase genes. This study also pointed out that IS*Ecp1B* acted as one of the strong positive factors in controlling the $bla_{\text{CTX-M-19}}$ gene encoding (Poirel et al. 2010).

In another similar study on multi-drug resistant clinical isolates of *E. coli*, linking drug-resistance genes with insertion sequence was carried out in Kenya, and surprisingly occurrence of Tn21, *ISEcp1*, *ISCR1*, and *IS26* was found in 22%, 10%, 15%, and 7% of the isolates, respectively (Kiiru et al. 2013).

Insertion element, *ISAba1* plays a key factor in the transmission of genes expressing metallo-beta-lactamases such as carbapenemases. This insertion element, ISAba1 has been described among *A. baumannii* isolates in association with carbapenem resistance genes $bla_{\text{OXA-51}}$ like, $bla_{\text{OXA-23}}$ like, and $bla_{\text{OXA-58}}$ and thus exposing its major contribution in the transfer as well as expression of genes responsible for carbapenem resistance (Pagano et al. 2016; Turton et al. 2006).

Vijayakumar et al. noted high frequency of $bla_{\text{OXA-23}}$ (29%) in imipenem and meropenem drug-resistant *Acinetobacter baumannii* clinical isolates and all these extensively drug-resistant *A. baumannii* isolates were positive for ISAba1 as well for ISAba1 insertion elements along with $bla_{\text{OXA-23}}$ like, and $bla_{\text{OXA-5}}$ 1 like gene. Thus, this study also demonstrates the association of ISs with beta-lactamase genes (Vijayakumar et al. 2020).

In south India, another similar study was conducted on resistant gram-negative bacterial isolates, and it was found that 39 $bla_{\text{NDM-1}}$ gene positive isolates harbored at least one of the ISs (*ISAba125*, *ISEc33*, *ISSen4*), with *ISAba125* (53%) being the most commonly encountered insertion sequence (Jose et al. 2017).

Notably, Shahid et al. in their study on *Escherichia coli* and *Klebsiella pneumoniae* isolates from an Indian tertiary care center uniquely demonstrated the association of $bla\text{a}_{\text{mpC}}$ families and $bla_{\text{CTX-M}}$ genogroups with Mobile Genetic Elements IS*Ecp1*, IS*26*, IS*CR1*, and *sul1*-type class 1 integrons (Shahid et al. 2012).

Similar research work was carried out on Citrobacter spp. isolates and interestingly, coexistence of bla_{TEM}, bla_{SHV}, bla_{ampC}, and $bla_{\text{CTX-M}}$ on class 1 Integrons was described. *sul1*-type integrons were commonly detected in isolates positive for both $bla_{\text{CTX-M}}$ and bla_{ampC} genes (69.2%; 9/13), followed by isolates housing only $bla_{\text{CTX-M}}$ and bla_{ampC} is absent (46.2%; 6/13). All the isolates harboring bla_{ampC} solely were devoid of *sul1* integrons. Same study also reported the possible modification in the genetic constitution of $bla_{\text{CTX-M-15}}$ via IS*26* and *orf513* insertion (Shahid 2010).

11.10 Conclusions

Acquisition of drug-resistant genes with the help of mobile genetic elements such as integrons and insertion sequences is the most evolutionary measure developed by bacteria to survive under various environmental challenges including exposure to current antimicrobials. It is a matter of great concern that there is a strong relationship between these mobile genetic elements in gram-negative bacteria and

beta-lactam resistance and thus facilitating the dissemination of antibiotic resistance. To combat life-threatening sequelae arising due to multi-drug resistant bacterial infections, there is need that whole world comes together to put more emphasis on genetic based research work in this field on priority basis, particularly at the level of diagnostics, so that more effective measures can be taken to monitor and control spread of this global problem of antibiotic resistance.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Arakawa Y, Murakami M, Suzuki K, Ito H, Wacharotayankun R, Ohsuka S, Kato N, Ohta M (1995) A novel integron-like element carrying the metallo-β-lactamase gene blaIMP. Antimicrob Agents Chemother 39:1612–1615

Arduino SM, Roy PH, Jacoby GA, Betina E, Pineiro SA, Centron D, Arduino SM, Roy PH, Jacoby GA, Orman BE et al (2002) blaCTX-M-2 is located in an unusual class 1 integron (In35) which includes Orf513 blaCTX-M-2 is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrob Agents Chemother 46:2303–2306

Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS (2004) Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrob Agents Chemother 48: 838–842

Berg DE, Howe MM (eds) (1989) Mobile DNA. American Society for Microbiology, Washington, DC

Böhm ME, Razavi M, Flach CF, Larsson DG (2020a) A novel, integron-regulated, class C β-lactamase. Antibiotics 9:123

Böhm ME, Razavi M, Marathe NP, Flach CF, Larsson DG (2020b) Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities. Microbiome 8:41

Bukhari AI, Shapiro JA, Adhya SL (1997) DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor

Cheng C, Sun J, Zheng F, Lu W, Yang Q, Rui Y (2016) New structures simultaneously harboring class 1 integron and ISCR1-linked resistance genes in multidrug-resistant Gram-negative bacteria. BMC Microbiol 16:71

Clark CA, Purins L, Kaewrakon P, Focareta T, Manning PA (2000) The vibrio cholerae O1 chromosomal integron. Microbiology 146:2605–2612

Collis CM, Kim MJ, Partridge SR, Stokes HW, Hall RM (2002) Characterization of the class 3 integron and the site-specific recombination system it determines. J Bacteriol 184:3017–3026

Cullik A, Pfeifer Y, Prager R, Von Baum H, Witte W (2010) A novel IS26 structure surrounds blaCTX-M genes in different plasmids from German clinical Escherichia coli isolates. J Med Microbiol 59:580–587

Davies J (2007) Microbes have the last word. EMBO Rep 8:616–621

Diestra K, Juan C, Curiao T, Moya B, Miro E, Oteo J, Coque TM, Perez-Vazquez M, Campos J, Canton R et al (2008) Characterization of plasmids encoding blaESBL and surrounding genes in Spanish clinical isolates of Escherichia coli and Klebsiella pneumoniae. J Antimicrob Chemother 63:60–66

Eckert C, Gautier V, Arlet G (2006) DNA sequence analysis of the genetic environment of various blaCTX-M genes. J Antimicrob Chemother 57:14–23

Escudero JA, Loot C, Nivina A, Mazel D (2015) The integron: adaptation on demand. Microbiol Spectr 3:19

Essen-Zandbergen A, Smith H, Veldman K, Mevius D (2007) Occurrence and characteristics of class 1, 2 and 3 integrons in Escherichia coli, Salmonella and Campylobacter spp. in the Netherlands. J Antimicrob Chemother 59:746–750

Fiandt MW, Szybalski W, Malamy MH (1972) Polar mutations in lac, gal and phage lambda consist of a few IS-DNA sequences inserted with either orientation. Mol Gen Genet 119:223–231

Fluit AC, Schmitz FJ (2004) Resistance integrons and super-integrons. Clin Microbiol Infect 10: 272–288

Galani I, Souli M, Koratzanis E, Chryssouli Z, Giamarellou H (2006) Molecular characterization of an Escherichia coli clinical isolate that produces both metallo-β-lactamase VIM-2 and extended spectrum-lactamase GES-7: identification of the In8 integron carrying the blaVIM-2 gene. J Antimicrob Chemother 58:432–433

Hall RM (2012) Integrons and gene cassettes: hotspots of diversity in bacterial genomes. Ann N Y Acad Sci 1267:71–78

Hirsch HJ, Starlinger P, Brachet P (1972) Two kinds of insertions in bacterial genes. Mol Gen Genet 119:191–206

Jose J, Santhiya K, Jayanthi S, Ananthasubramanian M (2017) Insertion sequence based analysis of clinical isolates with NDM (blaNDM-1) resistance. Indian J Biotechnol 16:182–188

Jove T, Da Re S, Denis F, Mazel D, Ploy MC (2010) Inverse correlation between promoter strength and excision activity in class 1 integrons. PLoS Genet 6:e1000793

Kaushik M, Kumar S, Kapoor RK, Virdi JS, Gulati P (2018) Integrons in Enterobacteriaceae: diversity, distribution and epidemiology. Int J Antimicrob Agents 51:167–176

Kiiru J, Butaye P, Goddeeris BM, Kariuki S (2013) Analysis for prevalence and physical linkages amongst integrons, ISEcp1, ISCR1, Tn21 and Tn7 encountered in Escherichia coli strains from hospitalized and non-hospitalized patients in Kenya during a 19-year period (1992–2011). BMC Microbiol 13:109

Machado E, Canto R, Baquero F, Gala J, Coque TM (2005) Integron content of extended-spectrum β-lactamase-producing Escherichia coli strains over 12 years in a single hospital in Pseudomonas aeruginosa. Antimicrob Agents Chemother 49:1823–1829

Mahillon J, Chandler M (1998) Insertion sequences. Microbiol Mol Biol Rev 62:725–774

Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VSC, Verhoef J, Jones ME (1998) Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. J Antimicrob Chemother 42:689–696

Maurya AP, Talukdar AD, Chanda DD, Chakravarty A, Bhattacharjee A (2014) Integron-borne transmission of VEB-1 extended-spectrum β-lactamase in Pseudomonas aeruginosa in a tertiary care hospital in India. Antimicrob Agents Chemother 58:6966–6969

Mavroidi A, Tzelepi E, Tsakris A, Miriagou V, Sofianou D, Tzouvelekis LS (2001) An integron-associated β-lactamase (IBC-2) from Pseudomonas aeruginosa is a variant of the extended-spectrum β-lactamase IBC-1. J Antimicrob Chemother 48:627–630

Mazel D (2006) Integrons: agents of bacterial evolution. Nat Rev Microbiol 4:608–620

Mehdipour Moghaddam MJ, Mirbagheri AA, Salehi Z, Habibzade SM (2015) Prevalence of class 1 integrons and extended spectrum beta lactamases among multi-drug resistant Escherichia coli isolates from north of Iran. Iran Biomed J 19:233–239

Mohanam L, Menon T (2017) Coexistence of metallo-beta-lactamase-encoding genes in Pseudomonas aeruginosa. Indian J Med Res 146:46–52

Montaña S, Almuzara M, Pennini M et al (2017) ISCR2 and IS26: two insertion sequences highly dispersed among Acinetobacter spp. clinical strains. J Bacteriol Mycol Open Access 4:33–36

Odetoyin BW, Labar AS, Lamikanra A, Aboderin AO, Okeke IN (2017) Classes 1 and 2 integrons in faecal Escherichia coli strains isolated from mother-child pairs in Nigeria. PLoS ONE 12: e0183383

Pagano M, Martins AF, Barth AL (2016) Mobile genetic elements related to carbapenem resistance in Acinetobacter baumannii. Braz J Microbiol 47:785–792

Partridge SR (2011) Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev 35:820–855

Partridge SR, Tsafnat G, Coiera E, Iredell JR (2009) Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev 33:757–784

Pérez-Etayo L, Berzosa M, González D, Vitas AI (2018) Prevalence of integrons and insertion sequences in ESBL-producing E. coli isolated from different sources in Navarra, Spain. Int J Environ Res Public Health 15:2308

Peters JE, Craig NL (2001) Tn7: smarter than we thought. Nat Rev Mol Cell Biol 2:806–814

Petrovski S, Stanisich VA (2010) Tn502 and Tn512 are res site hunters that provide evidence of resolvase-independent transposition to random sites. J Bacteriol 192:1865–1874

Ploy MC, Chainier D, Tran Thi NH, Poilane I, Cruaud P, Denis F, Collignon A, Lambert T (2003) Integron-associated antibiotic resistance in Salmonella enterica serovar typhi from Asia. Antimicrob Agents Chemother 47:1427–1429

Poirel L, Carattoli A, Bernabeu S, Bruderer T, Frei R, Nordmann P (2010) A novel IncQ plasmid type harbouring a class 3 integron from Escherichia coli. J Antimicrob Chemother 65:1594–1598

Ramírez MS, Piñeiro S, Centrón D (2010) Novel insights about class 2 integrons from experimental and genomic epidemiology. Antimicrob Agents Chemother 54:699–706

Ramírez MS, Morales A, Vilacoba E, Márquez C, Centrón D (2012) Class 2 integrons dissemination among multidrug resistance (MDR) clones of Acinetobacter baumannii. Curr Microbiol 64:290–293

Rowe-Magnus DA, Mazel D (2001) Integrons: natural tools for bacterial genome evolution. Curr Opin Microbiol 4:565–569

Rowe-Magnus DA, Guerout AM, Mazel D (1999) Super-integrons. Res Microbiol 150:641–651

Rowe-Magnus DA, Guerout AM, Ploncard P, Dychinco B, Davies J, Mazel D (2001) The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc Natl Acad Sci U S A 98:652–657

Rowe-Magnus D, Guerot A-M, Mazel D (2002) Bacterial resistance evolution by recruitment of super-integron gene cassettes. Mol Microbiol 43:1657–1669

Saenz Y, Brinas L, Dominguez E, Ruiz J, Zarazaga M, Vila J, Torres C (2004) Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origins. Antimicrob Agents Chemother 48:3996–4001

Sedighi M, Halajzadeh M, Ramazanzadeh R, Amirmozafari N, Heidary M, Pirouzi S (2017) Molecular detection of β-lactamase and integron genes in clinical strains of Klebsiella pneumoniae by multiplex polymerase chain reaction. Rev Soc Bras Med Trop 50:321–328

Shahid M (2010) Citrobacter spp. simultaneously harboring bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{ampC}, and insertion sequences IS26 and orf513: an evolutionary phenomenon of recent concern for antibiotic resistance. J Clin Microbiol 48:1833–1838

Shahid M, Sobia F, Singh A, Khan HM (2012) Concurrent occurrence of bla_{ampC} families and bla_{CTX-M} genogroups and association with mobile genetic elements ISEcp1, IS26, ISCR1, and sul1-type class 1 integrons in Escherichia coli and Klebsiella pneumoniae isolates originating from India. J Clin Microbiol 50:1779–1782

Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M (2006) ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:32–36

Sobia F, Shahid M, Jamali S, Khan HM, Niwazi S (2016) Molecular profiling and characterization of integrons and genotyping of Escherichia coli and Klebsiella pneumoniae isolates obtained from north Indian Tertiary Care Hospital. Trop Med J 1:1003

Stokes HW, Hall RM (1989) A novel family of potentially mobile DNA elements encoding site specific gene-integration functions: integrons. Mol Microbiol 3:1669–1683

Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM et al (2006) The role of IS Aba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett 258:72–77

Vijayakumar S, Anandan S, Prabaa D, Kanthan K, Vijayabaskar S, Kapil A et al (2020) Insertion sequences and sequence types profile of clinical isolates of carbapenem-resistant A. baumannii collected across India over four year period. J Infect Public Health 13:1022–1028

Waddell CS, Craig NL (1998) Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Dev 2:137–149

Wolkow CA, DeBoy RT, Craig NL (1996) Conjugating plasmids are preferred targets for Tn7. Genes Dev 10:2145–2157

Zhao WH, Hu ZQ (2013) Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. Crit Rev Microbiol 39:79–101

Part III

Methods for Detection of Beta-Lactam-Resistance in Gram-Negative Bacteria

Phenotypic Methods of Detection of Beta-Lactamases

12

Nusrat Perween

Abstract

Extended-spectrum beta-lactamases have the capability to hydrolyze oxyimino-cephalosporins such as Ceftazidime, Cefotaxime, and Monobactams; however, they may not hydrolyze cephamycins or the carbapenems. There are three major types of beta-lactamases commonly encountered in our laboratories while processing Gram-negative bacteria and these are extended-spectrum beta-lactamases, AmpC beta-lactamases, and Carbapenemases. The previous chapters have described about beta-lactamases and their different classes. Disk diffusion and MIC methods are commonly used methods for the detection of beta-lactamases. This chapter will discuss various phenotypic detection methods to identify producers of different types of beta-lactamases.

Keywords

Phenotypic detection · ESBL · AmpC · Beta-lactamases · MIC method · Carbapenemase detection

12.1 Introduction

Disk diffusion and MIC methods are the methods which are commonly used methods for the detection of beta-lactamases. Here, this chapter will also discuss various other phenotypic detection methods to identify producers of different types of beta-lactamases including some CLSI recommended methods as well as research based methods.

N. Perween (✉)
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_12

12.2 Detection of ESBLs

12.2.1 Screening Methodologies

12.2.1.1 By Disk Diffusion Method

The production of ESBL can be screened via disk diffusion methods by screening specific zone diameters of third generation Cephalosporins like Ceftazidime/Cefpodoxime/Cefotaxime and Aztreonam; using more than one antibiotics will improve the sensitivity of ESBL detection. Once screening raises suspicion for ESBL production based on zone diameters, phenotypic confirmation is performed. Therefore, the CLSI 2020 recommends isolates with a Ceftazidime zone diameter of $\leq$22 mm or Cefpodoxime $\leq$17 mm or Aztreonam and Cefotaxime $\leq$27 mm or Ceftriaxone $\leq$25 mm should undergo phenotypic confirmation (Clinical and Laboratory Standards Institute 2020).

12.2.1.2 By Dilution Method

CLSI has also recommended dilution method for screening of ESBL production. Cefotaxime, Ceftriaxone, Ceftazidime, Aztreonam may be used in a concentration of 1 μg/ml. Appearance of bacterial growth at this antibiotic concentration, i.e., MIC of the Cephalosporins $\geq$2 μg/ml is considered as potential ESBL producers (Clinical and Laboratory Standards Institute 2020).

12.2.2 Phenotypic Confirmation of the ESBLs

12.2.2.1 By Cephalosporin–Clavulanate Combined Disk Method

Thirty microgram of Ceftazidime or Cefotaxime disks are used with and without 10 μg of Clavulanate for phenotypic confirmation of the presence of ESBLs. As per the CLSI recommendation, this method is performed on a confluent bacterial growth cultured on Mueller-Hinton agar (MHA) and an increase of $\geq$5 mm in the zone diameter of Cephalosporin–Clavulanate combined disks, as opposed to Cephalosporin disks alone, is considered as potential producers of ESBLs (Clinical and Laboratory Standards Institute 2020).

12.2.2.2 By Broth Microdilution Method

Phenotypic confirmation of ESBL production may be done via broth microdilution procedure by using Cefotaxime (0.25–64 μg/ml) and Cefotaxime plus clavulanate (0.25/4 to 64/4 μg/ml), Ceftazidime (0.25–128 μg/ml), Ceftazidime plus clavulanate (0.25/4 to 128/4 μg/ml). Using both the Cephalosporins is suggested for better results. Broth microdilution method should be performed using the standard protocol. A decrease of $\geq$3 twofold serial dilution in MIC of either Cephalosporins in the presence of clavulanic acid when compared to their MICs (when tested alone) is considered as phenotypically confirmed ESBL producers (Clinical and Laboratory Standards Institute 2020).

12.2.3 Other Methods

Furthermore, some other methods to confirm ESBL producers are as follows:

12.2.3.1 By Double-Disk Synergy Method

On the inoculated MHA plates, third generation Cephalosporins and Augmentin disks (having Clavulanate as an ESBL inhibitor) are placed about 30 mm apart from their centers (Jarlier et al. 1988). Enhancement of the edges of zone of inhibition of Cephalosporin disks towards Augmentin disks is indicative of ESBL production. Sometimes, false-negative results can be observed in the isolates harboring SHV-2 (Thomson and Sanders 1992; Ho et al. 1998; Randegger et al. 2001; MacKenzie et al. 2002), SHV-3 (Ho et al. 1998), or TEM-12 (Vercauteren et al. 1997). Repeat testing should be done in such isolates suspicious of ESBL production but are false negative with the standard spacing (Ho et al. 1998; Randegger et al. 2001; MacKenzie et al. 2002).

12.2.3.2 By Three-dimensional Method

In this method, the culture sensitivity plate (MHA) is inoculated with standard *E. coli* ATCC strain. Then, a slit is cut in agar homocentric with the margin of the culture plate and a test inoculum measuring around 10^9 to 10^{10} CFU/ml is poured in the slit with the help of a pipette. Beta-lactam disks are then put on the agar, approximately 3 mm away from the inoculated slit. A distortion in the usual circular inhibitory zone or the production of discrete colonies in the vicinity of the inoculated slit is indicative of beta-lactamase (ESBL) production (Thomson and Sanders 1992; Rawat and Nair 2010).

12.2.3.3 By Inhibitor Potentiated Disk Diffusion Method

Another disk diffusion methodology for phenotypic detection of ESBL producers is suggested by the British Society for Antimicrobial Chemotherapy (BSAC) where Ceftazidime-Clavulanate and Cefotaxime-Clavulanate combination disks are used with semiconfluent growth on iso-sensitest agar. If the ratio of inhibition zone size of Cephalosporin-Clavulanate with the zone size of Cephalosporin alone is 1.5 or greater, it is considered as ESBL producer (M'Zali et al. 2000).

12.2.3.4 By Disk Approximation Method

An inducer disk (usually Cefoxitin) is put at a distance of 2.5 cm from a third generation Cephalosporin disk (Revathi and Singh 1997). Flattening of >1 mm of the zone of inhibition of Cephalosporin towards the inducer disk is suggestive of inducible ESBL production.

12.2.4 Commercially Available Test Methods for ESBL Detection

12.2.4.1 By Vitek ESBL Test

An FDA approved automated Vitek card test that tests for ESBLs has now been commercially available. The Vitek ESBL test utilizes Cefotaxime or Ceftazidime alone (at 0.5 μg/ml) and in combination with Clavulanate (at 4 μg/ml). Inoculation of this card is done as it is done for other routine Vitek cards. Analysis is performed automatically when the growth control well reaches a threshold set value (usually 4–15 h of incubation). A reduction in the bacterial growth of the wells that contained Cephalosporins plus Clavulanate as compared to the bacterial growth in the wells with Cephalosporin alone is indicative of the presence of ESBL (Sanders et al. 1996).

12.2.4.2 By E-Test

The E-test ESBL strip carrying two gradients: Ceftazidime alone on the one end and Ceftazidime plus Clavulanate on the opposing end is used (Vercauteren et al. 1997). Minimum inhibitory concentration (MIC) is determined by locating the point of intersection of the zone of inhibition with the E-test strip edge. If ratio of the MIC of Ceftazidime to the MIC of Ceftazidime-clavulanate is ≥ 8, then it indicates the presence of ESBL.

12.2.4.3 By MicroScan Panels

MicroScan panels comprise dehydrated panels for microdilution antibiotic susceptibility testing using combinations of Cefotaxime or Ceftazidime plus beta-lactamase inhibitors. It has received FDA approval and has appeared to be highly reliable in large numbers of ESBL-producing isolates (Paterson and Yu 1999; Pagani et al. 2002; Komatsu et al. 2003).

12.2.4.4 By BD Phoenix Automated Microbiology System

To detect the production of ESBLs, the Phoenix ESBL test system uses growth response to third generation Cephalosporins with or without clavulanic acid. In 2003, the test algorithm has been delineated (Sanguinetti et al. 2003).

12.3 Detection of AmpC Producers

AmpC β-lactamase producers can show positive results on ESBL screening tests but come negative on its confirmation (Bell et al. 2007; Steward et al. 2001). There is no any recent CLSI recommended method at present for detection of AmpC β-lactamases. However, there are some study-based methods suggested for its detection. Cefoxitin-resistance test has been suggested for the screening of AmpC β-lactamase producers in the *Enterobacteriaceae.* And for confirmation, disk assays with combinations of beta-lactams (for example, Cloxacillin) with inhibitors such as boronic acid were suggested (Coudron et al. 2003; Qin et al. 2004; Yagi et al. 2005; Brenwald et al. 2005). D69C, which is a commercially available kit, has also been

validated for the detection of plasmid- and chromosomal-mediated AmpC β-lactamases in the *Enterobacteriaceae* (Halstead et al. 2012).

Some of the suggested methods for the detection of AmpC producers are as follows:

12.3.1 Screening Method for AmpC Detection

Screening of the *Enterobacterales* for AmpC β-lactamases is performed with Cefoxitin (Ratna et al. 2003; Coudron et al. 2003). Those isolates that yield a zone diameter of <18 mm are considered AmpC screen-positive. Both screen-positive and screen-negative isolates are then subjected to phenotypic confirmation.

Resistance to Cefoxitin or Oxyimino-β-lactams is suggestive of an AmpC enzyme. However, its specificity is low as certain Carbapenemase producing bacteria, a few class A β-lactamases, some strains of *K. pneumoniae* and *E. coli* due to decreased levels of production of outer membrane porins may also show cefoxitin resistance (Martínez-Martínez et al. 1996, 1999; Hernández-Allés et al. 1991, 2000; Poirel et al. 2000; Wachino et al. 2004). So, various other confirmatory tests designed for AmpC detection will be discussed as follows:

12.3.2 Phenotypic Confirmation of AmpC Beta-lactamases

12.3.2.1 AmpC Disk Test

Tris-EDTA permeabilizes a bacterial cell leading to release β-lactamase enzymes externally into the environment. This AmpC Disk test is solely based on the use of Tris-EDTA. Commercially available filter paper disks containing Tris-EDTA can be used or can also be made by pouring 20 µl of a 1:1 mixture of saline and 100× Tris-EDTA (catalog code T-9285; Sigma-Aldrich Corp. as recommended by NCCLS, 2003) onto sterile filter paper disks. These disks are then air-dried and further stored at 2–8 °C. Subsequently, MHA plates are inoculated as a carpet culture of cefoxitin-susceptible *E. coli* ATCC strain 25922 (NCCLS 2003). AmpC disks are rehydrated with 20 µl of saline and sufficient number of colonies of each test organism is applied to a disk just before its use. A disk of cefoxitin (30 µg) is placed on respective inoculated plates of MHA. Subsequently, the inoculated AmpC disk is then placed just adjacent to Cefoxitin disk with the inoculated side of disk in contact with the agar surface. The plate is then incubated overnight at 35 °C in ambient air. After incubation, an indentation or a flattening of the zone of inhibition indicates enzymatic inactivation of Cefoxitin by AmpC producer test organism (positive result) or absence of any distortion indicates absence of AmpC enzyme in the test organism (negative result).

12.3.2.2 Inhibition Based Method Using Boronic Acid (BA)

AmpC beta-lactamase production can be detected by IBM method using disk containing BA as per the method used by Hemalatha et al. (2007). A 30 µg Cefoxitin

(CX) disk and a disk containing 30 μg of CX plus 400 μg of BA are placed on the MHA agar plate 30 mm apart. Inoculated plates are then incubated overnight at a temperature of 37 °C. An increase of ≥5 mm in zone size around the disk containing CX plus BA than the zone of the disk containing CX alone is considered as AmpC producer.

12.3.2.3 Three-Dimensional Test (3D Test)

This 3D test was designed for detection of AmpC as well as ESBL. In the Conventional 3D test for the detection of AmpC, a disk diffusion test is carried out with *E. coli* ATCC 25922 and a suspension of test organism is poured in a circular slit in the agar which is 3 mm apart from the Cefoxitin disk. As Cefoxitin gets hydrolyzed by an AmpC enzyme. So, any distortion in the zone of inhibition indicates a positive 3D test (Thomson and Sanders 1992).

However, a variation in the existing three-dimensional test was done by Kuwabara and Abraham in which the plate was inoculated with a sensitive standard strain on an agar plate inoculated with 4 μg/ml of Cefoxitin and then the cell extract of the test organism derived by repeated freeze-thawing was added to a circular slit in the plate. Any growth around the well after incubation was suggestive of production of a Cefoxitin hydrolyzing AmpC enzyme by the bacteria (Kuwabara and Abraham 1967). Multiple samples can be tested per plate via this method.

Further modifications were done in this method by Manchanda and Singh in 2003. In place of intact cells of the test organisms, centrifuged and concentrated pellets were used. The pellets were then freeze-thawed approximately 5–7 times in order to release β-lactamase and were added to a radial slit rather than the circular slit (Coudron et al. 2000).

In 2005 another modification was also given by Lee et al. (2005), in which a heavy inoculum of the test organism was radially streaked from the edge of the Cefoxitin disk onto the surface of agar plate without using any type of slit.

12.3.2.4 E-Strip Test Method for AmpC Detection

E-strips having a gradient of Cephamycin and Cephamycin combined with a gradient of Cloxacillin on respective half of the E-strips are used for the detection of AmpC producers (Bolmstrom et al. 2006). A reduction in the MIC of Cephamycin for at least three dilutions or deformation of its zone of inhibition or a "Phantom zone" suggests the presence of AmpC enzyme producers.

12.3.2.5 Spot Inoculation Method

On a lawn culture of *E. coli* ATCC 25922, bacterial cells and freeze-thawed preparations of bacterial cells as the source of beta-lactamases were strategically applied as "spots" on the surface of culture plate near Ceftazidime or Cefoxitin disks at a distance of 5 mm. Plate is then incubated overnight at 37 °C. Enhanced growth of the surface organisms of the test spot at the point of intersection with the zone of inhibition of Cefoxitin/Ceftazidime is interpreted as the evidence for the presence of AmpC beta-lactamases (Shahid et al. 2004).

12.4 Detection of Carbapenemases

12.4.1 For Routine Laboratories Procedures

As per CLSI guidelines, Gram-negative isolates are subjected to Kirby Bauer disk diffusion susceptibility testing (Clinical and Laboratory Standards Institute 2020). The isolates with zone of inhibition ≥23 for Imipenem, Meropenam, and Doripenem or ≥22 mm for Ertapenem and also resistant to Cephalosporins subclass III are considered as Carbapenemase producers.

12.4.2 Older Methods for the Confirmation of MBL (Carbapenemases)

12.4.2.1 Imipenem and EDTA Combined Disk Synergy Test

In this test two Imipenem disks (10 ug) are placed on MHA inoculated with the test organism. Then 10 µl of a 0.5M EDTA solution is added to one of them so as a desired concentration of 750 ug is obtained. Plates are then incubated for 16–18 h at 37 °C. It is suggestive of MBL producer if the zone of inhibition of Imipenem—EDTA disk is ≥7 mm in comparison with Imipenem disk alone (Yong et al. 2002).

12.4.2.2 Imipenem and EDTA Double-Disk Synergy Test

An imipenem disk (10 ug) and a disk (as blank) containing 10 µl 0.5M EDTA (750 ug) are placed 20 mm apart center to center. Plates are incubated for 16–18 h at 37 °C. Test strain showing enhancement of the zone of inhibition between Imipenem and EDTA disks is considered as MBL producer (Yong et al. 2002).

12.4.2.3 Ceftazidime and EDTA Combined Disk Synergy Test

Method and interpretation of this test are similar to Imipenem and EDTA combined disk synergy test except using Ceftazidime (30 ug) disk in place of Imipenem (Galani et al. 2008).

12.4.2.4 Ceftazidime and EDTA Double-Disk Synergy Test

Method and interpretation of this test are similar to that of the double-disk synergy test performed using Imipenem and EDTA except using Ceftazidime (30 ug) disks in place of Imipenem (Galani et al. 2008).

The above methods are obsolete nowadays. According to CLSI, just the Kirby Bauer susceptibility testing is enough for the detection of Cabapenemases. But for the epidemiological purposes, infection, prevention, and research procedures, CLSI has recommended some newer methods for the detection of Carbapenemases. These are as follows:

12.4.3 For Epidemiological and Infection Prevention Purposes

Isolates those are screened positive for Carbapenemases via Kirby Bauer disk diffusion testing as discussed above are then confirmed phenotypically with additional tests like Carba NP test, eCIM, and mCIM (Clinical and Laboratory Standards Institute 2020).

mCIM is done to detect Carbapenemases in *Enterobacterales* and *Pseudomonas aeruginosa*, whereas eCIM is done in addition to mCIM to differentiate between metallobetalactamases and Serine Carbapenemases in Enterobacterales. eCIM is valid only if mCIM comes positive.

12.4.3.1 CarbaNP Test

The Procedure is described in detail in the document of Clinical and Laboratory Standards Institute (2020). Briefly, it is summarized below:

- Two microcentrifuge tubes are labeled (for instance, "a" and "b") for each patient isolate, uninoculated reagent control, and QC organism.
- 100 μL of bacterial protein extraction reagent is added to each tube.
- A loopful of 1-μl loop of individual bacteria to be tested is emulsified from an overnight blood agar plate in respective tubes ("a" and "b"). Each tube is vortexed for 5 s. Kindly note that uninoculated reagent control tube does not contain any organism (rather contains only bacterial protein extraction reagent).
- 100 μL of solution A of CarbaNP test is added in first labeled tube and 100 μL of solution B is added in second labeled tube.
- The tubes are vortexed well and then incubated at 35 °C ± 2 °C for up to 2 h.

Isolates that demonstrate positive results before 2 h can be reported as "Carbapenemase producers" (Table 12.1).

12.4.3.2 mCIM Test (Modified Carbapenem Inactivation Method)

Procedure: As described by Clinical and Laboratory Standards Institute (2020)

Table 12.1 Test interpretations: as stated by Clinical and Laboratory Standards Institute (2020)

Tube "a": solution A which acts as an internal control	Tube "b": solution B	Interpretation
Showing red or red-orange color	Showing red or red-orange color	Negative test, i.e., non-Carbapenemase-producer
Showing red or red-orange color	Showing light orange, dark yellow, or yellow color	Positive test, i.e., Carbapenemase-producer
Showing red or red-orange color	Showing orange color	Invalid test
Showing orange, light orange, dark yellow, or yellow color	Showing any color	Invalid test

1. Loop full of bacteria for each isolate of Enterobacterales or 10 μl loop full of *Pseudomonas aeruginosa* isolate is emulsified from overnight cultured blood agar plate in 2 ml of TSB.
2. It is vortexed approximately 10–15 s.
3. A 10 μg antibiotics disk of Meropenem is added to each tube by using sterile disk dispenser or forceps ensuring that the disk is completely immersed in the tube.
4. They are incubated at 35 ± 2 °C for 4 h ± 15 min.
5. Before incubation of Meropenem disk suspension, a 0.5 McFarland turbid inoculum of *E. coli* ATCC (25922) is prepared in saline or nutrient broth.
6. MHA plates are inoculated with the above *E. coli* suspension as is done for routine antibiotics disk diffusion procedure. The plates are allowed to dry for 5–10 min.
7. Meropenem disks are removed from its disk suspension using 10 μl loop and are placed on the MHA plates that are prior inoculated with *E. coli* ATCC Strain. 4 disks are placed on a 100 mm plate or 8 disks may be placed on a 150 mm plate.
8. The MHA plates are kept inverted and incubated at 37 °C in air for 18–24 h.
9. Following the incubation, the zone size around each Meropenem disks is measured and then interpreted accordingly.

Interpretation

a. A 6–15 mm zone diameter or the presence of colonies (pinpoint) within the area of zone of 16-18 mm is considered as Carbapenemase positive, i.e., because of the presence of Carbapenemase the Meropenem disk gets inactivated.
b. A clear zone of 19 mm or more is considered as Carbapenemase Negative, i.e., the strain is not producing Carpapenemase enzyme that's why the disk did not get inactivated.
c. Zone diameter of 16–18 mm or zone ≥19 mm with presence of pinpoint colonies inside the zone is considered as Carbapenemase intermediate test, i.e., presence/absence of Carbapenemase cannot be confirmed.

12.4.3.3 eCIM Test (EDTA-Modified Carbapenem Inactivation Method)

Procedure:

- For any isolate to be tested for this test, another tube (tube number 2) of 2 ml having TSB is labeled for this test (eCIM test).
- 20 μl of 0.5M EDTA is added to this 2 ml TSB tube so that a final suspension of 5 mM EDTA is prepared.
- Steps from 1 to 9 are followed as above for mCIM test procedure above. The mCIM and eCIM tests are processed in parallel.
- The disks of Meropenem from these two tubes (from mCIM and eCIM tubes) are placed on the same MHA plate which is inoculated with *E. coli* ATCC Strain susceptible to Meropenem.

Interpretation

a. An increase of 5 mm (or more) of zone diameters of Carbapenems in eCIM test as compared to mCIM test is considered as metallo-beta-lactamase positive (because the test isolate produces metallo-beta-lactamases, hence, the activity of MBL Carbapenemases gets inactivated with the use of EDTA).
b. An increase of 4 mm or less in the zone diameters of Carbapenems in eCIM test as compared to mCIM test is considered as metallo-beta-lactamase negative (the test isolate produces a Serine Carbapenemases so its activity is not affected with EDTA).

Reporting after eCIM and mCIM tests

a. If both mCIM and eCIM tests are negative, it will be reported as Carbapenemase not detected.
b. If mCIM is positive but eCIM is negative, it will be reported as Serine Carbapenemase detected.
c. If both mCIM and eCIM are positive, it will be reported as metallo-beta-lactamase detected.
d. If mCIM is intermediate, test is considered as inconclusive for the detection of Carbapenemase. Repeat testing can be done to recheck. If again intermediate result comes, a different phenotypic test can be done for detecting Carbapenemase production.

References

Bell JM, Chitsaz M, Turnidge JD et al (2007) Prevalence and significance of a negative extended-spectrum β-Lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of *Escherichia coli* and *Klebsiella pneumoniae*: results from the SENTRY Asia-Pacific surveillance program. J Clin Microbiol 45(5):1478–1482

Bolmstrom A, Engelhardt A, Bylund L et al (2006) Evaluation of two new Etest strips for AmpC detection, abstr. D-0451. Abstr 46th Intersci Conf Antimicrob Agents Chemother

Brenwald NP, Jevons G, Andrews J et al (2005) Disk methods for detecting AmpC β-lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. J Antimicrob Chemother 56(3):600–601

Clinical and Laboratory Standards Institute (2020) Performance standards for antimicrobial susceptibility test, 29th edn. Committee for Clinical Laboratory Standards, Wayne

Coudron PE, Moland ES, Thomson KS (2000) Occurrence and detection of AmpC beta-lactamases among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates at a veterans medical center. J Clin Microbiol 38:1791–1796

Coudron PE, Hanson ND, Climo MW (2003) Occurrence of extended-spectrum and AmpC beta-lactamases in bloodstream isolates of *Klebsiella pneumoniae*: isolates harbor plasmid-mediated FOX-5 and ACT-1 AmpC beta-lactamases. J Clin Microbiol 41(2):772–777

Galani I, Rekatsina PD, Hatzaki D et al (2008) Evaluation of different laboratory tests for the detection of metallo-ß-lactamase production in Enterobacteriaceae. J Antimicrob Chemother 61: 548–553

Halstead FD, Vanstone GL, Balakrishnan I (2012) An evaluation of the mast D69C AmpC detection disk set for the detection of inducible and derepressed AmpC β-lactamases. J Antimicrob Chemother 67(9):2303–2304

Hemalatha V, Padma M, Sekar U et al (2007) Detection of Amp C beta lactamases production in *Escherichia coli* & *Klebsiella* by an inhibitor based method. Indian J Med Res 126:220–223

Hernández-Allés S, Albertí S, Álvarez D et al (1991) Porin expression in clinical isolates of Klebsiella pneumoniae. Microbiology 145(3):673–679

Hernández-Allés S, del Conejo MC, Pascual A et al (2000) Relationship between outer membrane alterations and susceptibility to antimicrobial agents in isogenic strains of Klebsiella pneumoniae. J Antimicrob Chemother 46(2):273–277

Ho PL, Chow KH, Yuen KY et al (1998) Comparison of a novel, inhibitor-potentiated disk-diffusion test with other methods for the detection of extended-spectrum beta-lactamases in Escherichia coli and Klebsiella pneumoniae. J Antimicrob Chemother 42:49–54

Jarlier V, Nicolas MH, Fournier G et al (1988) ESBLs conferring transferable resistance to newer-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 10:867–878

Komatsu M, Aihara M, Shimakawa K et al (2003) Evaluation of MicroScan ESBL confirmation panel for Enterobacteriaceae-producing, extended-spectrum beta-lactamases isolated in Japan. Diagn Microbiol Infect Dis 46:125–130

Kuwabara S, Abraham EP (1967) Some properties of two extracellular beta-lactamases from Bacillus cereus 569/H. Biochem J 103(3):27–30

Lee K, Hong SG, Park YJ et al (2005) Evaluation of phenotypic screening methods for detecting plasmid-mediated AmpC β-lactamases–producing isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Diagn Microbiol Infect Dis 53(4):319–323

M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM (2000) Detection of extended-spectrum beta-lactamases in members of the family *Enterobacteriaceae:* comparison of the MAST DD test, the double disc and the Etest ESBL. J Antimicrob Chemother 45:881–885

MacKenzie FM, Miller CA, Gould IM (2002) Comparison of screening methods for TEM- and SHV-derived extended-spectrum beta-lactamase detection. Clin Microbiol Infect 8:715–724

Martínez-Martínez L, Hernández-Allés S, Albertí S et al (1996) In vivo selection of porin-deficient mutants of Klebsiella pneumoniae with increased resistance to cefoxitin and expanded-spectrum-cephalosporins. Antimicrob Agents Chemother 40(2):342–348

Martínez-Martínez L, Pascual A, Hernández-Allés S et al (1999) Roles of β-lactamases and porins in activities of carbapenems and cephalosporins against Klebsiella pneumoniae. Antimicrob Agents Chemother 43(7):1669–1673

National Committee for Clinical Laboratory Standards (2003) Performance standards for antimicrobial disk susceptibility testing; eighth informational supplement. Approved standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne

Pagani L, Migliavacca R, Pallecchi L et al (2002) Emerging extended-spectrum beta-lactamases in Proteus mirabilis. J Clin Microbiol 40:1549–1552

Paterson DL, Yu VL (1999) Extended-spectrum beta-lactamases: a call for improved detection and control. Clin Infect Dis 29:1419–1422

Poirel L, Naas T, Nicolas D et al (2000) Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and Integron-Borne gene from a Pseudomonas aeruginosa clinical isolate in France. Antimicrob Agents Chemother 44(4):891–897

Qin X, Weissman SJ, Chesnut MF (2004) Kirby-Bauer disk approximation to detect inducible third-generation cephalosporin resistance in Enterobacteriaceae. Ann Clin Microbiol Antimicrob 15(3):13

Randegger C, Boras A, Haechler H (2001) Comparison of five different methods for detection of SHV extended-spectrum beta-lactamases. J Chemother 13:24–33

Ratna AK, Menon I, Kapur I et al (2003) Occurrence & detection of AmpC beta-lactamases at a referral hospital in Karnataka. Indian J Med Res 118:29–32

Rawat D, Nair D (2010) Extended-spectrum β-lactamases in gram negative bacteria. J Global Infect Dis 2(3):263–274

Revathi G, Singh S (1997) Detection of expanded spectrum cephalosporin resistance due to inducible lactamases in hospital isolates. Indian J Med Microbiol 15:113–115

Sanders CC, Barry AL, Washington JA, Shubert C, Moland ES, Traczewski MM et al (1996) Detection of extended-spectrum-beta-lactamase-producing members of the family Enterobacteriaceae with Vitek ESBL test. J Clin Microbiol 34:2997–3001

Sanguinetti M, Posteraro B, Spanu T et al (2003) Characterization of clinical isolates of Enterobacteriaceae from Italy by the BD Phoenix extended-spectrum beta-lactamase detection method. J Clin Microbiol 41:1463–1468

Shahid M, Malik A, Agrawal M et al (2004) Phenotypic detection of extended-spectrum and AmpC β-lactamases by a new spot-inoculation method and modified three-dimensional extract test: comparison with the conventional three-dimensional extract test. J Antimicrob Chemother 54(3):684–687

Steward CD, Rasheed JK, Hubert SK et al (2001) Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the National Committee for clinical laboratory standards extended-spectrum β-lactamase detection methods. J Clin Microbiol 39(8):2864–2872

Thomson KS, Sanders CC (1992) Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. Antimicrob Agents Chemother 36(9):1877–1882

Vercauteren E, Descheemaeker P, Ieven M et al (1997) Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of Escherichia coli and Klebsiella spp. in a Belgian teaching hospital. J Clin Microbiol 35(9): 2191–2197

Wachino J, Doi Y, Yamane K et al (2004) Molecular characterization of a cephamycin-hydrolyzing and inhibitor-resistant class A β-lactamase, GES-4, possessing a single G170S substitution in the Ω-loop. Antimicrob Agents Chemother 48(8):2905–2910

Yagi T, Wachino J, Kurokawa H et al (2005) Practical methods using boronic acid compounds for identification of class C β-lactamase-producing Klebsiella pneumoniae and Escherichia coli. J Clin Microbiol 43(6):2551–2558

Yong D, Lee K, Yum JH (2002) Imipenem–EDTA disk method for differentiation of metallo-ß-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 40:3798–3801

Molecular Methods for Detection of Beta-Lactamases

13

Asfia Sultan and Fatima Khan

Abstract

Gram-negative bacterial infections are a substantial cause of health care associated morbidity and mortality among patients. β-lactam antibiotics are proven effective agents against these infections. However, with the increased use of these agents, bacteria acquired and developed resistant mechanisms to inactivate these antibiotics, most common being production of extended spectrum β-lactamases (ESBLs) which leads to hydrolysis of β-lactam ring. Numerous types of ESBLs exist and tend to evolve rapidly showing huge diversity due to point mutations, leading to various gene subtypes. Phenotypic methods are generally used for detection of ESBLs as they are cheap and easy to perform, but these lack coverage on mechanism and patterns of resistance. The basis of employing molecular method is to recognize the epidemiological diversity of different types, their patterns and presence on chromosome or plasmids.

Vast majority of methods are available for profiling of these ESBLs ranging from simplex PCR to randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP), etc. Commonest is PCR and its modifications in the form of RAPD, PCR-SSCP, etc. Tests based on combination of various molecular principal techniques are now being used, i.e.: PCR-RFLP, restriction site insertion PCR (RSI-PCR). Latest in the list are line probe assays, multilocus gene sequencing, and microarrays. This chapter will discuss about the currently available molecular techniques involved in study of ESBLs along with their advantages and disadvantages.

A. Sultan (✉) · F. Khan
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_13

Keywords

ESBLs · Molecular techniques · PCR · Plasmids · SHV

13.1 Introduction

Gram-negative bacteria are substantial cause of infections throughout the world and also have a vital role in healthcare-associated infections. β-lactam antibiotics are the most commonly used agents to treat these infections. Emergence of resistance to these antimicrobial agents leading to increased rate of morbidity and mortality especially in the intensive care unit is of major concern. The most common resistance mechanism to β-lactams involves hydrolysis by β-lactamases resulting in the inactivation of the antibiotic (Nordmann et al. 2012). ESBLs are the most abundant enzymes in *Enterobacteriaceae*, with approximately 600 natural variants (http://www.lahey.org/Studies/). Among ESBLs the CTX-M family is the most predominant and widely distributed group (Canton 2008; CLSI 2020).

Other β-lactam resistant mechanisms such as decreased porin function or increased efflux may also increase the level of antimicrobial resistance. In many Gram-negative pathogens, a combination of β-lactamase production and porin deletions contributes to the overall resistance profile (Blair et al. 2015). The most common ESBL types are TEM, SHV, and CTX-M (Pitout and Laupland 2008).

However, numerous other types also exist and tend to evolve rapidly showing huge diversity due to point mutations, leading to various gene subtypes. The genes of resistance towards antimicrobial agents can either be coded on the chromosome or on mobile genetic elements. These could be plasmids, transposons, or integrons. The most agonizing part is that these mobile elements often harbor resistance determinants to multiple classes of antibiotic leading to the emergence of progeny virtually resistant to almost all antibiotics (Bush 2010).

For detection of antimicrobial resistance, traditional phenotypic methods are commonly employed in most microbiology laboratories (Rood and Li 2017). Even though these methods are comparatively cheap and easy to perform, but turnaround time is generally long and does not give any information regarding mechanism and pattern of resistance. For better and improved understanding of antimicrobial resistance development mechanisms various genotypic and molecular detection methods are developed.

The basis of employing molecular method is to recognize the epidemiological diversity of different types. These can either be present on chromosome or plasmid, significance of being on chromosome or plasmid is the likely spread of these ESBLs to other organisms. Their presence on plasmids is often convoy with resistance to several other antibiotic classes as well.

Vast majority of methods have been developed for profiling of these ESBLs ranging from simplex PCR to RAPD and restriction fragment length polymorphism (RFLP), etc. Following are description of techniques developed over course of time.

13.2 Isoelectric Point and DNA Probes

Early studies on ESBLs used determination of the isoelectric point to identify the ESBL. However, with several types and subtypes of β-lactamases with identical isoelectric points, determination on the basis of isoelectric point is no longer possible (Bradford 2001). *DNA probes* are stretches of single-stranded *DNA* used to detect the presence of complementary nucleic acid sequences by hybridization. DNA probes were initially developed to detect β-lactamase genes specific for TEM and SHV enzymes but use of this method was sometimes labor intensive (Huovinen and Jacoby 1988).

13.3 Oligotyping

The oligotyping method was earlier developed by Ouellette et al., to discriminate between TEM-1 and TEM-2. This method used oligonucleotide probes labeled with radioisotope or biotin molecule that are designed to detect point mutations under stringent hybridization conditions (Ouellette et al. 1998). Several new TEM variants were identified using this method, but this method is less sensitive for the detection of mutations (Bradford 2001).

13.4 Polymerase Cain Reaction (PCR)

PCR is a revolutionary technique developed by Kary Mullis in 1983. It is an in vitro method which exploits DNA polymerase to synthesize complementary strand of DNA to the offered template strand in the presence of primers and nucleotides. Through this method, it is possible for a researcher to isolate and amplify a specific region (amplicons) of template sequence (Polymerase chain reaction (PCR): https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/).

Conventional/simplex PCR with oligonucleotide primers specific to β-lactamase gene is the easiest and most common molecular method used for detection of β-lactamases (Wu et al. 2001), whereas multiplex PCR is the technique where two or more genes of interest are amplified and detected simultaneously. Various studies have utilized these techniques for detection of β-lactamases (Lalzampuia et al. 2013; Bijllaardt et al. 2018). Most commonly detected β-lactamases by this technique are CTX-M, SHV, TEM, etc. However, PCR does not discriminate between different variants of ESBLs and non-ESBL enzymes (Fluit et al. 2001; Bradford 2001). Another disadvantage is that it does not give any idea about the quantification and the exact location of amplicon. However, chromosomal and plasmid locations can be detected by separating chromosomal and plasmid DNA before the procedure and then carrying out the PCR on separate DNA template (Lalzampuia et al. 2013).

Real-time PCR is the modification where simultaneous quantification of gene of interest is done along with amplification. Researchers have employed combination

of multiplex PCR along with real-time PCR to quantify and detect ESBLs from clinical samples (Alfaresi and Elkoush 2010; Reid and Samaras 2018).

13.5 Molecular Beacons (MB)

MB are oligonucleotide hybridization probeswith an internal quencher molecule. These are hairpin like structure in native state and fluoresce upon hybridization to the target nucleic acid sequence. Use of DNA probes and molecular beacons is the recent development in making real-time PCR more sensitive and quick for detection of ESBLs (Willemsen et al. 2014). Recent study employed the combination of molecular beacon probes, multiplex PCR and real-time PCR techniques for rapid detection of ESBLs (Chavda et al. 2016). In the early twenty-first century, Randeggar and Haechler developed a technique using real-time PCR monitored with fluorescently labeled hybridization probes for differentiation of SHV variants and to discriminate between non-ESBLs and ESBLs (Randegger and Hachler 2000).

13.6 PCR-Single-Strand Conformation Polymorphism (PCR-SSCP)

This method was developed by M'Zali et al. in the late 1990s for detection of SHV subtypes (M'Zali et al. 1996). Single-strand conformational polymorphism (SSCP) analysis is a simple and sensitive technique for detection of mutation. This method relies on the observation that point mutations can lead to changes in the migration of small single-stranded DNA molecules in non-denaturing gels. Therefore wild-type and mutant DNA samples display different band patterns on electrophoresis (Dong and Zhu 2005). In this technique, amplified product of PCR is denatured by heating, then cooled for self-annealing and lastly mobilized on electrophoresis for detection of mobility differences due to mutations (Dong and Zhu 2005). This method has been used to detect a single base mutation at specific location within the beta-lactamase genes with satisfactory results (Kim and Lee 2000). This technique allows detection of single base mutations but fails to give information about the nature of alteration in the genetic code (M'Zali et al. 1996). Later, researcher utilizes the combination of PCR-SSCP with PCR-restriction fragment length polymorphism (PCR-RFLP) for the rapid identification of newer SHV variants (Chanawong et al. 2000).

13.7 Randomly Amplified Polymorphic DNA (RAPD)

Several modifications of PCR have been applied to the typing of β-lactamases. One of this is randomly amplified polymorphic DNA (RAPD), which is also known as arbitrarily primed PCR (Kumari and Thakur 2014). In this method simple arbitrary sequence of DNA (8–12 nucleotide long) is used as primers and can hybridize

randomly to the complementary sites of target DNA molecule with sufficient affinity to permit the initiation of polymerization. The template is thus amplified and subjected to electrophoresis. The quantity and position of these random sites (and therefore the number and sizes of fragments) will vary among different strains of the same species (Arbeit 1999). This is the most popular method used to evaluate the genetic relatedness of ESBL-producing strains (Paterson and Bonomo 2005). Whole lot of literature is available on exploit RAPD in profiling of ESBLs like SHV, TEM, and KPCs (Eftekhar and Nouri 2015; Farivar et al. 2017). The major advantage of RAPD is its utility in identifying genetic variation without the need for pre-sequencing of DNA (Kumari and Thakur 2014). The limitation is the reproducibility of results as it depends on quality and concentration of template DNA, PCR components, and cycling conditions. Thus, the RAPD is a laboratory dependent technique and needs extensively validated laboratory protocols to be reproducible (Kumari and Thakur 2014).

13.8 PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)

Another advancement in characterization of β-lactamases is the combination of restriction fragment length polymorphism analysis to PCR (PCR-RFLP). In this, amplified DNA segment is subjected to restriction enzymes and then the product is analyzed by gel electrophoresis. The sizes of the fragments generated by each restriction enzyme indicate point mutations. This method has been employed in detection of TEM and SHV mutations (Sharma et al. 2010a, b; Chroma et al. 2007). Although this method cannot determine which SHV-type ESBL is present but can detect the specific position of mutation (Arlet et al. 1995). It has good reproducibility of results and is highly specific but costly as compared to RAPD and other PCR based techniques.

13.9 Plasmid Profile Analysis

It is already known that vast majority of ESBL genes are coded on plasmid, plasmid profile analysis has been utilized to study ESBL-producing organisms (Motayo et al. 2013; Olukoya and Oni 1990; Sharma et al. 2010a, b). It is a simple method which distinguishes the number and size of the plasmids carried by the organism by extracting plasmid from bacterial cell and subjecting it to agarose gel electrophoresis. This method can be improved by digesting plasmid extracts by restriction enzymes before performing agarose gel electrophoresis. This modification and the analysis of the size and number of the resulting restriction fragment of plasmids are referred as restriction enzyme analysis of plasmids. A drawback in plasmid profile analysis is that plasmids may be lost after storage, so extraction method should be standardized for better results (Paterson and Bonomo 2005).

13.10 Pulsed-Field Gel Electrophoresis

In 1984, David C. Schwartz and Charles Cantor developed this technique which is a variation of standard gel electrophoresis. Since larger DNA molecules move irrespective of their size in presence of constant unidirectional current, the resolution of larger molecules can be improved by introducing an alternating voltage gradient (Schwartz and Cantor 1984). In this process the extracted DNA is exposed to restriction enzymes followed by subjecting these restriction fragments to electrophoresis in which the voltage is periodically switched among three directions. Main advantage is that it yields stable and reproducible DNA patterns and can be successfully utilized in epidemiological typing. It is time-consuming and inferior to genome sequencing (PFGE 2016). Pulsed-field gel electrophoresis of chromosomal DNA is probably the most widely used molecular method to determine the relatedness of ESBL-producing organisms (Liu et al. 1998; Nemoy et al. 2005; Kao et al. 2016).

13.11 Ligase Chain Reaction (LCR)

The LCR is based on the principle of ligation of two adjacent synthetic oligonucleotide primers, which uniquely hybridize to one strand of the target DNA (Wiedmann et al. 1994). LCR is a chain reaction that differs from polymerase chain reaction in the involvement of two thermostable enzymes, ligase along with polymerase to carry out the amplification. This reaction was first developed by Barany (1991b). In this process first a thermostable ligase is utilized to join two probes or molecules together flowed by amplification by standard PCR technique of the joined product which serves as template (Barany 1991a). LCR was originally developed to detect point mutations (Kim and Lee 2000). If the mutation is present in the form of single base mismatch at the junction of the two probe molecules it prevents ligation. LCR will thus amplify template molecules that have been successfully ligated and result in a large amount of product with even greater specificity than PCR. The absence of the ligated product therefore indicates at least a single base-pair change in the target sequence (Wiedmann et al. 1994). Thus, LCR is not necessarily an alternative, but rather a complement, to PCR. The ligase chain reaction (LCR) is used for the identification of SHV genes. Ligase chain reaction (LCR) allows the discrimination of DNA sequences that differ by a single base pair (Wiegand et al. 2007). Ligase detection reaction (LDR) is similar to LCR where only one pair of adjacent primers is used which hybridizes to one of the target strands in order to achieve a linear amplification. LDR coupled with PCR has been utilized to detect multiple mutations at a time (Barany 1991a LCR). Niederhauser et al. used LDR-PCR for identification of SHV variants (Niederhauser et al. 2000).

13.12 Nucleotide Sequencing

DNA sequencing is the process of determining the nucleic acid sequence via determination of physical order of bases in a DNA molecule (Behjati and Tarpey 2013). Through this technique the sequence of individual genes, larger regions (cluster of genes), or entire genome of any organism (whole-genome sequencing) can be determined. In order to determine the presence of specific β-lactamase gene in an organism, nucleotide sequencing is the standard method. However, results may vary with the difference in technique used. This variability in results may occur due to compressions or difficulty in reading in older sequencing methods (Bradford 2001). This technique has been employed in typing relationship of β-lactamases of Gram-positive and Gram-negative bacteria (Wang and Novick 1987; Zscheck and Murray 1991). Other researchers have utilized this technique for characterization of specific β-lactamase type (Oliver et al. 2001; Wu et al. 2018; Barnaud et al. 1997).

13.13 Multilocus Gene Sequencing (MLST)

MLST is based on principle of multilocus enzyme electrophoresis in which high levels of discrimination are achieved through analysis of various patterns of housekeeping loci of an organism. In this method alleles are assigned for multiple housekeeping loci through DNA sequencing. This approach overcame the difficulty in comparisons of results of two different laboratories or sources, etc. (Maiden et al. 1998). This has been used recently for typing and characterization of β-lactamases (Nemoy et al. 2005; Seenama et al. 2019). Although MLST discriminates well but is expensive and labor intensive. Whole-genome multilocus sequence typing (wgMLST) is the recent modification which is more specific for discrimination of β-lactamases as compared to MLST (Kluytmans-van den Bergh et al. 2016).

13.14 Ribotyping

Ribotyping has also been utilized in study of CTX-M, SHVs, and other ESBLs (Shen et al. 2001; Costa et al. 2000). It is a modification of RFLP in which after digestion with restriction endonucleases and separation by electrophoresis, the separated fragments are hybridized to nitrocellulose membrane labeled with ribosomal RNA (Grimont and Grimont 1986). It is potentially a very useful tool in typing of β-lactamases.

13.15 Microarray

It is an advanced tool developed nearly a decade ago which enables the detection of thousands of genes at the same time. These are microchips with multiple spots, each coded with DNA sequences at specific locations. To these spots the extracted cDNA

labeled with fluorophore is hybridized and development of fluorescence is read (Nature 2014). This technology is helpful in rapid detection of various ESBLs, AmpC, KPCs and other carbapenemases, etc. in an organism (Cuzon et al. 2012; Naas et al. 2010; Stuart et al. 2010).

13.16 Line Probe Assay

It is a kind of hybridization method which utilizes the mobilization of PCR product to nitrocellulose membranes coded with designed probes for detection of particular gene. Various line probe assays have been developed for rapid detection of ESBLs, i.e.: AID line probe assay for detection of SHV, TEM, CTX-M, etc. (Bloemberg et al. 2014).

13.17 Combination Techniques

Restriction site insertion PCR is a recently developed technique for rapid and reliable detection of point mutations. This technique was first utilized for discrimination between various SHV variants by Chanawong et al. (2001). The author also combined RSI-PCR with PCR-RFLP for quick and valid characterization of different SHV variants. This is an efficient tool for epidemiological typing of SHV types and has a potential for determination of other resistance determinants (Chanawong et al. 2001).

Earlier same researchers used successfully the combination of PCR-single strand conformational polymorphism and PCR-restriction fragment length polymorphism (Chanawong et al. 2000). Using this combination, the genes encoding for 12 SHV types were distinguished (Chanawong et al. 2000).

Another approach could be a sequence-specific peptide nucleic acid (PNA)-based multiplex PCR detection which allows a precise identification of bla (GES-2) (Bauernfeind et al. 1992). Recently peptide nucleic acid (PNA) has been combined with multiplex real-time PCR method for accurate and simultaneous evaluation of various carbapenemases (Jeong et al. 2015).

Variety of methods are available and being used for characterization of β-lactamases. The major advantage of using molecular method is quick and accurate results as compared to phenotypic methods. However, molecular methods cannot replace conventional methods for antimicrobial resistance detection. These methods could potentially be utilized as a tool in improving infection control practices because of rapid turnaround time leading to early containment of resistant organisms (Ducomble et al. 2015; Suzuki et al. 2015). Molecular methods are mainly a tool for epidemiological studies. For effective utilization of molecular techniques in clinical practice there should be a correlation with clinical scenario. However, a good knowledge of the local gene pool is really important in implementing a molecular method.

References

Alfaresi MS, Elkoush AA (2010) Real-time polymerase chain reaction for rapid detection of genes encoding SHV extended-spectrum β-lactamases. Indian J Med Microbiol 28(4):332–336

Arbeit RD (1999) Laboratory procedures for the epidemiologic analysis of microorganisms. In: Murray PR (ed) Manual of clinical microbiology, 7th edn. ASM Press, Washington, DC

Arlet G, Brami G, Dècrère D, Flippo A, Galtolot O, Lagrange PH, Philippon A (1995) Molecular characterization by PCR-restriction fragment length polymorphism of TEM β-lactamases. FEMS Microbiol Lett 134:1498–1500

Barany F (1991a) Genetic disease detection and DNA amplification using cloned thermostable ligase. Proc Natl Acad Sci U S A 88(1):189–193

Barany F (1991b) The ligase chain reaction in a PCR world. PCR Methods Appl 1:5–16

Barnaud G, Arlet G, Danglot C, Philippon A (1997) Cloning and sequencing of the gene encoding the AmpC β-lactamase of *Morganella morganii*. FEMS Microbiol Lett 148(1):15–20

Bauernfeind A, Casellas JM, Goldberg M, Holley M, Jungwirth R, Mangold P, Rohnisch T, Schweighart S, Wilhelm R (1992) A new plasmidic cefotaximase from patients infected with *salmonella typhimurium*. Infection 20:158–163

Behjati S, Tarpey PS (2013) What is next generation sequencing? Arch Dis Childhood Educ Pract Ed 98(6):236–238

Bijllaardt W, Janssens MM, Buiting AG, Muller AE, Mouton JW, Verweij JJ (2018) Extended-spectrum β-lactamase (ESBL) polymerase chain reaction assay on rectal swabs and enrichment broth for detection of ESBL carriage. J Hosp Infect 98(3):264–269

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13(1):42–51

Bloemberg GV, Polsfuss S, Meyer V, Bo¨ttger EC, Hombach M. (2014) Evaluation of the AID ESBL line probe assay for rapid detection of extended-spectrum b-lactamase (ESBL) and KPC carbapenemase genes in Enterobacteriaceae. J Antimicrob Chemother 69:85–90

Bradford PA (2001) Extended spectrum betalactamases in the 21st century: characterization, epidemiology and the detection of this important resistance threat. Clin Microbiol Rev 14(4): 933–951

Bush K (2010) Alarming beta-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol 13(5):558–564

Canton R (2008) Epidemiology and evolution of beta-lactamases. In: Baquero FNC, Cassell G, Gutiérrez-Fuentes J (eds) Evolutionary biology of bacterial and fungal pathogens. ASM Press, Washington, DC, pp 249–270

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM (2000) Characterisation of extended-spectrum beta-lactamases of the SHV family using a combination of PCR-single strand conformational polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (PCR-RFLP). FEMS Microbiol Lett 184:85–89

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM (2001) Discrimination of SHV beta-lactamase genes by restriction site insertion-PCR. Antimicrob Agents Chemother 45(7): 2110–2114. https://doi.org/10.1128/AAC.45.7.2110-2114.2001

Chavda KD, Satlin MJ, Chen L, Manca C, Jenkins SG, Walsh TJ, Kreiswirth BN (2016) Evaluation of a multiplex PCR assay to rapidly detect Enterobacteriaceae with a broad range of -lactamases directly from perianal swabs. Antimicrob Agents Chemother 60:6957–6961

Chroma M, Kolar M, Marek O, Koukalova D, Sauer P (2007) Molecular characterization of ESBL-producing Klebsiella pneumoniae isolates from intensive care patients. Klin Mikrobiol Infekc Lek 13(5):206–212

CLSI (2020) Performance standards for antimicrobial susceptibility testing. 30th informational supplement. CLSI documentM100-S22. Clinical and Laboratory Standards Institute, Wayne

Costa SF, Woodcock J, Gill M, Wise R, Barone AA, Caiaffa H, Levin ASS (2000) Outer-membrane proteins pattern and detection of β-lactamases in clinical isolates of imipenem-resistant Acinetobacter baumannii from Brazil. Int J Antimicrob Agents 13(3):175–182

Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P (2012) Evaluation of a DNA microarray for the rapid detection of extended-spectrum β-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother 67(8):1865–1869

Dong Y, Zhu H (2005) Single-strand conformational polymorphism analysis: basic principles and routine practice. Methods Mol Med 108:149–157

Ducomble T, Faucheux S, Helbig U, Kaisers UX, Konig B, Knaust A et al (2015) Large hospital outbreak of KPC-2-producing Klebsiella pneumoniae: investigating mortality and the impact of screening for KPC-2with polymerase chain reaction. J Hosp Infect 89(3):179–185

Eftekhar F, Nouri P (2015) Correlation of RAPD-PCR profiles with ESBL production in clinical isolates of Klebsiella pneumoniae in Tehran. J Clin Diagn Res 9(1):DC01–DDC3

Farivar AZ, Nowroozi J, Eslami G, Sabokbar A (2017) RAPD PCR profile, antibiotic resistance, prevalence of *armA* gene, and detection of KPC enzyme in *Klebsiella pneumoniae* isolates. Can J Infect Dis Med Microbiol 1:1–7

Fluit AC, Visser MR, Schmitz FJ (2001) Molecular detection of antimicrobial resistance. Clin Microbiol Rev 14(4):836–871

Grimont F, Grimont PA (1986) Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur Microbiol 137B(2):165–175

Huovinen SP, Jacoby GA (1988) Detection of plasmid-mediated β-lactamases with DNA probes. Antimicrob Agents Chemother 32:175–179

Jeong S, Kim JO, Jeong SH, Bae IK, Song W (2015) Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in gram-negative clinical isolates. J Microbiol Methods 113:4–9. https://doi.org/10.1016/j.mimet.2015.03.019

Kao CY, Udval U, Huang Y, Wu H, Huang A, Bolormaa E, Yan JJ, Urangoo Z, Batbaatar G, Khosbayar T, Wu JJ (2016) Molecular characterization of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella spp. isolates in Mongolia. J Microbiol Immunol Infect 49(5):692–700

Kim J, Lee HJ (2000) Rapid discriminatory detection of genes coding for SHV beta-lactamases by ligase chain reaction. Antimicrob Agents Chemother 44:1860–1864

Kluytmans-van den Bergh MFQ, Rossen JWA, Bruijning-Verhagen PCJ, Bonten MJM, Friedrich AW, Vandenbroucke-Grauls CMJE, Willems RJL, Kluytmans JAJW, on behalf of the SoM Study Group (2016) Whole-genome multilocus sequence typing of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol 54:2919–2927

Kumari N, Thakur SK (2014) Randomly amplified polymorphic DNA-a brief review. Am J Anim Vet Sci 9(1):6–13. ISSN: 1557-4555

Lalzampuia H, Dutta TK, Warjri I, Chandra R (2013) PCR-based detection of extended-spectrum β-lactamases ($bla_{CTX\text{-}M\text{-}1}$ and bla_{TEM}) in *Escherichia coli*, *salmonella* spp. and *Klebsiella pneumoniae* isolated from pigs in north eastern India (Mizoram). Indian J Microbiol 53(3): 291–296

Liu PY, Tung JC, Ke SC, Chen SL (1998) Molecular epidemiology of extended-spectrum beta-lactamase-producing *Klebsiella pneumonia* isolates in a district hospital in Taiwan. J Clin Microbiol 36:2759–2762

M'Zali FH, Gascoyne-Binzi DM, Heritage J, Hawkey PM (1996) Detection of mutations conferring extended-spectrum activity on SHV -lactamase using polymerase chain reaction single strand conformational polymorphism (PCR-SSCP). J Antimicrob Chemother 37:797–802

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95(6):3140–3145

Motayo BO, Akinduti PA, Adeyakinu FA, Okerentugba PO, Nwanze JC, Onoh CC, Innocent-Adiele HC, Okonko IO (2013) Antibiogram and plasmid profiling of carbapenemase and

extended spectrum Beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in Abeokuta, Southwestern, Nigeria. Afr Health Sci 13(4):1091–1097

Naas T, Cuzon G, Truong H, Bernabeu S, Nordmann P (2010) Evaluation of a DNA microarray, the checkpoints ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. Antimicrob Agents Chemother 54(8): 3086–3092

https://www.nature.com/scitable/definition/microarray-202/#:~:text=A%20microarray%20is%20a%20laboratory,known%20DNA%20sequence%20or%20gene. © 2014 Nature Education

Nemoy LL, Kotetishvili M, Tigno J, Keefer-Norris A, Harris AD, Perencevich EN, Johnson JA, Torpey D, Sulakvelidze A, Morris JG, Stine O (2005) Multilocus sequence typing versus pulsed-field gel electrophoresis for characterization of extended-Spectrum Beta-lactamase-producing Escherichia coli isolates. J Clin Microbiol 43(4):1776–1781

Niederhauser C, Kaempf L, Heinzer L (2000) Use of the ligase detection reaction-polymerase chain reaction to identify point mutations in extended-spectrum beta-lactamases. Eur J Clin Microbiol Infect Dis 19(6):477–480

Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 18(5):263–272

Oliver A, Pérez-Díaz JC, Coque TM, Baquero F, Rafael CN (2001) Nucleotide sequence and characterization of a novel cefotaxime-hydrolyzing b-lactamase (CTX-M-10) isolated in Spain. Antimicrob Agents Chemother 45(2):616–620

Olukoya DK, Oni O (1990) Plasmid profile analysis and antimicrobial susceptibility patterns of Shigella isolates from Nigeria. Epidemiol Infect 105(1):59–64

Ouellette M, Paul GC, Philippon AM, Roy PH (1998) Oligonucleotide probes (TEM-1, OXA-1) versus isoelectric focusing in ß-lactamase characterization of 114 resistant strains. Antimicrob Agents Chemother 32:397–399

Paterson DL, Bonomo RA (2005) Extended-spectrum-lactamases: a clinical update. Clin Microbiol Rev 18(4):657–686

Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8(3):159–166

Pulsed-field Gel Electrophoresis (PFGE). https://www.cdc.gov/pulsenet/pathogens/pfge.html. Page last reviewed: 16 Feb 2016. Content source: National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)

Randegger CC, Hachler H (2000) Real-time PCR and melting curve analysis for reliable and rapid detection of SHV extended spectrum betalactamases. Antimicrob Agents Chemother 45:1730–1736

Reid R, Samaras S (2018) The development and evaluation of a multiplex real-time PCR assay for the detection of ESBL genes in urinary tract infections. Int J Clin Microbiol 1(1):16–24

Rood IGH, Li Q (2017) Review: molecular detection of extended spectrum-β-lactamase- and carbapenemase-producing Enterobacteriaceae in a clinical setting. Diagn Microbiol Infect Dis 89:245–250

Schwartz DC, Cantor CR (1984) Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37(1):67–75

Seenama C, Thamlikitkul V, Ratthawongjirakul P (2019) Multilocus sequence typing and bla_{ESBL} characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from healthy humans and swine in Northern Thailand. Infect Drug Resist 12:2201–2214. https://doi.org/10.2147/IDR.S209545

Sharma J, Ray P, Sharma M (2010a) Plasmid profile of ESBL producing Gram-negative bacteria and correlation with susceptibility to beta-lactam drugs. Indian J Pathol Microbiol 53(1):83–86. https://doi.org/10.4103/0377-4929.59190

Sharma J, Sharma M, Ray P (2010b) Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res 132:332–336

Shen D, Winokur P, Jones RN (2001) Characterization of extended spectrum beta-lactamase-producing Klebsiella pneumoniae from Beijing, China. Int J Antimicrob Agents 18(2): 185–188. https://doi.org/10.1016/s0924-8579(01)00351-x. PMID: 11516943

Stuart JC, Dierikx C, Al Naiemi N, Karczmarek A, Van Hoek AHAM, Vos P, Fluit AC, Scharringa J, Duim B, Mevius D, Leverstein-Van Hall MA (2010) Rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemother 65(7):1377–1381

Suzuki H, Hitomi S, Yaguchi Y, Tamai K, Ueda A, Kamata K et al (2015) Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. J Infect Chemother 21(12):849–856

Wang PZ, Novick RP (1987) Nucleotide sequence and expression of the beta-lactamase gene from Staphylococcus aureus plasmid pI258 in Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. J Bacteriol 169(4):1763–1766. https://doi.org/10.1128/jb.169.4.1763-1766.1987

Wiedmann M, Wilson WI, Czajka J, Luo J, Barany F, Batt CA (1994) Ligase chain reaction (LCR) --overview and applications. Manual supplement. 3:SS1-S649 by Cold Spring Harbor Laboratory ISSN 1054-9805/94. Genome Res 3:S51–S64

Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H et al (2007) Detection of extended-spectrum betalactamases among Enterobacteriacea by use of semiautomated microbiology systems and manual detection procedures. J Clin Microbiol 45(4):1167–1174

Willemsen I, Hille L, Vrolijk A, Bergmans A, Kluytmans J (2014) Evaluation of a commercial real-time PCR for the detection of extended spectrum b-lactamase genes. J Med Microbiol 63:540–543

Wu TL, Siu LK, Su LH, Lauderdale TL, Lin FM et al (2001) Outer membrane protein change combined with co-existing TEM-1 and SHV-1 beta-lactamases lead to false identification of ESBL-producing Klebsiella pneumoniae. J Antimicrob Chemother 47(6):755–761

Wu C, Lin C, Zhu X, Liu H, Zhou W, Lu J, Zhu L, Bao O, Cheng C, Hu Y (2018) The β-lactamase gene profile and a plasmid-carrying multiple heavy metal resistance genes of Enterobacter cloacae. Int J Genomics 1(1):1–12

Zscheck KK, Murray BE (1991) Nucleotide sequence of the beta-lactamase gene from Enterococcus faecalis HH22 and its similarity to staphylococcal beta-lactamase genes. Antimicrob Agents Chemother 35(9):1736–1740. https://doi.org/10.1128/aac.35.9.1736

Part IV

Scenario of Antibiotic Resistance in Environment with Focus on Beta-Lactamases

14 Epidemiology and Prevalence of Beta-Lactamases and Recent Resistance Pattern in Gram-Negative Bacteria from Environmental Reservoirs

Uzma Tayyaba and Shariq Ahmed

Abstract

Antibiotic resistance has largely been studied in the context of clinical settings, but subsequently with time it is being realized that bacteria present in the natural environment can be resistant to a large number of antibiotics, which make us ponder that antibiotic resistance in bacteria encountered clinically may have originated in environmental bacteria and transferred overtime. Globally, among animals, environment, and humans there has been a rapid emergence and dissemination of resistant bacteria and genes, therefore antibiotic resistance is now considered as a One Health Challenge. Prevalence of ESBL-producing *Enterobacterales* has seen a rapid increase in water, wastewater, fresh vegetables, food-producing animals and soil, and is not limited to only hospital environments. The environment sources have become potential reservoir of resistant bacteria harboring resistant genes that can be mobilized to the microbes dangerous for human health. Study of this reservoir could provide an early warning system for future clinically relevant antibiotic resistance mechanisms.

Keywords

Environment · Water · Food animals · Dairy farm · Livestock

U. Tayyaba (✉) · S. Ahmed
Department of Microbiology, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_14

14.1 Introduction

Use, misuse, irrational use, and overuse of antimicrobial agents in a given environment ultimately lead to antimicrobial drug resistance and it has gained a particular importance among β-lactam family of antimicrobial agents.

Antibiotic resistance has largely been studied in the context of clinical settings, but subsequently with time it's being realized that bacteria present in the natural environment can be resistant to a large number of antibiotics, which make us ponder that antibiotic resistance in bacteria encountered clinically may have originated in environmental bacteria and transferred overtime to humans (D'Costa et al. 2006; Riesenfeld et al. 2004).

Worldwide, among humans, animals, and environment there has been a rapid emergence and dissemination of resistant bacteria and genes, therefore antibiotic resistance is now considered as a One Health Challenge (Robinson et al. 2016). The One Health Approach is defined as "the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals, and our environment." It concedes that health of humans is related to health of animals and the environment (American Veterinary Medical Association 2008). The conceptual framework of One Health and its interrelatedness is depicted in Fig. 14.1.

Apart from hospital environments, ESBL-producing *Enterobacteriaceae* are becoming increasingly prevalent in water, wastewater, soil, and food such as fresh vegetables and meat (Abayneh et al. 2019; Bréchet et al. 2014; Kluytmans et al. 2013). All food-producing animals have shown to be carrying ESBL/AmpC-producing isolates (Carattoli 2008a), including all kinds of meats sold at retail

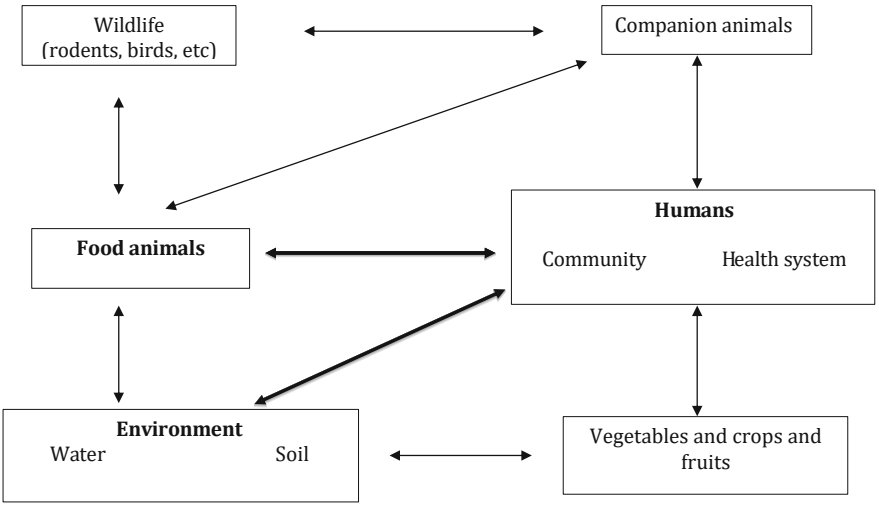


Fig. 14.1 Interrelationship and complexity of various sectors (human, animal, and environment) involved in One Health Approach

(Cohen Stuart et al. 2012; Leverstein-van Hall et al. 2011; Overdevest et al. 2011a) and in vegetables (Egea et al. 2011).

14.2 Water Environment and Antimicrobial Resistance

Very few studies are there that have examined role of water environments such as wastewaters, sludge, freshwater, and aquaculture as "mixing pots" and transporters of genes from environmental bacteria to the bacteria of human diseases as these environmental water sources are prime sites for gene exchange (Marshall et al. 2009).

Pharmaceutical waste and *hospital effluents* which are released in the water bodies without treatment seem to be the major cause of antimicrobial resistance in the environment. Presence of antibiotic residue in the water bodies is a matter of concern which may lead to toxicity to aquatic communities, development of resistance in microorganisms and ultimately impact public health. Several studies have reported bacteria having genes coding for resistance to numerous antibiotics from different water sources of India. Irrational dumping and spillover of antibiotics in the environment via pharmaceutical waste and hospital effluents have accelerated the development, selection and/or horizontal plasmid-mediated transfer of antibiotic resistance genes among environmental bacteria and bacteria of human importance (Allen et al. 2010; Taneja and Sharma 2019).

From Indian perspective, this problem can be huge as most of the wastewater in India does not undergo any pretreatment before being discharged and ultimately reaching river, lakes, reservoirs, etc. It can be argued that antibiotics do not reach the therapeutic concentration in these water bodies as they are diluted during the course. However, subtherapeutic exposure over a long period of time may be the ideal environment for the bacteria to acquire resistance genes and their genetic transfer to the other bacteria (Diwan et al. 2010).

Akiba et al. in 2016 reported more than half *E. coli* isolated from various water sources all over India were resistance to eight or more antimicrobials. 66.3% of *E. coli* isolates had $bla_{\text{CTX-M}}$ gene of which (108/112) were $bla_{\text{CTX-M-15}}$ and (4/112) $bla_{\text{CTX-M-55}}$. Carbapenem-resistant *E. coli* isolates with $bla_{\text{NDM-1}}$ in two isolates, $bla_{\text{NDM-5}}$ in seven isolates, and $bla_{\text{NDM-7}}$ in five isolates were also seen. Distributions of other resistance genes like TEM, OXA, and CMY were 43.8%, 39.6%, and 40.2%, respectively. On comparison of the DNA sequences of the bla_{NDM}-positive plasmids detected in this study with known sequences of related plasmids suggested that various mutation events facilitated the evolution of the plasmids and that plasmids with similar genetic backgrounds have widely disseminated in India (Akiba et al. 2016).

Diwan et al. from Ujjain district of Madhya Pradesh in 2010 reported presence of fluoroquinolones in hospital effluents. High residue levels of these antibiotics in aquatic environment can modify bacterial strains like *Salmonella typhimurium* and also lead to genotoxic effects. They also found $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$ genes in *E. coli* isolated from the hospital waste water (Diwan et al. 2010).

Another study from Ujjain district of central India reported 44% were resistant to both cephalosporins and quinolones and 3% to imipenem in the wastewater of two hospitals. Phenotypically 96% of the *E. coli* isolates were ESBLs having genes $bla_{\text{CTX-M}}$ (87%) and bla_{TEM} (63%) (Chandran et al. 2014). Plasmid-mediated $bla_{\text{NDM-1}}$ gene that encode NDM-1 metallo-β-lactamases resulting in resistance to carbapenems, which is one of the last resort drugs, can be readily transferred from one bacterium to other. Presence to bacteria having these notorious genes in the headwaters of Upper Ganges River is matter of concern (Ahammad et al. 2014). Also, $\text{bla}_{\text{NDM-1}}$ was reported in seepage water (i.e., water pools in streets or rivulets) and public tap water samples from New Delhi, India (Walsh et al. 2011).

From river Yamuna traversing through Delhi extended-spectrum β-lactamase (ESBL) and AmpC-producing *E. coli* was reported having $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} genes. They reported bla_{TEM} as the most widespread (100%) gene followed by $bla_{\text{CTX-M}}$ (16%), and plasmid-mediated *ampC* (3%). $bla_{\text{CTX-M-15}}$ and $bla_{\text{CMY-42}}$ were identified as the genes encoding CTX-M type ESBL and CIT type AmpC β-lactamases, respectively. CTX-M-15 ESBL phenotype was most common in phylogroup D (50%), followed by phylogroups B1 (30%), and A (20%) (Bajaj et al. 2015).

Resistance to cefadroxil among *E. coli* isolates from drinking water and recreational sources, e.g., hand pumps, ponds, river, kunds, dug wells and piped supply in Ayodhya-Faizabad district of Eastern Uttar Pradesh, India was 88.89%, while in *Enterobacter, Klebsiella,* and *Salmonella* it was 86.75%, 83.33%, and 100% respectively. Also, resistance to cefaclor and cefuroxime was seen 94% in Shigella spp. Authors suggested the transmission of bacterial isolates from one to other water sources on the basis of RAPD pattern analysis (Kumar et al. 2013).

Shahid M. et al. among isolates from environmental samples (drinking water, drain, sewage) in Aligarh region of western Uttar Pradesh reported the maximum resistance was noticed for ceftazidime (52%), resistance to a fourth-generation cephalosporin, cefepime, was noticed in 24% isolates. Prevalence of *bla* genes were as follows bla_{ampC} (48.15%), bla_{SHV} 18.52%, $bla_{\text{CTX-M}}$ (11.11%), and bla_{TEM} (11.11%) respectively (Shahid et al. 2014). Resistance to cefotaxime was seen 7% in *E. coli* and 16.7% in *Salmonella* isolated from different sources of water supply (Dal Lake, streams, community supply water and tube wells) in Kashmir, India (Rather et al. 2012).

Isolates of Salmonella and Shigella from environmental water source in Sikkim were resistant to cefixime (34.5%), ceftazidime (26.3%), however no resistance to imipenem and piperacillin/tazobactam was seen (Poonia et al. 2014).

Contamination from antimicrobials were seen among all the water samples collected from Hyderabad and nearby villages, the Musi River, and direct environment of pharmaceutical facilities, and the two sewage treatment plants vicinity. Also, more than 95% of the samples had ESBL and Carbapenemase-producing *Enterobacteriaceae* and non-fermenters (carrying mainly *bla* OXA-48, *bla* NDM, and *bla* KPC) (Lübbert et al. 2017).

To summaries prevalence of antibiotic resistance bacteria from various water sources like river, ponds, lake, hand pump, waste water from hospitals,

pharmaceutical industry etc. all over India, 17.4% of gram-negative bacteria isolated from Ganges and Yamuna river were ESBL producer (Ahammad et al. 2014), resistance to third-generation cephalosporin among *E. coli* isolates were 100% from river Cauvery in Karnataka (Skariyachan et al. 2015), 100% from Hyderabad (South India) (Lübbert et al. 2017), 7% from Kashmir (North India) (Rather et al. 2012), 17% from Faizabad (North-Central India) (Kumar et al. 2013), 52% from Aligarh (North-Central India) (Shahid et al. 2014), 50% from Sikkim (East India) (Poonia et al. 2014), and 44% from Ujjain (central India) (Akiba et al. 2016).

Antimicrobial resistance among environmental isolates has been reported all around the world. A very high (78.8%) resistance to cefoxitin and meropenem followed by 73.2% to cefoxitin, cefotaxime, and 72% to piperacillin/Tazobactam, respectively was reported from Colombia. Also, at least one type of *bla* gene was detected (75% and 88.4%) in the dry and wet seasons, respectively, among all the isolates identified. On genotyping which were found to be (49.4%) *bla*VIM-2, (45.2%) *bla*TEM-1, (22.9%) *bla*IMP-1, (20.5%) bla_{ampC}, (14.5%) *bla*CTXM-9, respectively (Chavez et al. 2019).

A study from Ethiopia reported 9.4% as overall prevalence of ESBL-producing *Enterobacteriaceae* in drinking water (Abera et al. 2016). Treated wastewater from 12 different treatment plants in Tokyo, Japan showed 5.7% resistance to cefotaxime and 5.3% of ESBL-producing *E. coli* in total E. coli isolates (Urase et al. 2020).

Factors responsible for development of antimicrobial resistance in environment

1. India hosting a large pharmaceutical Industry (Rehman et al. 2015). Antibiotic residue contaminating the environment via industrial wastewater is a potential source of development of antimicrobial resistance (Ashbolt et al. 2013; Taneja and Sharma 2019).
2. Treatment of the Municipal wastewater is not effective enough to eliminate the resistant organisms. These wastewater on reaching the nearby water bodies contaminate them with antimicrobial resistant organisms (Lundborg and Tamhankar 2017; Taneja and Sharma 2019).
3. Hospital effluents carrying patient secretions or discarded unused medications are important source of generation of antimicrobial waste (Mutiyar and Mittal 2014; Taneja and Sharma 2019). It's a greater threat in India as only less than 45% of health care facilities have adequate wastewater treatment (WHO and UNCF 2015).
4. Animal excreta mainly from food-producing animals like cattle, poultry, pig etc. can contaminate the environment either with antimicrobial resistant bacteria directly or with presence of antimicrobial residue (Taneja and Sharma 2019; Wichmann et al. 2014).
5. Agricultural manure and sludge rich microorganism and undegraded antimicrobials when used in farms can provide an environment of development and transfer of resistant genes among bacteria (McClellan and Halden 2010; Taneja and Sharma 2019).

6. Sea food industry—irrational/overuse of antimicrobials in farmed sea food is an emerging source of antimicrobial resistance in the environment (Taneja and Sharma 2019).

14.3 Food-Producing Animals and Antimicrobial Resistance

Although for sustainable production of food-producing animals and for the control of infections in animals that can affect humans also, antimicrobials have proved to be critical, however irrational use has also lead to concern of antimicrobial resistance (World Health Organization Division of Emerging and other Communicable Diseases Surveillance and Control 1997).

Ideally, antimicrobial susceptibility test should accompany the therapeutic use of antimicrobials in animal husbandry. However, in the presence of clinical symptoms of infection in few animals, the whole flock is often treated to prevent the dissemination of illness in the flock for infectious diseases. This is termed as METAPHYLAXIS, in which usually high doses of antibiotics are administered for a short duration. However there is no clear cut red line between use of antibiotics for treatment or prevention ("Antibiotics in Animal Farming" 2011; Center for Veterinary Medicine and FDA 2012).

However, this surplus and uncontrolled use of antibiotics in animals lead to serious consequences on public health, via spreading of antibiotic resistant strains of nonpathogenic and pathogenic bacteria into the surrounding habitat and in turn leading to their further transmission to humans via food chain (Apata 2009).

14.3.1 Antimicrobial Consumption in Food Animals

Rise in demand of food animals worldwide has also led to rise in use of antimicrobials in food animals worldwide. Due to low productivity of livestock animals, protests and riots took place in America in 1910, which in turn led to start of antimicrobials use in animal production (Ogle 2013). Use of antimicrobials enabled the production of more meat at a relatively cheaper cost (Dibner and Richards 2005).

However, antibiotic usage was banned in some countries as global threat of antibiotic resistance and treatment failure increased day by day. Sweden was the first country to implement the ban followed by Denmark, the Netherlands, the United Kingdom, and other European Union countries (Castanon 2007; Choct 2001; Cogliani et al. 2011). To combat the problem of antimicrobial resistance, structures have been set up to regulate the use of selected antibiotics in animal production and some classes of antibiotic have been withdrawn from several countries (Choct 2001).

Even after enforcing all the precautionary measures it is estimated that use of antibiotics in livestock production, including poultry, is over 60% of all antibiotics produced (Boeckel et al. 2014; Van Boeckel et al. 2015), as it is favorable for farmers and for economy of the country. According to Food and Drug Administration (FDA), Antimicrobial Usage in food animals in USA was estimated to account

for 80% of the total nations annual antimicrobial consumption (Center for Veterinary Medicine 2019).

Van Boeckel et al. in a global analysis of antimicrobial usage, estimated the consumption of antimicrobials in food animal production at ≥57,000 tonnes worldwide (1 tonne = 1000 kg) and projected a 67% increase in total usage by 2030 to ≥95,000 tonnes. Van Boeckel et al. projected that by 2030, the top countries consuming antimicrobials in livestock production will be China, the US, India, Brazil, and Germany, with 23% of global consumption by China only (Van Boeckel et al. 2015).

Almost every class of antimicrobials are used in livestock animals, even those antimicrobials which are abundantly required for human usage, like penicillins, cephalosporins, sulphonamides, tetracyclines, fluoroquinolones, and aminoglycosides (Marshall and Levy 2011; Schwarz et al. 2001; Silbergeld et al. 2008).

As antibiotics consumption are more in food animals than in humans, animal gut microbiomes have been shown to harbor higher amount and more diversified antibiotic resistance genes (Berendonk et al. 2015; Boeckel et al. 2019; Liu et al. 2016; Munk et al. 2018; Xiao et al. 2017).

14.3.2 Problem Statement in India

As per the 2015 data, India was at second in the production of fish, while leading in the production of milk worldwide. Further, a 577% enhancement is estimated between the year 2000 and 2030 in consumption of poultry in India. With such a huge potential of food animal industry, overuse of antimicrobial agents to increase the productivity is more likely (Taneja and Sharma 2019).

India accounts for 3% of global consumption of antibiotic use in poultry and cattle as estimated by a study in 2010. Along with China, the United States, Brazil, and Germany; India is among the top consumers of antibiotics use in animals. Van Boeckel et al. 2015 has further estimated that by 2030 there will be increase of about two-thirds consumption of antibiotic worldwide in the animals. In India, 82% increase in use of antibiotics in animal feed by 2030 is estimated. Penicillins, tetracyclines and quinolones are most widely used antibiotics globally in animal feeds (Van Boeckel et al. 2015).

Surplus use of antimicrobial in food animal in India have led to presence of antimicrobial residues in food animal products like chicken, meat, and milk (Basnyat 2014; Brower et al. 2017a; Kakkar and Rogawski 2013; Maron et al. 2013).

For estimation of AMR in livestock, samples of milk from cows and buffaloes of West Bengal were analyzed, and it was found that 48% of gram-negative bacilli isolated were extended-spectrum β-lactamases (ESBL) producers (Mesa et al. 2006a).

14.3.3 Antibiotic Resistance Dissemination from Food-Producing Animals to the Environment

The potential pathways for transmission of antimicrobial resistance from dairy farm environment to humans have been identified but they are complex in nature (Collis et al. 2018). Some of the theoretical pathways for AMR transmission from dairy cattle and their environment to humans are through food chain, feco-oral route, and clinical contact, between farmers and animals (Boerlin and Reid-Smith 2008). However, the actual contribution of each pathway is undetermined. Evidence of this transmission is still equivocal; however, poultry and swine seem to be more likely source compared to cattle (Lazarus et al. 2015), probably because of the routine use of antimicrobials in these production systems. Human to human transmission is also a possibility as ESBL-producing Enterobacteriaceae were also isolated from humans and human sewage (Mesa et al. 2006a).

Dahms et al. 2015 assessed risk of animal and human direct contact for the transmission of ESBL-producing Enterobacteriaceae indicated potential zoonotic transfer (Dahms et al. 2015). However, Wu et al. 2013 showed little similarity between ESBL and AmpC-producing *E. coli* from animal derived food products, animal gut microbiota and human clinical isolates (from Germany, Netherland, and the UK) using Multilocus sequence typing (MLST) and virulence and resistance gene microarrays, only 1.2% of animal isolates shared same MLST with human ones (Wu et al. 2013).

Also, a study done in North-Indian district region by Shahid et al. 2014, on clinical isolates and environmental samples (water, sewage, drain). On comparing the banding profile of the environmental and clinical isolates by RAPD typing, the isolates could not be genetically related as they showed the diversity in the banding profile (Shahid et al. 2014).

Karanika et al. 2016 in a systemic and meta-analytic review reported that no apparent association was found with animal contact. However, they also concluded that colonization rate of ESBL is increasing over time and healthy individuals are an important reservoir of ESBLs (Karanika et al. 2016).

Lazarus et al. 2015 in its systematic review studied whether food-producing animals (FPAs) are a source of extraintestinal expanded-spectrum cephalosporin-resistant Escherichia coli (ESCR-EC) infections in humans and found that six molecular epidemiology studies were in support while 17 did not support the whole bacterium transfer of resistance. Similarly, 13 molecular epidemiology studies were in support of transfer of resistance by mobile genetic elements, while two were not in support. Zoonotic transmission was backed up by four observational epidemiology studies. Overall, they concluded that FPAs are a source of origin for a proportion of human extraintestinal ESCR-EC infections, with special emphasis on Poultry being the most important culprit. However further investigation and large scale studies are required to determine the quantitative and geographical extent of the problem (Lazarus et al. 2015).

14.3.4 Prevalence of Beta-Lactamases in Food Animals

All food-producing animals have shown to be carrying ESBL/AmpC-producing isolates (Carattoli 2008a), and all kinds of meats sold at retail (Cohen Stuart et al. 2012; Leverstein-van Hall et al. 2011; Overdevest et al. 2011a) and in vegetables (Egea et al. 2011).

ESBL/AmpC-producing isolates from poultry and poultry meat are highly prevalent for quite some time now (Cohen Stuart et al. 2012; Dierikx et al. 2013; Mesa et al. 2006b; Smet et al. 2008). Isolates carrying similar ESBL genes have been found in clinical isolates in humans and isolates from broiler and broiler meat (Leverstein-van Hall et al. 2011; Overdevest et al. 2011a), moreover similar plasmid (mainly incI1) carrying ESBL genes are found in both broiler and human clinical isolates (Leverstein-van Hall et al. 2011). This suggests that contamination of broilers and broiler meat with ESBL/AmpC-producing isolates lead to human colonization resulting in human infection with ESBL/AmpC-producing pathogens. Broiler farms in Spain, Belgium, and the Netherlands have shown a strong evidence of ESBL/AmpC-producing *E. coli* shedding (Dierikx et al. 2013; Mesa et al. 2006b; Smet et al. 2008). Food-producing animals like broilers may pose a human health hazard due to the presence of ESBL/AmpC genes in commensal *E. coli* (Laube et al. 2013).

However, India being a developing countries, where sanitation standards are low and self-medication is quite high, and malpractices like inappropriate use of veterinary antibiotics and easy availability of over-the-counter drugs without prescription, these could be considered high source of antibiotic resistance (Falgenhauer et al. 2019; Mainda et al. 2015). But contrasting findings have been also seen in a recent review where developed countries like Netherland (77%) and Spain (84–93%) have shown high prevalence of ESBL/AmpC in E. coli among poultry meat products as compared to African countries (average 16.3%) (Alonso et al. 2017; Falgenhauer et al. 2019). It can be assumed that inter host transmission in rural areas of sub-Saharan Africa is more likely to happen as they are mainly agricultural based communities and people live in close contact with animals when compared to industrialized countries (Alonso et al. 2017; Falgenhauer et al. 2019). However, large scale studies are required to adequately address the bacterial transmission of poultry and human in this region (Alonso et al. 2017; Falgenhauer et al. 2019).

Kar et al. 2015 from Orissa showed 6% prevalence of ESBL-producing *E. coli* in food-producing animals (poultry and cattle) (Kar et al. 2015). However, Brower et al. 2017 from Punjab showed a very high prevalence of ESBL-producing *Enterobacteriaceae*. Also, they compared broiler farms and layers farms with layer farms further categorized into contracted and independent. They depicted prevalence of ESBL-producing *Enterobacteriaceae* was higher for broiler farms (87%) than layer farms (42%), and for contracted layer farms (49%) than independent layer farms (38%) (Brower et al. 2017b).

Kola et al. 2012 from Germany detected 43.9% ESBL *Enterobacteriaceae* predominantly *E. coli* from poultry meat samples (Kola et al. 2012). Friese et al. (2013) from Northern and eastern part of Germany, showed prevalence of ESBL

producers as 60% from cattle and 100% in broiler (Friese et al. 2013). Other studies also showed a high prevalence of ESBL-producing *E. coli* (93.3%, 94%) (Cohen Stuart et al. 2012; Egea et al. 2012). Gay Noellie et al. 2018 from Madagascar showed prevalence of ESBL-producing *Enterobacteriaceae* to be high (71%) in poultry than in cattle (46.2%) (Gay et al. 2018). Madec et al. 2008 from France showed a prevalence of ESBL-producing *Enterobacteriaceae* in fecal isolates as 6.2% in sick and 5.8% in healthy cattle (Madec et al. 2008). Schmidt et al. 2013 found ESBL-producing *E.coli* even on farms that did not use the antibiotics of these groups (Schmid et al. 2013). One possible reason could be that since the resistant determinants against cephalosporins, aminoglycosides, tetracycline and sulphonamide are often situated in same plasmid (Jacoby and Sutton 1991). Use of non β-lactam antibiotics can co-select for other ones.

14.3.5 Distribution of ESBL Types in Food-Producing Animals

Several studies has delineated the occurrence of ESBL/AmpC-producing *E. coli* and strains pertinent to human health from food-producing animals and companion animals (Overdevest et al. 2011b; Ewers et al. 2011; Hasman et al. 2005; Carattoli 2008b; Smet et al. 2010).

Briñas et al. (2003) was first to report CTX-M producing, SHV-12 producing and CMY-2 producing *E.coli* in healthy chicken from Spain between 2000 and 2001 (Briñas et al. 2003). Similarly Kojima et al. 2005 depicted CTX-M 14, CTX-M 2 and CMY-2 from healthy poultry in Japan (Kojima et al., 2005). Ewers et al. 2012 in its review article observed the prevalence of various ESBL/AmpC types ranging between 0.6% and 44.7% (studies mostly from European countries), from Asia it was 1.7% to 11.8% of ESBL/AmpC-producing E.coli and Salmonella species (Ewers et al. 2012). As we can see Asia had relatively low prevalence than Europe. They also depicted CTX-M 14 and CTX-M 15 to be the most common types regardless of the geographical origin. However European countries also showed high prevalence of CTX-M 1 which was rarely reported from other regions. In Asia, most prevalent type reported was CTX-M 14 (30–33%) in poultry and around (14%) in cattle (Ewers et al. 2012).

In contrast Schmid et al. 2013 showed a higher prevalence of $bla_{\text{CTX-M}}$ gene (93.4%) in isolates from cattle farms (Schmid et al. 2013). Madec et al. 2008 from farm showed presence of genes $bla_{\text{CTX-M}}$ highest followed by bla_{TEM} and bla_{SHV} in healthy cattle fecal isolates (Madec et al. 2008). Karanika et al. 2016 in a systematic and meta-analysis review depicted CTX-Ms were the prevalent ESBL enzyme (69%) (Karanika et al. 2016). Dandachi et al. 2018 from Lebanon reported that CTX-M type beta-lactamases followed by CMY-ampC type beta-lactamases are the most dominant genes in Labanese chickens (Dandachi et al. 2018). Hordijk et al. 2019 from dairy farms in Netherland noted that the most frequently observed ESBL/AmpC gene variants were $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTXM-32}}$ and $bla_{\text{CMY-2}}$. Less frequently observed gene variants were $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{TEM-52c}}$ and an inhibitor resistant TEM, $bla_{\text{TEM-79}}$ (Hordijk et al. 2019). The

most frequent genes associated with this resistance among livestock and companion animals encode various CTX-M enzymes, followed by $bla_{\text{TEM-52}}$ and $bla_{\text{SHV-12}}$; other TEM and SHV types are also observed (Ewers et al. 2011, 2012; Overdevest et al. 2011b; Smet et al. 2010).

Valentin et al. 2014 reported that more than 70% of the animal isolates and more than 50% of the human isolates contained the broadly distributed ESBL genes $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, or the combinations $bla_{\text{SHV-12}}$+ bla_{TEM} or $bla_{\text{CTX-M-1}}$+ bla_{TEM}. $bla_{\text{CTX-M-1}}$ was in majority seen in 37.5% of animal isolates and the combination $bla_{\text{CTX-M-1}}$+ bla_{TEM} was seen in 25.8% of isolates. However, majority of human isolates carried $bla_{\text{CTX-M-15}}$ (28.2%) and only 10.8% of the animal isolates had $bla_{\text{CTX-M-15}}$ gene (Valentin et al. 2014).

Valentin et al. 2014 showed presence of $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$ in animals (livestock + companion) and human isolates as 63.3% and 29.3%, 17.3% and 48%, 5.3% and 8.7%, respectively. They also depicted resistant rates in isolates from livestock animals were below that from human isolates but among livestock, cattle isolates yielded the highest resistance rates. Valentin et al. 2014 showed that $bla_{\text{CTX-M-1}}$ was the dominating ESBL gene in German cattle and pig feces (Valentin et al. 2014).

Falgenhauer et al. (2019) from Ghana depicted $bla_{\text{CTX-M-15}}$ as the most predominant ESBL-producing genotype both in broiler (96%) and human (97%), while *bla*SHV-12 was exclusively found in broiler and $bla_{\text{CTX-M-14}}$ in human isolates (Falgenhauer et al. 2019). Alonso et al. 2017 from Africa showed predominance of $bla_{\text{CTX-M-15}}$ in poultry fecal isolates (Alonso et al. 2017).

Valentin et al. 2014 also found that $bla_{\text{CTX-M-15}}$ is one of the most frequent ESBL type causing human infection (Valentin et al. 2014). $bla_{\text{CTX-M-15}}$ is mainly identified gene in human both in hospital and community, its presence in poultry and cattle probably indicate a common past source of contamination with introduction of ESBL-producing Enterobacteriaceae carriers and diffusion due to close contact in livestock.

14.3.6 Action Plan to Control the Menace of Antibiotic Resistance

Various international organizations, such as the World Health Organization (WHO), Infectious Diseases Society of America (IDSA), Centers for Disease Control and Prevention (CDC), and World Economic Forum have already declared antibiotic resistance as "global public health concern." (Michael et al. 2014; Spellberg et al. 2016). The World Health Assembly have requested WHO to propose a global action plan to fight the antibiotic resistance problem (Hoffman et al. 2015). To combat AMR India has also framed its National Action Plan (NAP) (Govt of India (NAP-AMR) 2017). The National Health Policy 2017 highlights the problem of antimicrobial resistance and calls for a rapid standardization of guidelines regarding antibiotic use, limiting the use of antibiotics as over-the-counter medications, banning or restricting the use of antibiotics as growth promoters in animal livestock, and

pharmacovigilance including prescription audits inclusive of antibiotic usage—in the hospital and community.

References

Abayneh M, Tesfaw G, Woldemichael K, Yohannis M, Abdissa A (2019) Assessment of extended-spectrum β-lactamase (ESBLs) – producing Escherichia coli from minced meat of cattle and swab samples and hygienic status of meat retailer shops in Jimma town, Southwest Ethiopia. BMC Infect Dis 19:897. https://doi.org/10.1186/s12879-019-4554-6

Abera B, Kibret M, Mulu W (2016) Extended-spectrum beta (β)-lactamases and antibiogram in Enterobacteriaceae from clinical and drinking water sources from Bahir Dar City, Ethiopia. PLoS One 11:e0166519. https://doi.org/10.1371/journal.pone.0166519

Ahammad ZS, Sreekrishnan TR, Hands CL, Knapp CW, Graham DW (2014) Increased waterborne blaNDM-1 resistance gene abundances associated with seasonal human pilgrimages to the upper Ganges River. Environ Sci Technol 48:3014–3020. https://doi.org/10.1021/es405348h

Akiba M, Sekizuka T, Yamashita A, Kuroda M, Fujii Y, Murata M, Lee K, Joshua DI, Balakrishna K, Bairy I, Subramanian K, Krishnan P, Munuswamy N, Sinha RK, Iwata T, Kusumoto M, Guruge KS (2016) Distribution and relationships of antimicrobial resistance determinants among extended-spectrum-cephalosporin-resistant or carbapenem-resistant Escherichia coli isolates from rivers and sewage treatment plants in India. Antimicrob Agents Chemother 60:2972–2980. https://doi.org/10.1128/AAC.01950-15

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 8:251–259. https://doi.org/10.1038/nrmicro2312

Alonso CA, Zarazaga M, Sallem RB, Jouini A, Slama KB, Torres C (2017) Antibiotic resistance in Escherichia coli in husbandry animals: the African perspective. Lett Appl Microbiol 64:318–334. https://doi.org/10.1111/lam.12724

American Veterinary Medical Association (2008) One health - OHITF final report (2008) [WWW document]. Am Vet Med Assoc. https://www.avma.org/resources-tools/reports/one-health-ohitf-final-report-2008. Accessed 22 April 2021

Antibiotics in Animal Farming [WWW Document] 2011. https://www.ciwf.org.uk/research/food-and-human-health/antibiotics-in-animal-farming/. Accessed 23 April 2021

Apata DF (2009) Antibiotic resistance in poultry. Int J Poult Sci 8:404–408. https://doi.org/10.3923/ijps.2009.404.408

Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T, Lawrence JR, Larsson DGJ, McEwen SA, Ryan JJ, Schönfeld J, Silley P, Snape JR, Van den Eede C, Topp E (2013) Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect 121:993–1001. https://doi.org/10.1289/ehp.1206316

Bajaj P, Singh NS, Kanaujia PK, Virdi JS (2015) Distribution and molecular characterization of genes encoding CTX-M and AmpC β-lactamases in Escherichia coli isolated from an Indian urban aquatic environment. Sci Total Environ 505:350–356. https://doi.org/10.1016/j.scitotenv.2014.09.084

Basnyat B (2014) Antibiotic resistance needs global solutions. Lancet Infect Dis 14:549–550. https://doi.org/10.1016/S1473-3099(14)70800-X

Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons M-N, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13:310–317. https://doi.org/10.1038/nrmicro3439

Boeckel TPV, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis 14:742–750. https://doi.org/10.1016/S1473-3099(14)70780-7

Boeckel TPV, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Gilbert M, Bonhoeffer S, Laxminarayan R (2019) Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science 365. https://doi.org/10.1126/science.aaw1944

Boerlin P, Reid-Smith RJ (2008) Antimicrobial resistance: its emergence and transmission. Anim Health Res Rev 9:115–126. https://doi.org/10.1017/S146625230800159X

Bréchet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P, Guyeux C, Hocquet D, Bertrand X (2014) Wastewater treatment plants release large amounts of extended-spectrum β-lactamase-producing Escherichia coli into the environment. Clin Infect Dis 58:1658–1665. https://doi.org/10.1093/cid/ciu190

Briñas L, Moreno MA, Zarazaga M, Porrero C, Sáenz Y, García M, Dominguez L, Torres C (2003) Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in Escherichia coli fecal-sample isolates from healthy chickens. Antimicrob Agents Chemother 47:2056–2058. https://doi.org/10.1128/AAC.47.6.2056-2058.2003

Brower CH, Mandal S, Hayer S, Sran M, Zehra A, Patel SJ, Kaur R, Chatterjee L, Mishra S, Das BR, Singh P, Singh R, Gill JPS, Laxminarayan R (2017a) The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant Escherichia Coli in poultry chickens and variation according to farming practices in Punjab, India. Environ Health Perspect 125. https://doi.org/10.1289/EHP292

Brower CH, Mandal S, Hayer S, Sran M, Zehra A, Patel SJ, Kaur R, Chatterjee L, Mishra S, Das BR, Singh P, Singh R, Gill JPS, Laxminarayan R (2017b) The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant Escherichia coli in poultry chickens and variation according to farming practices in Punjab, India. Environ Health Perspect 125:077015. https://doi.org/10.1289/EHP292

Carattoli A (2008a) Animal reservoirs for extended spectrum β-lactamase producers. Clin Microbiol Infect 14:117–123. https://doi.org/10.1111/j.1469-0691.2007.01851.x

Carattoli A (2008b) Animal reservoirs for extended spectrum beta-lactamase producers. Clin Microbiol Infect 14(suppl 1):117–123. https://doi.org/10.1111/j.1469-0691.2007.01851.x

Castanon JIR (2007) History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci 86:2466–2471. https://doi.org/10.3382/ps.2007-00249

Center for Veterinary Medicine (2019) FDA releases annual summary report on antimicrobials sold or distributed in 2017 for use in food-producing animals showing declines for past two years. FDA

Center for Veterinary Medicine, FDA (2012) The judicious use of medically important antimicrobial drugs in food-producing animals, CVM GFI #209 [WWW Document]. US Food Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-209-judicious-use-medically-important-antimicrobial-drugs-food-producing-animals. Accessed 23 April 2021

Chandran SP, Diwan V, Tamhankar AJ, Joseph BV, Rosales-Klintz S, Mundayoor S, Lundborg CS, Macaden R (2014) Detection of carbapenem resistance genes and cephalosporin, and quinolone resistance genes along with oqxAB gene in Escherichia coli in hospital wastewater: a matter of concern. J Appl Microbiol 117:984–995. https://doi.org/10.1111/jam.12591

Chavez MV, Caicedo LD, Castillo JE (2019) Occurrence of β-lactamase-producing gram-negative bacterial isolates in water sources in Cali City, Colombia. Int J Microbiol 2019:1375060. https://doi.org/10.1155/2019/1375060

Choct M (2001) Alternatives to in-feed antibiotics in monogastric animal industry. ASA Tech Bull 30:1–7

Cogliani C, Goossens H, Greko C (2011) Restricting antimicrobial use in food animals: lessons from Europe. Microbe Mag 6:274–279. https://doi.org/10.1128/microbe.6.274.1

Cohen Stuart J, van den Munckhof T, Voets G, Scharringa J, Fluit A, Hall ML-V (2012) Comparison of ESBL contamination in organic and conventional retail chicken meat. Int J Food Microbiol 154:212–214. https://doi.org/10.1016/j.ijfoodmicro.2011.12.034

Collis RM, Burgess SA, Biggs PJ, Midwinter AC, French NP, Toombs-Ruane L, Cookson AL (2018) Extended-spectrum beta-lactamase-producing Enterobacteriaceae in dairy farm environments: a New Zealand perspective. Foodborne Pathog Dis 16:5–22. https://doi.org/10.1089/fpd.2018.2524

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377. https://doi.org/10.1126/science.1120800

Dahms C, Hübner N-O, Kossow A, Mellmann A, Dittmann K, Kramer A (2015) Occurrence of ESBL-producing Escherichia coli in livestock and farm workers in Mecklenburg-Western Pomerania. Germany PLoS One 10. https://doi.org/10.1371/journal.pone.0143326

Dandachi I, Sokhn ES, Dahdouh EA, Azar E, El-Bazzal B, Rolain J-M, Daoud Z (2018) Prevalence and characterization of multi-drug-resistant gram-negative bacilli isolated from Lebanese poultry: a Nationwide study. Front Microbiol 9:550. https://doi.org/10.3389/fmicb.2018.00550

Dibner JJ, Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634–643. https://doi.org/10.1093/ps/84.4.634

Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D (2013) Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing Escherichia coli in Dutch broilers and broiler farmers. J Antimicrob Chemother 68:60–67. https://doi.org/10.1093/jac/dks349

Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, Iyer RV, Sundblad-Tonderski K, Stålsby- Lundborg C (2010) Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Public Health 10:414. https://doi.org/10.1186/1471-2458-10-414

Egea P, López-Cerero L, Navarro MD, Rodríguez-Baño J, Pascual A (2011) Assessment of the presence of extended-spectrum beta-lactamase-producing Escherichia coli in eggshells and ready-to-eat products. Eur J Clin Microbiol Infect Dis 30:1045. https://doi.org/10.1007/s10096-011-1168-3

Egea P, López-Cerero L, Torres E, del Carmen Gómez-Sánchez M, Serrano L, Navarro Sánchez-Ortiz MD, Rodriguez-Baño J, Pascual A (2012) Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing Escherichia coli in the south of Spain. Int J Food Microbiol 159:69–73. https://doi.org/10.1016/j.ijfoodmicro.2012.08.002

Ewers C, Grobbel M, Bethe A, Wieler LH, Guenther S (2011) Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted! Berl Munch Tierarztl Wochenschr 124:94–101

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH (2012) Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18:646–655. https://doi.org/10.1111/j.1469-0691.2012.03850.x

Falgenhauer L, Imirzalioglu C, Oppong K, Akenten CW, Hogan B, Krumkamp R, Poppert S, Levermann V, Schwengers O, Sarpong N, Owusu-Dabo E, May J, Eibach D (2019) Detection and characterization of ESBL-producing Escherichia coli from humans and poultry in Ghana. Front Microbiol 9:3358. https://doi.org/10.3389/fmicb.2018.03358

Friese A, Schulz J, Laube H, von Salviati C, Hartung J, Roesler U (2013) Faecal occurrence and emissions of livestock-associated methicillin-resistant Staphylococcus aureus (laMRSA) and ESbl/AmpC-producing E. coli from animal farms in Germany. Berl Munch Tierarztl Wochenschr 126:175–180

Gay N, Leclaire A, Laval M, Miltgen G, Jégo M, Stéphane R, Jaubert J, Belmonte O, Cardinale E (2018) Risk factors of extended-spectrum β-lactamase producing Enterobacteriaceae occurrence in farms in Reunion, Madagascar and Mayotte Islands, 2016–2017. Vet Sci 5:22. https://doi.org/10.3390/vetsci5010022

Government of India (2017) National action plan on antimicrobial resistance (NAP-AMR) 2017–2021. http://www.searo.who.int/india/topics/antimicrobial_ esistance/nap_amr.pdf

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM (2005) Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant salmonella from poultry, poultry products and human patients in the Netherlands. J Antimicrob Chemother 56:115–121. https://doi.org/10.1093/jac/dki190

Hoffman SJ, Caleo GM, Daulaire N, Elbe S, Matsoso P, Mossialos E, Rizvi Z, Røttingen J-A (2015) Strategies for achieving global collective action on antimicrobial resistance. Bull World Health Organ 93:867–876. https://doi.org/10.2471/BLT.15.153171

Hordijk J, Fischer EAJ, van Werven T, Sietsma S, Van Gompel L, Timmerman AJ, Spaninks MP, Heederik DJJ, Nielen M, Wagenaar JA, Stegeman A (2019) Dynamics of faecal shedding of ESBL- or AmpC-producing Escherichia coli on dairy farms. J Antimicrob Chemother 74:1531–1538. https://doi.org/10.1093/jac/dkz035

Jacoby GA, Sutton L (1991) Properties of plasmids responsible for production of extended-spectrum beta-lactamases. Antimicrob Agents Chemother 35:164–169

Kakkar M, Rogawski L (2013) Antibiotic use and residues in chicken meat and milk samples from Karnataka and Punjab, India. Research Scheme 34. New Delhi Public Health Foundation

Kar D, Bandyopadhyay S, Bhattacharyya D, Samanta I, Mahanti A, Nanda PK, Mondal B, Dandapat P, Das AK, Dutta TK, Bandyopadhyay S, Singh RK (2015) Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing Escherichia coli isolated from poultry and cattle in Odisha, India. Infect Genet Evol 29:82–90. https://doi.org/10.1016/j.meegid.2014.11.003

Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E (2016) Fecal colonization with extended-spectrum beta-lactamase–producing Enterobacteriaceae and risk factors among healthy individuals: a systematic review and metaanalysis. Clin Infect Dis 63:310–318. https://doi.org/10.1093/cid/ciw283

Kluytmans JAJW, Overdevest ITMA, Willemsen I, Kluytmans-van den Bergh MFQ, van der Zwaluw K, Heck M, Rijnsburger M, Vandenbroucke-Grauls CMJE, Savelkoul PHM, Johnston BD, Gordon D, Johnson JR (2013) Extended-spectrum β-lactamase-producing Escherichia coli from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clin Infect Dis 56:478–487. https://doi.org/10.1093/cid/cis929

Kojima A, Ishii Y, Ishihara K, Esaki H, Asai T, Oda C, Tamura Y, Takahashi T, Yamaguchi K (2005) Extended-spectrum-β-lactamase-producing Escherichia coli strains isolated from farm animals from 1999 to 2002: report from the Japanese veterinary antimicrobial resistance monitoring program. Antimicrob Agents Chemother 49:3533–3537. https://doi.org/10.1128/AAC.49.8.3533-3537.2005

Kola A, Kohler C, Pfeifer Y, Schwab F, Kühn K, Schulz K, Balau V, Breitbach K, Bast A, Witte W, Gastmeier P, Steinmetz I (2012) High prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany. J Antimicrob Chemother 67:2631–2634. https://doi.org/10.1093/jac/dks295

Kumar S, Tripathi VR, Garg SK (2013) Antibiotic resistance and genetic diversity in water-borne Enterobacteriaceae isolates from recreational and drinking water sources. Int J Environ Sci Technol 10:789–798. https://doi.org/10.1007/s13762-012-0126-7

Laube H, Friese A, von Salviati C, Guerra B, Käsbohrer A, Kreienbrock L, Roesler U (2013) Longitudinal monitoring of extended-spectrum-beta-lactamase/AmpC-producing Escherichia coli at German broiler chicken fattening farms. Appl Environ Microbiol 79:4815–4820. https://doi.org/10.1128/AEM.00856-13

Lazarus B, Paterson DL, Mollinger JL, Rogers BA (2015) Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin Infect Dis 60:439–452. https://doi.org/10.1093/cid/ciu785

Leverstein-van Hall MA, Dierikx CM, Stuart JC, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJM, Mevius DJ (2011) Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17:873–880. https://doi.org/10.1111/j.1469-0691.2011.03497.x

Liu H, Fu M, Jin X, Shang Y, Shindell D, Faluvegi G, Shindell C, He K (2016) Health and climate impacts of ocean-going vessels in East Asia. Nat Clim Chang 6:1037–1041. https://doi.org/10.1038/nclimate3083

Lübbert C, Baars C, Dayakar A, Lippmann N, Rodloff AC, Kinzig M, Sörgel F (2017) Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens. Infection 45:479–491. https://doi.org/10.1007/s15010-017-1007-2

Lundborg CS, Tamhankar AJ (2017) Antibiotic residues in the environment of South East Asia. BMJ 358:j2440. https://doi.org/10.1136/bmj.j2440

Madec J-Y, Lazizzera C, Châtre P, Meunier D, Martin S, Lepage G, Ménard M-F, Lebreton P, Rambaud T (2008) Prevalence of fecal carriage of acquired expanded-Spectrum cephalosporin resistance in Enterobacteriaceae strains from cattle in France. J Clin Microbiol 46:1566–1567. https://doi.org/10.1128/JCM.02299-07

Mainda G, Bessell PB, Muma JB, McAteer SP, Chase-Topping ME, Gibbons J, Stevens MP, Gally DL, de C Bronsvoort BM (2015) Prevalence and patterns of antimicrobial resistance among Escherichia coli isolated from Zambian dairy cattle across different production systems. Sci Rep 5:12439. https://doi.org/10.1038/srep12439

Maron DF, Smith TJ, Nachman KE (2013) Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Glob Health 9:48. https://doi.org/10.1186/1744-8603-9-48

Marshall BM, Ochieng DJ, Levy SB (2009) Commensals: underappreciated reservoirs of resistance. Microbe 4:231–238

Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24:718–733. https://doi.org/10.1128/CMR.00002-11

McClellan K, Halden RU (2010) Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA National Sewage Sludge Survey. Water Res 44:658–668. https://doi.org/10.1016/j.watres.2009.12.032

Mesa RJ, Blanc V, Blanch AR, Cortés P, González JJ, Lavilla S, Miró E, Muniesa M, Saco M, Tórtola MT, Mirelis B, Coll P, Llagostera M, Prats G, Navarro F (2006a) Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). J Antimicrob Chemother 58:211–215. https://doi.org/10.1093/jac/dkl211

Mesa RJ, Blanc V, Blanch AR, Cortés P, González JJ, Lavilla S, Miró E, Muniesa M, Saco M, Tórtola MT, Mirelis B, Coll P, Llagostera M, Prats G, Navarro F (2006b) Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). J Antimicrob Chemother 58:211–215. https://doi.org/10.1093/jac/dkl211

Michael CA, Dominey-Howes D, Labbate M (2014) The antimicrobial resistance crisis: causes, consequences, and management. Front Public Health 2:145. https://doi.org/10.3389/fpubh.2014.00145

Munk P, Knudsen BE, Lukjancenko O, Duarte ASR, Van Gompel L, Luiken REC, Smit LAM, Schmitt H, Garcia AD, Hansen RB, Petersen TN, Bossers A, Ruppé E, EFFORT Group, Lund O, Hald T, Pamp SJ, Vigre H, Heederik D, Wagenaar JA, Mevius D, Aarestrup FM (2018) Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat Microbiol 3:898–908. https://doi.org/10.1038/s41564-018-0192-9

Mutiyar PK, Mittal AK (2014) Risk assessment of antibiotic residues in different water matrices in India: key issues and challenges. Environ Sci Pollut Res Int 21:7723–7736. https://doi.org/10.1007/s11356-014-2702-5

Ogle M (2013) In meat we trust: an unexpected history of carnivore America. Houghton Mifflin Harcourt Publishing, New York

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J (2011a) Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands. Emerg Infect Dis 17:1216–1222. https://doi.org/10.3201/eid1707.110209

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J (2011b)

Extended-Spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands. Emerg Infect Dis 17:1216–1222. https://doi.org/10.3201/eid1707.110209

Poonia S, Singh TS, Tsering DC (2014) Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. Indian J Community Med 39:156–160. https://doi.org/10.4103/0970-0218.137152

Rather T, Hussain S, Bhat S, Nazir S, Arshid S, Shahnawaz M (2012) Antibiotic sensitivity of E. coli and Salmonella isolated from different water sources in Kashmir, India. Comp Clin Pathol 22. https://doi.org/10.1007/s00580-012-1474-x

Rehman MSU, Rashid N, Ashfaq M, Saif A, Ahmad N, Han J-I (2015) Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere 138:1045–1055. https://doi.org/10.1016/j.chemosphere.2013.02.036

Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525–552. https://doi.org/10.1146/annurev.genet.38.072902.091216

Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S, Laxminarayan R, Lubroth J, Magnusson U, Thi Ngoc P, Van Boeckel TP, Woolhouse MEJ (2016) Antibiotic resistance is the quintessential one health issue. Trans R Soc Trop Med Hyg 110:377–380. https://doi.org/10.1093/trstmh/trw048

Schmid A, Hörmansdorfer S, Messelhäusser U, Käsbohrer A, Sauter-Louis C, Mansfeld R (2013) Prevalence of extended-Spectrum β-lactamase-producing Escherichia coli on Bavarian dairy and beef cattle farms. Appl Environ Microbiol 79:3027–3032. https://doi.org/10.1128/AEM.00204-13

Schwarz S, Kehrenberg C, Walsh TR (2001) Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents 17:431–437. https://doi.org/10.1016/S0924-8579(01)00297-7

Shahid M, Khan H, Sobia F, Singh A, Khan F, Shah M, Shukla I, Malik A, Khan I (2014) Molecular epidemiology of various antibiotics resistance genes, including blaNDM-1, in Indian environmental and clinical bacterial isolates: a comparative study. N Z J Med Lab Sci 68:09–14

Silbergeld EK, Graham J, Price LB (2008) Industrial food animal production, antimicrobial resistance, and human health. Annu Rev Public Health 29:151–169. https://doi.org/10.1146/annurev.publhealth.29.020907.090904

Skariyachan S, Mahajanakatti AB, Grandhi NJ, Prasanna A, Sen B, Sharma N, Vasist KS, Narayanappa R (2015) Environmental monitoring of bacterial contamination and antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River, a major drinking water source in Karnataka, India. Environ Monit Assess 187:279. https://doi.org/10.1007/s10661-015-4488-4

Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P (2008) Diversity of extended-spectrum β-lactamases and class C β-lactamases among cloacal Escherichia coli isolates in Belgian broiler farms. Antimicrob Agents Chemother 52:1238–1243. https://doi.org/10.1128/AAC.01285-07

Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L, Haesebrouck F, Butaye P (2010) Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiol Rev 34:295–316. https://doi.org/10.1111/j.1574-6976.2009.00198.x

Spellberg B, Srinivasan A, Chambers HF (2016) New societal approaches to empowering antibiotic stewardship. JAMA 315:1229–1230. https://doi.org/10.1001/jama.2016.1346

Taneja N, Sharma M (2019) Antimicrobial resistance in the environment: the Indian scenario. Indian J Med Res 149:119. https://doi.org/10.4103/ijmr.IJMR_331_18

Urase T, Okazaki M, Tsutsui H (2020) Prevalence of ESBL-producing Escherichia coli and carbapenem-resistant Enterobacteriaceae in treated wastewater: a comparison with nosocomial infection surveillance. J Water Health 18:899–910. https://doi.org/10.2166/wh.2020.014

Valentin L, Sharp H, Hille K, Seibt U, Fischer J, Pfeifer Y, Michael GB, Nickel S, Schmiedel J, Falgenhauer L, Friese A, Bauerfeind R, Roesler U, Imirzalioglu C, Chakraborty T, Helmuth R,

Valenza G, Werner G, Schwarz S, Guerra B, Appel B, Kreienbrock L, Käsbohrer A (2014) Subgrouping of ESBL-producing Escherichia coli from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. Int J Med Microbiol 304:805–816. https://doi.org/10.1016/j.ijmm.2014.07.015

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R (2015) Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 112:5649–5654. https://doi.org/10.1073/pnas.1503141112

Walsh TR, Weeks J, Livermore DM, Toleman MA (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 11:355–362. https://doi.org/10.1016/S1473-3099(11)70059-7

WHO, UNCF (2015) Water, sanitation and hygiene in health care facilities status in low- and middle-income countries and way forward 2015. WHO, Switzerland

Wichmann F, Udikovic-Kolic N, Andrew S, Handelsman J (2014) Diverse antibiotic resistance genes in dairy cow manure. mBio 5:e01017. https://doi.org/10.1128/mBio.01017-13

World Health Organization Division of Emerging and other Communicable Diseases Surveillance and Control (1997) The medical impact of the use of antimicrobials in food animals: report of a WHO meeting, Berlin, Germany, 13–17 Oct 1997

Wu G, Day MJ, Mafura MT, Nunez-Garcia J, Fenner JJ, Sharma M, van Essen-Zandbergen A, Rodríguez I, Dierikx C, Kadlec K, Schink A-K, Wain J, Helmuth R, Guerra B, Schwarz S, Threlfall J, Woodward MJ, Woodford N, Coldham N, Mevius D (2013) Comparative analysis of ESBL-positive Escherichia coli isolates from animals and humans from the UK, The Netherlands and Germany. PLoS One 8:e75392. https://doi.org/10.1371/journal.pone.0075392

Xiao Z-N, Li D-L, Zhou L-M, Zhao L, Huo W-J (2017) Interdisciplinary studies of solar activity and climate change. Atmospheric Ocean Sci Lett 10:325–328. https://doi.org/10.1080/16742834.2017.1321951

Environmental Biofilms as Reservoir of Antibiotic Resistance and Hotspot for Genetic Exchange in Bacteria

15

Iqbal Ahmad, Shirjeel Ahmad Siddiqui, Samreen, Kirti Suman, and Faizan Abul Qais

Abstract

The global emergence and spread of antimicrobial resistance (AMR) within bacterial species have reached an alarming stage. These drug resistant bacteria are often resistant to variety of antimicrobial drugs and hence, referred to as multidrug resistant (MDR). The problem from drug resistant bacteria is not only the matter of concern for human health but also to the environment and food industry. The development of AMR is favored by the biofilms. Bacteria conventionally inhabit as communities usually attached to solid surface. Once attached, they establish themselves to form biofilms that provide protection from environmental threats thereby acting as an extremely beneficial survival strategy. Bacterial species exhibit distinct features in biofilms that are not present in a planktonic state among which enhanced tolerance to antibiotics is a very prominent phenomenon. Biofilms through horizontal gene transfer leads to the enhanced spread of antibiotic resistance genes including to β-lactam antibiotics and development of resistance among bacterial populations have been documented. The biofilms formed in environment are unique in diversity and exchange of genetic material coding for resistance and other straits with in participating compatible microbial populations becomes easier. In bacterial biofilms, the emergence and spread of antibiotic resistance whether through horizontal transfer of genes or mutations, is a serious threat to public health that requires rapid attention. In this chapter, the above aspects of biofilms have been reviewed in the light of current understanding on biofilms as hotspot for gene exchange with special reference to β-lactam antibiotics.

I. Ahmad (✉) · S. A. Siddiqui · Samreen · K. Suman · F. A. Qais
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_15

Keywords

Biofilms · Antibiotics · β-lactam antibiotics · Antimicrobial resistance · Horizontal gene transfer

15.1 Introduction

The continuous rise in emergence and transmission of multiple drug resistant bacteria has created immense clinical issue in the management of bacterial infections. In the treatment of bacterial infections, there is a clinical issue. It takes a long time to develop new antibiotics with novel modes of action (Ahmad et al. 2019). Common community acquired infections which were earlier treatable by antibiotics have now become unresponsive to available recommended drugs resulting in threat to human health and well-being. Patients receiving chemotherapy for cancer treatment, organ transplants and other advanced therapies are particularly more susceptible to such infections (Ahmad and Aqil 2008). In the last few decades rise in nosocomial infections caused by many problematic MDR bacteria belonging from the group ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) has recently attracted worldwide attention (Ma et al. 2020). In additional, drug resistance has also grown among veterinary pathogens, notably those related from poultry production and livestock farming. For example, *Campylobacter* spp. and *Salmonella enterica* are major zoonotic pathogen of concern (Uruén et al. 2020). Because of drug resistant bacterial infections, 700,000 people die each year globally which may surpass to ten million deaths worldwide by 2050 (O'Neill 2016; Aslam et al. 2018). The resistance emergence of quinolones, carbapenems and third generation of cephalosporin antibiotics in community has become a major cause of concern (Colpan et al. 2013).

Besides the well-established mechanisms of antibiotic resistance and horizontal transfer of genes for the transmission between closely or distantly related bacterial species, other strategy such as biofilm development further enhanced the resistance capacity to many folds to evade the action of antimicrobial drugs (Uruén et al. 2020). The formation of biofilm is an ancient practice for the adaptation of bacterial species that directly leads to the survival of the bacterium because of their recalcitrance to antimicrobial treatments (Jorge et al. 2019). Many mechanisms, including stress responses, metabolic heterogeneity, efflux pump regulation, antibiotic entrapment and inactivation in extracellular matrix, increased mutability, and inter-bacterial communication are involved in biofilm recalcitrance, which lead to a rise in the number of antibiotic resistant strains (Jorge et al. 2019). Horizontal gene transfer and or hypermutability are more favorable within the biofilms and therefore are known as storehouse of antibiotic resistant genes (Olsen 2015; Maheshwari et al. 2017). Biofilm engaged in a wide spectrum of infections, the most of which result in high death and morbidity rates, notably those caused by gram-negative bacteria (Jorge et al. 2012). Bacterial biofilms are known to be shown extreme resistant toward

antibiotic therapy. Despite this, using antibiotics in microbial infections is the most effective method in curbing infections, antibiotic treatments have negligible effect on the established biofilms (Høiby et al. 2011). A considerable amount of data on environmental biofilms and its significance have been documented, however our understanding on the role of biofilms as reservoir of AMR and genetic exchange is poorly understood in the environmental context. Therefore, in this chapter, we have given the significance of biofilm as reservoir of the AMR especially in environmental settings, genetic exchange across bacterial species, transforming biofilms into antibiotic resistance hotspots with specific reference toward antibiotics including β-lactam drugs in gram-negative bacteria.

15.2 General Characteristics of Bacterial Biofilms

Bacteria may grow in virtually any environmental conditions and often get attached to the surfaces they grow upon. Biofilm development not only permits cells to survive in adverse conditions but also allow microorganisms to disperse from their clusters and colonize to new niches representing a protected way of lifestyle. This habitat enables microorganisms to communicate as functional unit to perform tasks that are not achievable when microorganisms are either outside of biofilms or in planktonic state (Ahmad et al. 2017). Formation of biofilm appears to be an ancient and basic feature of life cycles in many microbes, and it is required for survival in a variety of environmental settings (Hall-Stoodley et al. 2004). Bacteria can establish biofilms on various surfaces like riverbeds, soil, deep-sea vents, and within the plants and animal including humans. The cell aggregates formed embedded in self-produced ECM (extracellular matrix) is termed as a biofilm (Wimpenny and Gass 2000). Biofilm can be formed by single type of bacteria (mono-biofilm) or can be polymicrobial in nature (mixed biofilm) (O'Toole et al. 2000; Stoodley et al. 2002). When planktonic cells are subjected to stress conditions such antibiotic pressure, adverse environmental conditions, nutrient unavailability or exposure to heavy metals, organic compounds and other chemical entities etc., the process of biofilm formation is initiated via gene expression and regulation (Lopez et al. 2010). Factors like nutrient availability, oxygen concentration, the age of biofilm, heterogeneous microbial interactions, ECM aggregates, waste products accumulation, mechanical signals, signals of host cells, antimicrobial drugs, concentration of metal ions, etc. greatly influence biofilm formation (McDougald et al. 2012; Beauregard et al. 2013; Velmourougane et al. 2017). Biofilm formation and their inhibition or eradication are serious matter of concern in food, environment and biomedical fields (Islam et al. 2008; Cha et al. 2013). More than 80% of chronic infections are associated with biofilm-forming bacteria which leads to increased mortality and morbidity in humans besides elevated healthcare cost (Davies 2003; Fey 2010). Biofilm formation also takes place on medical equipment such as orthopedic prostheses, artificial heart valves, intravascular and urinary catheters, neurosurgical, cochlear, dentures, breast implants, ophthalmic devices, etc. (Jorge et al. 2012). Surface coatings, in addition to surface composition, can influence biofilm growth. The fibrin sheath that

promotes adherent growth mode across and into the air-deprived lumen of central venous catheters is composed of blood components (collagen, fibrin, fibronectin, laminin, and immunoglobulins) (CVC). Although results from in vitro and in vivo investigations on fibrin sheath-coated surfaces are inconclusive, reports of increased incidence of persistent bacteremia for select species indicate the biofilm formation related to fibrin coating (Jamal et al. 2018). Biofilms can cause infection in upper and lower respiratory tracts, ocular region, chronic wounds, urinary tracts, periodontitis, etc. (Wu et al. 2015).

15.2.1 Biogenesis of Biofilm

The biogenesis of bacterial biofilms relies on the interaction between the bacterial cells and substrate (Van Houdt and Michiels 2010). Biofilm formation on a solid surface is a sequential process that starts from reversible adherence or attachment of bacterial cells and then production of extracellular matrix takes places, enabling the bacteria to attach on a surface followed by its maturation and detachment (Bogino et al. 2013; Laganà et al. 2018). Broadly, the following major steps are involved in the biofilm development.

1. Attachment: The first and foremost stage in the biofilm formation involves adhering and binding of the cells leading to the attachment on the surface/substratum. The process of attachment is generally favored by filamentous fibers such as pili flagella or fimbriae, that arise from surface of bacterial cell (Jamal et al. 2018).
2. Growth and maturation: Once the cells start to attach, they begin to produce extrapolymeric substances which forms the biofilm matrix. These extrapolymeric substances are termed as extracellular matrix (ECM). ECM serves as glue and helps in increasing and stabilizing interbacterial interactions. The cells grow from the microcolonial structures and further mature into cell clusters (Donlan 2002).
3. Detachment and dispersal: After maturation of biofilms, planktonic cells disseminate from the biofilm structure in order to release the cells and form biofilms in other settings. The shelf life of any mature biofilm depends on the extent of nutrient availability in surrounding environment. Once the nutrients start to exhaust, the outermost layer of biofilms begins to release planktonic cells, escaping the biofilms that are capable of colonizing other surfaces. Detachment processes are further classified into two types: active and passive (Kaplan 2010). In active mechanism, there is nutrient unavailability or cell mass increases up to a limit which is facilitated the production of biofilm degrading enzymes. On contrary, passive detachment is mediated by external forces like fluid shear, surface tension or intervention by humans and other organisms. The schematic diagram of biofilm lifestyle is represented in Fig. 15.1.

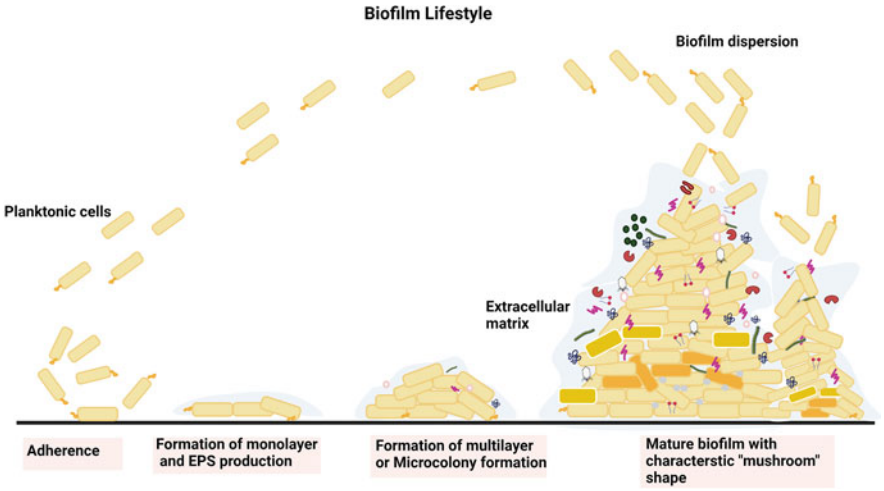


Fig. 15.1 Diagrammatic representation of different stages of biofilm formation

15.3 Evolution of Antimicrobial Resistance (AMR) in Biofilms

Despite the fact that the developmental phases leading to biofilm formation seems to be conserved across all bacterial species (or consortium of species in the case of polymicrobial biofilms). This secure network is capable of evading environmental hazards such as antimicrobial therapy and host defense mechanisms (Magana et al. 2018). Biofilms are made on numerous settings such as water treatment plants, microplastics rock surfaces, hot springs, and many others (Oberbeckmann et al. 2014; Besemer 2015; Michels et al. 2018). The antibiotic resistance genes such as for tetracycline tet(M), tet(S), genes for sulphonamide resistance sul1–sul3 and beta-lactam resistance genes such as *bla* NDM-1, *bla*OXA-32 moves and accumulate downstream of biofilms. A study performed by Sugimoto et al. (2017) documented the occurrence of floR, tet(B), tet(M), sul2, β-lactamase gene (blaCARB-9), and resistance toward macrolide genes mef(C) and mph(G) found in many aquatic habitats of Taiwan, Japan, and Thailand (Sugimoto et al. 2017). In another study biofilm-forming *Klebsiella pneumoniae and E. coli* isolated from cattle was found to express most frequently *bla*CTX-M gene followed by *bla*AmpC blaTEM1, *bla*CMY-6, *bla*OXA1, and *bla*PER. In addition, plasmid-mediated *qnrB*, *qnrS*, *qnrA*, and *qepA* were also detected in some bacterial isolates (Bandyopadhyay et al. 2021). Some findings of antibiotic resistance and their associated genes found in biofilms of different environmental settings is presented in Table 15.1.

AMR has been referred to as the ability of microorganism to sustain and survive at elevated antibiotic concentrations for prolonged periods, and is quantified by determining minimum inhibitory concentration (Kidd et al. 2018). AMR is one of biggest challenges that world is facing present time. In last two decades, emergence

Table 15.1 Major findings of antibiotic resistance and their associated genes found in biofilms of different environmental settings

S. No.	Established biofilm	Organism	Target ARGs	Main findings	References
1	Wastewater of burn Centre	*Pseudomonas aeruginosa*	*ps1A*	*pslA* associated with EPS production distributed among biofilm-producing isolates, linked to biofilm formation.	Emami et al. (2015)
2	Ground water	Multi-species biofilm	*Int1*	Integrons abundance was increased on the treatment of streptomycin and oxytetracycline	Huyan et al. (2020)
3	Urinary catheters	Mixed species, single biofilm	*Van*	*E. faecalis* cells expressing higher level of virulence expression and biofilm formation	Cui et al. (2020)
4	Hospital wastewater effluent	Enterobacteriaceae, *Acinetobacter baumannii,*, *P. aeruginosa*	bla_{IMP-1}, bla_{OXA-23}, bla_{oxa-48} and *NDM-1* $bla_{CTX-M-8}$ bla_{SFC-1}, bla_{VIM-1}, and bla_{VIM-13}	Relative occurrence of target genes was significantly higher in biofilm samples collected from downstream discharge point	Karkman et al. (2018), Khan (2020)
5	Lake	*E. coli, enterococcus* sp.	*bla*TEM, *aadA, tetA, cmlA, vacA*	Isolated biofilm matrices showed long lasting source of target genes	Eckert et al. (2018)
6	Veterinary hospitals	*S. aureus*	*clfA*, *clfB*, *fnbA,* and *sdC*	All the target genes are amplified from veterinary wastewater biofilms	Chen et al. (2020)
7	Yangtze Estuary	Mixed species	*sul*1, *sul*2, *tet*A, and *tet*W	Target genes relatively more expressed in biofilms than sediments and water. Most of the genes were contributed by eDNA	Guo et al. (2018)

8	River Toce		*tet*A, *erm*B, bla_{CTXM}, *sul*2, *qnr*S		Di Cesare et al. (2017)
9	River bed	Mixed species biofilm	qnrA, qnrB, qnrS blaTEM, blaCTX−M, blaSHV	The relative abundance of target genes was significantly higher in biofilm formers as compared to non-biofilm cells isolated from downstream of river.	Marti et al. (2014)
10	Lake	*Aeromonas punctata, Aeromonas media, E. coli*	QRDR mutations (quinolone resistant determining region)	The qnrS genes was predominant among all the qnr genes in biofilm cell	Balcázar et al. (2015)
11	Poultry	*E. coli*	*afa, iha, papC, aatA*, aggR, *FimH, luxs*, and *ahp*	Corelation was found in adhesion encoding genes and biofilm formation in *E. coli* isolates	Wang et al. (2016b)
12	Clinical sample	*K. pneumoniae*	CPS (capsular polysaccharide genes) *treC*, *sugE*	Enhanced expression of target genes as compared to mutant cells	Wu et al. (2011)
13	Urban stream	*Mixed biofilm*	*tetW, sulI, sulI*	The biofilm communities showed enhanced expression of target genes in association with metal resistance	Roberto et al. (2019)
14	Clinical source	*P. aeruginosa*	*Pmr, arn* and *rpoS*, *spoT*, *relA*, *dksA*, *dinG*, *spuC*, *algR*, *pilH*, *ycgM*, and *pheA*	Expression of target genes provide tolerance to biofilm through development of persisters cells and expression of efflux pump	De Groote et al. (2009), Beaudoin et al. (2012), Ciofu and Tolker-Nielsen (2019)
15	Chicken and meat sample	*S. aureus*	*eb, seg, sei, sem, sen seo, and agr*	Increased expression of target enterotoxin genes in biofilm cells	Wang et al. (2018)

(continued)

Table 15.1 (continued)

S. No.	Established biofilm	Organism	Target ARGs	Main findings	References
16	Milk sample	*S. aureus*	*icaA*, *icaB*, *icaC*, *icaD*,*bap blaZ vanC*, *tetK*, tetL, and *msr*	Some of the isolates showed corelation with expression of biofilm-forming genes and antibiotic resistance genes	Bissong and Ateba (2020)
17	Pharmaceutical wastewater	*Xanthomonas*, *Bacteroides, Burkholderia*, *Porphyromonas*, *Nitrosomonas*, *lactobacillus*, and *Kinetoplastibacterium*	*tet*X, *aph, vanA*	Increased expression of vancomycin resistant genes in microbial communities of aerobic system while aminoglycosides and tetracycline genes expressed in anaerobic system	Tao et al. (2016)
18	Hospital effluents	*Aeromonas*, *Pseudomonas*, *Stenotrophomonas* and *Acinetobacter*	bla_{VIM}, bla_{OXA48}, bla_{GES}, bla_{NDM}	Biofilm of all isolates showed increased expression of carbapenem mase encoding gens	Ory et al. (2019)
19		Mixed species	*strA*, *sul*2, *tet*A, *str*B, bla_{CTX}	Biofilm and suspended growth showed similarities in target gene expression, composition, and mobilization trends	Petrovich et al. (2018)
20	Agricultural watershed	*E. coli*	*Amp, tetA, str*	Highest frequency of resistance was observed in the agriculturally impacted soil as compared to headwaters	Maal-Bared et al. (2013)

and spread of AMR among pathogenic bacteria has been a major cause of concern for public health (Roca et al. 2015; Ferri et al. 2017). The most evident cause of antimicrobial resistance emergence and transmission, is the inappropriate or abrupt use of antibiotics in both in clinical settings and food industry (Laxminarayan and Chaudhury 2016).

In the spread of AMR, the mobile genetic elements or HGT mechanism also play a crucial role. Such mobile genetic elements containing drug resistant genes are called R-plasmids (Carattoli 2013). There are no solid boundaries separating animal microbiota from human microbiota. The external environment is another source of resistance, both as a pathogen transmission pathway and as a repository of resistance genes. Many resistant genes from harmless environmental bacteria end up through horizontal transfer of genes in bacterial pathogens (Tacconelli et al. 2018). The principle of AMR is quite simple whenever antibiotic stress conditions are created in an environment, growth of resistant microorganisms is favored over the sensitive ones by the evolutionary mechanism (Rawson et al. 2016).

15.3.1 Antibiotic Resistance Mechanisms

A resistance mechanism interacts with the drug and prevents its action on the cell via different ways. These reactions can either occur within the cell or outside the cell if the enzymes are secreted. A change in the chemical composition or thickness of the bacterial cell envelope reduces the rate of diffusion of antibiotics into the cell. Sometimes the diffusion of drugs is completely inhibited by this mechanism. Additionally, cell membranes often contain drug dedicated or general pumps called Efflux pump whose function is to propel out the drug from cell. Other protective mechanisms may prevent drug accumulation by chemically targeting the drug designated enzymes either by modifying them or even by hydrolyzing the drug itself. The last line of defense can be avoiding the toxic effect of target binding by bypassing the need for the chemical reaction in which the target is involved, or even by changing the chemical composition of the cell (Yelin and Kishony 2018; Peterson and Kaur 2018).

Antimicrobial resistance offers additive advantage to the bacterium with a means of surviving in a hostile environment, which making bacterial chemotherapy more difficult (Ahmad 1993; Reygaert 2018). A principal mechanism for the rapid spread of antibiotic resistance genes through bacterial population is that such genes get collected on plasmid that are independently replicated within and passed on to bacterial cells and species. The global spread of resistance is attributed to plasmids that enable horizontal transfer of plasmid-borne genes. Resistance plasmids (which confer antibiotic resistance) are generally conjugative and migratory. Conjugative plasmids have a wide host range (no host limitation within the division) as well as a confined host range (shifting limited to a few related bacterial taxa) (Nonaka et al. 2018).

Genes acquired from the host chromosome by homologous recombination, integration, and excision are conjugated from donor to recipient cells. These

plasmid-encoded complexes assist the contributor by connecting to a potential recipient, resulting in the creation of secured connection, which is essential prior to DNA translocation. Plasmids that are unable to be relocated using this method are transferred to conjugative elements via transitory or stable fusions known as co-integrates. Plasmids also promote cell contact growth by producing microfibrillar external covering components that are influenced by pheromones (San Millan 2018).

Gram-negative bacteria are often linked to ARGs that pose a severe threat to human medicine. Genes encoding extended spectrum-lactamases are among them. These include genes coding for extended spectrum β-lactamases (ESßL) (e.g., CTX-M), carbapenemases enzymes (like, NDM, KPC, and OXA-58) as well as colistin resistance like MCR-1 (Li and Yan 2021). Many gram-negative bacteria have been shown to produce beta-lactamase, which gives them acquired resistance toward antibiotics of beta-lactam class. Genes for β-lactamases can be passed around on plasmids (plasmid mediated) or encoded in the chromosome of an organism (chromosomally mediated) (Zhou and Zhong 2015). Transferrable plasmid-mediated genes frequently encode for a variety of resistance mechanisms, allowing them to display resistance to many drugs (Bello-López et al. 2019). The Ambler and Bush-Jacoby classification schemes are the most used schemes for classification. The Bush-Jacoby classification divides enzymes into four categories based on molecular class and functional groups, whereas the Ambler system classifies enzymes into four type of groups depending upon genetic amino acid sequences and phenotypic features (Bush and Jacoby 2010). ESBLs are among the lactamases that have attracted the interest of scientific community in recent decades. ESBLs are plasmid-borne bacteria that can hydrolyze cephalosporin antibiotics belonging to third and fourth generation (oxyimino-cephalosporins) including monobactams, excluding cephamycins like cefoxitin or carbapenems like imipenem, meropenem, doripenem, and ertapenem. The two main schemes of ESBL evolution involves first the ability to obtain and integrate novel resistant genes from the ecological metagenome, as well as the selection of enzyme mutants with the potential to extend substrate from already abundantly accessible plasmid-mediated TEM and SHV type -lactamases (Lambert et al. 2011). Because of these characteristics, ESBL is by far the most effective for disseminating in the environment and in clinical settings. AMR traits are incorporated into mobilizable genetic elements, allowing for the uniform spread of the AMR trait pool across ecosystems in many sectors such as human medicine, veterinary medicine, and the environment (ter Kuile et al. 2016; Gay et al. 2017). A recent study carried out by (Darphorn et al. 2021) represented that isolated *E. coli* strain from meat sample possess large plasmids with multiple antibiotic resistant genes including extended spectrum beta-lactamase genes belonging to $bla_{\text{CTX-M-1}}$, $bla_{\text{CMY-2}}$, and bla_{TEM} family genes (Darphorn et al. 2021). The epidemiology of ESBL is complicated by a number of factors, including geographical locations such as country, hospitals, communities, and so on, as well as the capacity of mobile resistant elements to travel across the environment, water, and wild animals, and even from food animals to human. The situation was made worse by coselection with other resistances, particularly to aminoglycosides, fluoroquinolones, and sulfonamides. The emergence of widespread clones that

hide multiple beta-lactamases (ESBLs, metallo-beta-lactamases, or cephamycinases) at the same time, as well as new mechanisms of resistance to fluoroquinolones and aminoglycosides, highlights the importance of controlled and concise future surveillance studies (Schrijver et al. 2018).

The intricacy of AMR plasmids contributes to the problems that they cause. Plasmids are notoriously plastic, with regular DNA insertions, deletions, and rearrangements, as well as modifications to specific ARGs. The blaCTX-M gene, for example, is extremely variable, and the CTX-M family of ESßLs is routinely coded for by numerous plasmids, including pCTX (Bevan et al. 2017).

Approximately 207 variants of $bla_{\text{CTX-M}}$ have been identified indicated by the Beta-Lactamase database. Another example of firstly identified in 2016 plasmid-mediated ARG is the *mcr-1* gene, on a transmissible plasmid, pHNSHP45. Since then, *mcr-1* and its related variants have been identified on multiple plasmid backbones and host strains. Among them, of concern are isolates carrying colistin and carbapenem ARGs, as few treatment options would remain for infections caused by such bacteria (Powell et al. 2021). In addition to these examples, plasmids can carry a variety of other resistance genes, including *qnr* variants aac(6′)-*lb-cr* and plasmid-mediated efflux pump genes like *qepA* and *oqxAB* which impart decreased level of resistance toward antimicrobial. Therefore, research on ARGS should be taken into consideration as they have frequent mobilization and transmission between bacteria (Wang et al. 2016a).

Since 2007, the National Veterinary Research and Quarantine Service (NVRQS) of South Korea has discovered a high incidence of multidrug resistant *E. fergusonii* in fecal samples of clinically unwell pigs at their laboratory. Antibiotic resistance is widespread among these isolates. In addition to antibiotic resistance genes, plasmids may also bear important toxin genes that could be maintained and disseminated to a wide range of microbes, especially members of the *Enterobacteriaceae*, from farm animals that share common environmental niches (Zhou et al. 2017).

15.3.1.1 Role of Extracellular Matrix (ECM) in Antibiotic Resistance

The extracellular matrix (ECM), which is formed by the microorganism in the biofilm, is the immediate habitat for the biofilm bacteria (Dragoš and Kovács 2017). The main purpose of this matrix is to impart stability and protection to the microorganism against a variety of environmental challenges. The extra roles of ECM components such as proteins, exopolysaccharides, and eDNA go beyond providing structural support have been widely explored (Yin et al. 2019). Antibiotic effectiveness on biofilm-forming cells is influenced by component of ECM. Although individual components have been shown to contribute to antibiotic resistance evolution, the interaction of matrix as a whole contributes to enhanced antibiotic resistance (Hall and Mah 2017). The ECM plays a major role in the biofilm architecture, which in turn prevents the entry of antibiotics in the biofilm structure by making dispersions (Lopez et al. 2010). The passage of chemicals through biofilm isn't always consistent. Antibiotics can thus pass quickly across the biofilm's channels, although they may be trapped locally in cell aggregates (Kour et al. 2020). The passage of chemicals through biofilm is not always consistent.

Antibiotics can thus pass quickly across the channels of biofilm, although they may be trapped locally in cell aggregates (Donlan 2002). Antimicrobial activities further encourage the emergence of extracellular DNA (eDNA) within the matrix. For example, treatment with vancomycin increased the level of eDNA in *S. epidermidis* biofilms. The eDNA that has been released binds to positively charged antibiotics which inhibit it to reach the cells thereby hindering the activity of antimicrobials (Uruén et al. 2020).

15.3.1.2 Cell-to-Cell Communication in Biofilms (Quorum Sensing)

QS is a cell–cell communication mechanism in which microbes coordinate the expression of and certain set of genes. Bacteria secrete curtain signal molecules and senses its concentration in surroundings. These signal molecules are called as auto-inducers (AIs). In gram-negative bacteria, acyl-homoserine lactones (AHLs) are the signal molecules. The short peptides or oligopeptide are the AIs of gram-positive bacteria (Mukherjee and Bassler 2019). The QS system mediated by AHLs controls genes that are essential for facilitating colonization in a high-cell-density environment, such as biofilms. The biofilms development is also believed to be influenced by QS. Moreover, it has been reported that the biofilms are closely connected with quorum sensing (QS) of bacteria (Parsek and Greenberg 2005). QS signals are known to regulate conjugation, transformation, and phage induction. Experimentally, it was determined that the transfer of model RP4 plasmid was strongly affected by QS between specific bacteria in the biofilms and shown the existence of AHLs and their producers in BAC biofilm. The study also explored the molecular mechanisms that contribute to conjugative transfer of ARGs for mRNA expression levels of conjugative transfer related genes and further investigated the effects of AHL-secreting bacteria and several QSIs. The results revealed that AHL-secreting bacteria facilitated the dissemination of ARGs, while QSIs inhibited this process (Papenfort and Bassler 2016).

15.3.2 Biofilms as Reservoir of Genetic Diversity

Genetic diversity provides bacterial adaptation, evolution, and survival in adverse environments. Biofilms are considered a reservoir of huge genetic diversity. In biofilm the genetic modifications can contain small variations like single point mutations or large structural rearrangements of genomic regions as in deletions, insertions, duplications, inversions, translocations, etc. (Aminov 2011).

In addition, bacteria also obtain a significant proportion of their genetic diversity through lateral acquisition of gene sequences from distantly related organisms. HGT occurs at an increased frequency in biofilm compared to planktonic cells and is carried out via different mechanisms, named as conjugation (direct mechanism of gene transfer between cells), transformation (acquisition of DNA from the environment), and transduction (gene transfer between cells via bacteriophages) (Maheshwari et al. 2017; Abe et al. 2020).

Conjugation: Discovered in 1946 by Lederberg requires living donor cells and cell-to-cell contact between two cells for transfer of the DNA. Conjugation is carried out through a proteinaceous apparatus called as conjugation pilus, which connects the donor and recipient cells physically. Following the retraction of the pilus, close contact between the donor and the recipient enables transfer of DNA. Conjugation occurs more intensely in biofilms than in free-living bacteria because of the attachment of cells to the matrix and thus enhancing the proximity between them (Nesse and Simm 2018). Under laboratory conditions, the transfer of resistance genes for gentamicin and trimethoprim present on conjugative plasmid (pGO1) has been increased in *Staphylococcus aureus* biofilms leading to increment in transfer rates of up to ~16,000-fold as compared to planktonic cells. Under aquatic settings, the horizontal transmission of a conjugative plasmid (pKJK5) containing a tetA and trimethoprim resistance gene (dfrA1) into microplastic-localized biofilms composed of diverse species was demonstrated in lake water. Similarly, in in vitro biofilm experiment conjugation of a blaNDM-1 gene producing carbapenemase from Enterobacteriaceae into *P. aeruginosa* and *A. baumannii* resulted in inter-family transmission (Uruén et al. 2020).

Transformation: Historically, it was the first HGT mechanism identified by Griffith and the process of natural transformation involves i) exogenous DNA taken up by the competence machinery, which is made from of transformation pilus and a DNA transporter ii) integration of incorporated DNA into the bacterial chromosome by homologous recombination, or the introduced DNA autonomously replicating and being able to function as an episome, and iii) phenotypic expression of the acquired genetic material (Hasegawa et al. 2018).

According to an experiment, the transfer efficiency of two resistance genes, ermC and aadA, was greater at early phases of biofilm development, comparing the transformation rate in planktonic and biofilm cells of *Neisseria gonorrhoeae*. Even conjugative transposons from the Tn916 family, which code for tetracycline resistance, were capable of serving as a donor during transformation in multispecies oral bacteria biofilms generated in vitro (Nonaka et al. 2018).

Transduction: Bacteriophage being the most abundant biological entity on this planet is an important DNA reservoir in natural environments. The DNA packaged in phages avoids digestion by nucleases and hence, remains stable (Calero-Cáceres et al. 2019). HGT by transduction occurs when a bacteriophage transfers gene from one bacterial cell to another. Phage-mediated HGT can occur through two mechanisms: generalized or specialized transduction. Phages can transfer non-viral DNA, chromosomes, transposons, and plasmids acquired from bacteria in addition to their own genomes. Metagenomic stud that various ARGs have been discovered in phage fractions recovered from environmental water samples, including gene products resistance to aminoglycosides, β-lactams, macrolides, and quinolones sulphonamide, and tetracycline antibiotics from sewages, river water, seawater, and WWTPs (von Wintersdorff et al. 2016).

15.4 Environmental Biofilms as a Cause of Concern in Spreading AMR

The ability of microorganisms to evolve and adapt to the changing environmental conditions has resulted in a public health concern, as microbes have developed resistance toward variety of commercial antibiotics. In developing countries like India, the prevalence of drug resistant bacteria is predicted to be significantly higher. This is mainly due to the lack of awareness about safe and judicious uses of antibiotic, problems like unhygienic conditions, overcrowding, malnutrition, poverty, lack of proper quality control further worsen the possibility of the control of infections (Bürgmann et al. 2018). As per the report of CDC 2019, majority of pathogens that falls under the category of urgent threats and serious threats of AMR are the one belonging to family of Enterobacteriaceae along with *A. baumannii*, *P. aeruginosa*, *S. aureus*, etc. and also include fungi typically *C. auris*. These microorganisms are readily found in environment and are also known to have strong to moderate biofilm-forming capabilities which makes the situation more complicated. Environment plays a significant role in the emergence and spread of multidrug resistant bacteria. Antibiotics used in hospitals, released into effluents either by patient's excreta or by direct deposition impose a selection pressure on bacteria (Wright 2016). Antibiotics or their residues can enter the water and land environment through various pathways such as the discharge of municipal sewage, pharmaceutical industry, animal husbandry, and landfill leachates of antibiotic disposal. Other sources may include runoff from agricultural field containing livestock manure, aquaculture ponds, and more commonly irrigation with treated waste water and fertilization with livestock manure is also the critical pathway of resistance genes transmission in agro ecosystem (Ashbolt et al. 2013). This results in evolution of new resistance mechanisms which can be easily transmitted into either susceptible bacteria or clinically relevant pathogens of human populations (Dantas 2017). Additionally, wastewater is a meeting point for antibiotics, disinfectants, and toxic substances such as metals which could favor evolution and emergence of co-resistance to metal and antibiotics even at low concentration. This increases the number and mobilization of resistance genes in the resistome, which is critical for the development of clinical resistance and the transfer of antibiotic resistance genes exchange between bacteria (Karkman et al. 2019). The burden of antibiotic resistance genes (ARGs) Due to the possible transmission of ARGs from environmental bacteria to human pathogens, in the environment has a major impact on public health, resulting in decreasing antibiotic efficacy and eventually threatening human life (Qiao et al. 2018).

15.5 Resistance Mechanism in Biofilm Communities and Their Genetic Transfer

The mechanisms that impart bacterial antibiotic resistance have been studied extensively. Biofilms not only function as barrier of antibiotic diffusion, but they also utilize other resistance mechanisms within the microbial populations (Gebreyohannes et al. 2019). Antibiotic penetration, antibiotic-modifying enzymes, efflux pumps, hypoxia, biofilm heterogeneity, and decreased growth rates are all examples of these processes. Effect of tobramycin on *P. aeruginosa* is a good illustration of decreased penetration of antibiotic becoming a determinant of biofilm tolerance. Tobramycin penetration was slowed by *P. aeruginosa* biofilms, but this obstacle to penetration could be resolved by adding cations to the growth medium (Tseng et al. 2013). The findings indicate that the tobramycin molecule (positively charged) combines with matrix components like phage particles and eDNA, and that decreased penetration can explain *P. aeruginosa* biofilm resistance toward aminoglycoside (Tseng et al. 2013; Secor et al. 2015). Bacterial strains that develop despite being given minimum inhibitory concentrations of bactericidal or bacteriostatic antibiotics are referred to as resistant (Gebreyohannes et al. 2019). Mutations in strains or the exchange of genetic elements conferring acquired resistance may be to account for this resistance. Resistance is gained as a result of genetic, environmental, and cultural factors (Wang et al. 2015). Antibiotics are unable to effectively kill the biofilm population because of the protection conferred by the biofilm (Olsen 2015). Various methods have been hypothesized to explain the establishment of this resistance; for example, bacteria deep within the biofilm proliferate at a slower rate, making them less sensitive to antibiotic treatment. Antibiotics that enter more slowly have been proposed as having the potential to increase tolerance by allowing time for an adaptive phenotypic response (Tseng et al. 2013). This is due in part to the EPS's structure and material. The change from planktonic to biofilm lifestyle depicts various physiological differences, including the appearance of efflux pumps and other mechanisms that respond to oxidative stress. The diagrammatic representation of resistance mechanism in biofilms and genetic transfer of resistance gene between the biofilm cells is represented in Fig. 15.2.

15.5.1 Extracellular DNA

The bacterial biofilm matrix contains DNA, which is an essential and universal component. In *P. aeruginosa*, the molecular function of eDNA involved in resistance and tolerance in biofilm have been intensively investigated, despite the fact that it is an integral ingredient of most, if not all, bacterial biofilms. At infection site, from polymorphonuclear leukocytes, eDNA can be produced from outer membrane vesicles (MVs), release via quorum-sensing mechanism, and altruistic or fratricidal lysis in subpopulations of biofilm cell. eDNA enhances biofilm tolerance to some antimicrobial agents irrespective of whether the origin of the eDNA is exogenous or endogenous (Chiang et al. 2013). In *P. aeruginosa* biofilms applied exogenous DNA

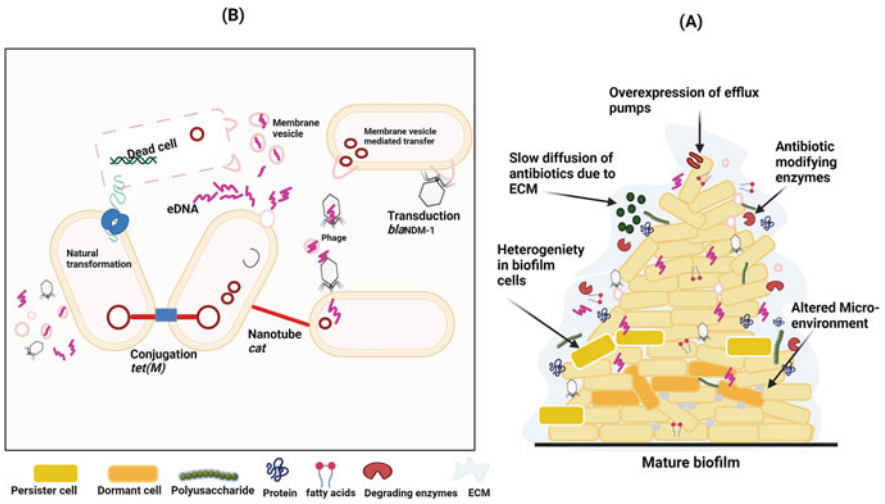


Fig. 15.2 (**a**) Different mechanism of antibiotic resistance in mature biofilm. (**b**) Mechanism of mechanism of gene transfer or antibiotic resistant genes in biofilm cells

to may be incorporated into the biofilm matrix, resulting in a threefold raise in tobramycin resistance and a two-fold rise in gentamicin resistance (Chiang et al. 2013). The alteration of the extracellular environment is one mechanism by which eDNA leads to biofilm resistance. Since eDNA is an anionic macromolecule, it also can chelate cations like magnesium ions, making decrease concentration of Mg^{2+} in the atmosphere (Okshevsky and Meyer 2015). Magnesium deficiency is an environmental signal that activates two-component systems in *P. aeruginosa* and *Salmonella enterica* serovar Typhimurium such as PhoPQ and PmrAB (Johnson et al. 2013). Recent research has also suggested that *P. aeruginosa* biofilms produce acidic microdomains (pH 6.0–6.2) that form as eDNA accumulates locally (Wilton et al. 2016). This low pH is a second signal generate in biofilm environmental that works in concert with deficiency of Mg^{2+} to stimulate the PhoPQ and PmrAB signaling pathways, resulting in antimicrobial resistance. eDNA has been linked to the horizontal transfer of antibiotic resistance genes within a biofilm of naturally competent cells, beside acting as physical role in antibiotic resistance. When compared to planktonic cultures, *Streptococcus pneumoniae* forms biofilms in the nasopharynx with eDNA formed by fratricide (Trappetti et al. 2011), and natural transformation of antibiotic resistance genes is facilitated in these biofilm communities (Cowley et al. 2018). Higher competence of *S. pneumoniae* cells in a biofilm has been linked to sustained overexpression of the competence genes comD and comX (Cowley et al., 2018). Additionally, capsule, which is known to prevent pneumococcal transformation, was significantly downregulated in *S. pneumoniae* biofilm cells compared to planktonic cells (Marks et al. 2012; Cowley et al. 2018). As a result of the exchange of resistance genes in eDNA between certain bacterial

species living in biofilms, antibiotic resistance can arise in surface-attached bacterial populations.

15.5.2 Antibiotic-Modifying Enzymes

Antimicrobials can be degraded by enzymes found in the biofilm matrix, such as secreted β-lactamases, preventing them from meeting their cellular targets. For example, the β-lactamase secreted in *K. pneumoniae* biofilms, has been shown to rapidly hydrolyze ampicillin and preventing to reach the biofilm cells (Murphy and Clegg 2012). *K. pneumoniae* biofilm cells, on the other hand, have many other mechanisms that minimize their resistance toward ampicillin, without having β-lactamase in the matrix they're still much more resistant to ampicillin as compared to planktonic counterparts (Landis, 2019). In *P. aeruginosa* biofilm matrix, secreted AmpC-lactamase, which is chromosomally encoded, is an essential and clinically significant resistance determinant of β-lactam antibiotic in this pathogen. ampC expression was insignificant in *P. aeruginosa* biofilms in the absence of imipenem and ceftazidime, but their presence promotes ampC expression, as assessed by a translational fusion of ampC with an unstable reporter protein (green-fluorescent protein). Despite the fact that the cells in the biofilm core and at base were physiologically active, ampC expression was limited to the biofilm periphery in the presence of a low concentration of imipenem. However, when the concentration of imipenem was increased, the reporter was fully inducted throughout the biofilm, showing that at high dosages, imipenem may be able to overcome the degradative capabilities of β-lactamases (Hall and Mah 2017). Mature *P. aeruginosa* biofilms are more resistant to ceftazidime and meropenem compared to newly produced biofilms due to the higher amount of β-lactamases in the matrix (Ciofu and Tolker-Nielsen 2019).

15.5.3 Efflux Pumps

Multidrug efflux pumps have been identified as important determinants of AMR in regulating the influx and efflux of a compound or drug. The efflux pumps prevent antimicrobial compounds from interacting with their target by reducing their influx. In addition to the traditional role of antibiotic efflux, these efflux pumps play a crucial role in the export of hazardous substrates and different quorum-sensing signaling chemicals into the biofilm milieu (Alav et al. 2018). While no direct link between efflux pumps expression and biofilm formation has been discovered, an investigation in *P. aeruginosa* suggested inhibition of efflux pumps reduced biofilm formation (Rampioni et al. 2017). The overexpression of multidrug efflux pump PA1875-1877 related with biofilm has been described by Zhang and Mah, is a good example of an efflux pump that contributes to biofilm resistance in *P. aeruginosa* (2008). PA1874-1877 is a four-gene operon that was found to be ten times more strongly expressed in biofilms of *P. aeruginosa* than in planktonic cells (Zhang and

Mah 2008). Even though PA1874, a large outer membrane protein has sequence similarity to the biofilm associated protein (Bap) involved in *Staphylococcus aureus* biofilm formation without any known role in *P. aeruginosa* (Valle et al. 2012; Zhang and Mah 2008), deletion of the PA1874-1877 operon had no effect on biofilm formation. In biofilms, deletion of PA1875, PA1876, or PA1877 resulted in two-to-four-fold rise of sensitivity to gentamicin, tobramycin, and ciprofloxacin, while planktonic susceptibility was unchanged. Furthermore, the presence of either the MexAB-OprM or MexCD-OprJ efflux pumps was needed for *P. aeruginosa* biofilm resistance to macrolide, azithromycin, and exposure of azithromycin triggered induction and expression of mexC in biofilm cells (Poole 2011). Tolerance to colistin was also reliant on multidrug efflux pumps in metabolically active cells in *P. aeruginosa* biofilms (Chiang et al. 2013). Multidrug efflux transporters are also essential for biofilm resistance in various other bacterial species. When compared to planktonic cells, the expression of four RND transporters was significantly higher in biofilm cells of *Helicobacter pylori*, and this elevated expression of efflux pump gene could form the basis in explaining why *H. pylori* biofilms are more resistant to clarithromycin antibiotic (Yonezawa et al. 2013). Major efflux associated proteins or pumps found in different bacterial pathogens during biofilm or planktonic state have been listed in Table 15.2.

15.5.4 Persister Cells

Usually in biofilm *Pseudomonas aeruginosa* cells are as vulnerable to antibiotics as planktonic cells, and the improved antibiotic tolerance of biofilms is attributed to higher quantities of persister cells retrieved from communities of biofilm compared planktonic state (Conlon et al. 2015). Toxins from redundant toxin-antitoxin (TA) modules have been related to the development of persister cells, such as MazF and RelE (Wang and Wood 2011). Persistent cells are frequently misunderstood for dormant cells. The difference between persistence and dormancy has been a point of debate. Despite common belief that persister cells are inactive, many studies show that this is not the case (Ayrapetyan et al. 2018; Kim and Wood 2016). Persister cells have demonstrated a very low level of metabolic activity. It's essential to remember that persisters are created by morphological changes rather than mutations. Toxins induce dormancy by interfering with a critical process such as protein synthesis, resulting in tolerance because antibiotics cannot contaminate the work of an inactive process (Kasari et al. 2013). In planktonic cells, the molecular mechanisms of persister cell production have been researched extensively in recent years. Since planktonic and biofilm cultures reflect different lifestyles, it is essential not to consider the mechanisms of persister generation in both states are the same. While more biofilm-specific research is needed, although persistent cell growth in biofilms has been studied in the literature. The yafQ gene, for example, codes for a toxin that is necessary for persister cell formation in *E. coli* biofilms (Harrison et al. 2009). Overexpression of yafQ in biofilm cells enhanced the number of persister cells sustaining elevated exposure of antibiotic, and the yafQ strain

Table 15.2 Common efflux pump in certain bacterial species and their resistance toward antibiotics

Bacteria	Associated efflux pump or genes	Resistance toward antibiotics	References
Pseudomonas aeruginosa	MexAB-OprM	β-Lactams, fluoroquinolones, macrolides, triclosan, acylated homoserine lactones tobramycin, ceftobiprole, ofloxacin, ciprofloxacin, gentamicin, novobiocin, macrolides	Poole (2011), Verchère et al. (2015)
	MexCD-OprJ	β-Lactams, fluoroquinolones, tetracycline, acriflavine	Jeannot et al. (2008)
	MexEF-OprN	Aromatic hydrocarbons, fluoroquinolones, chloramphenicol	Olivares et al. (2012)
	MexJK	Erythromycin, tetracycline	Poole (2011)
	Czm-OpmN	Zinc, cadmium	Kumar et al. (2008)
	PA1875-1877	Gentamycin, ciprofloxacin, tobramycin	Zhang and Mah (2008)
Staphylococcus aureus	Smr, QacH, QacG, MepA, NorA, NorC	Fluoroquinolones, glycylcyclines	Costa et al. (2013)
	NorB	Moxifloxacin	Ding et al. (2008), Costa et al. (2013)
	MdeA	Novobiocin, fusidic acid	Costa et al. (2013)
	Tet38	Tetracyclines	Costa et al. (2013)
Bacillus subtilis	Blt, Bmr3, Bmr, LmrB, ImrA	Lincosamides and fluoroquinolones	Sun et al. (2014)
Streptococcus pneumoniae	PmrA, Msr(D)	Macrolides, ketolides ciprofloxacin, Norfloxacin	Cherazard et al. (2017)
Staphylococcus haemolyticus	qacG, qacH, qacA, MdeA	Lincosamides, type A streptogramins, macrolides	Correa et al. (2008)
Burkholderia cepacia	BCAL1672-1676	Ciprofloxacin, tetracycline	Buroni et al. (2014)
Burkholderia pseudomallei	BPSL1661, BPSL1664, BPSL1665	Doxycycline, imipenem, ceftazidime	Kumar et al. (2008)
Helicobacter pylori	HP1327, HP971, HP1489, HP607	Clarithromycin	Yonezawa et al. (2013)
Clostridium botulinum	CdeA	Fluoroquinolone	Harnvoravongchai et al. (2017)
Listeria monocytogenes	FepA	Fluoroquinolone	Guérin et al. (2014)

formed biofilms that were significantly less resistant to cefazolin and tobramycin compared to wild type (Harrison et al. 2009). When comparing planktonic and biofilm cells in *Burkholderia cenocepacia*, the majority of toxin genes were transcriptionally increased in biofilm state (Van Acker and Coenye 2016). In *B. cenocepacia* biofilms, overexpression of certain toxins resulted in increased toxicity. In biofilms, persister cells, on the other hand, may not be mainly accountable for antibiotic tolerance, but rather play a supporting function (Aristizábal-Hoyos et al. 2019).

15.6 Role of Membrane Vesicles (MVs) and Nanotube in ARGs Exchange

Gram-negative bacteria produce OMVs by blabbing from the outer membrane, which are small spherical structures. Proteases, alkaline phosphatase, lipases, toxins, and some outer membrane proteins are common periplasmic and cytoplasmic contents (OMPs) (Jan 2017). MVs have been found biofilms, as well as planktonic cells. *Pseudomonas aeruginosa* (Murphy and Clegg 2012), *E. coli* (Tang et al. 2020), *Staphylococcus aureus* (Sugimoto et al. 2017), *Helicobacter pylori* (Yonezawa et al. 2013), and *B. subtilis* (Brown et al. 2014) have all been found to produce MVs in biofilms. In *Neisseria gonorrhoeae* the mobilization of R-plasmid having *bla* gene was the first evidence of MV-mediated gene delivery (Dorward et al. 1989). Various laboratory investigations have analyzed the role of MV-mediated mobilization of ARGs in number of bacteria since that first discovery (Domingues and Nielsen 2017). Though horizontal gene transfer mediated via MVs in natural environments has not completely understood. In experiments with *A. baumannii* with pMMA2 and pMMCU3 carrying blaOXA-2 gene (Rumbo et al. 2011), in *Thermus* spp. plasmid pMKPn carrying kanamycin resistance gene (Blesa et al. 2018), and pBBRMCS with chloramphenicol resistance gene in *Buttiauxella agrestis* (Tashiro et al. 2017). In gram-negative bacteria like *P. aeruginosa*, (Toyofuku et al. 2019; Morinaga et al. 2018), *Paracoccus* sp. and *Vibrio* spp. (Morinaga et al. 2018), MVs can transmit quorum-sensing (QS) signals. Conjugation, transformation, and phage induction are all known to be controlled by QS signals (Laganenka et al. 2019). Therefore, MVs have the potential to play key role in the control of both HGT and DNA transport.

Nanotubes, an elongated extracellular structure, have recently been identified as another mechanism of transfer of DNA in *Bacillus subtilis*; nanotubes are used in direct cellular interaction (Hurtig et al. 2010; Dubey et al. 2016).

The membranous structures called nanotubes differ from conjugation pili, that are protein-based structures. Nanotubes were often shaped in a cross-species manner, implying that they can be used to transport constituents both within and across species (Dubey and Ben-Yehuda 2011). Unlike conjugation pili, the cytoplasmic interchange of metabolites like nutrients and fluorescent marker proteins between different bacterial species has been documented, and it has been linked to nanotube-like structures (Shitut et al. 2019). As a result, nanotubes are likely to play an

important role in the localization and movement of biomaterials within bacterial communities in addition to ARGs alone (Fritts et al. 2021) s demonstrated that nanotubes act as gateways for intercellular exchange of cytoplasmic molecules and non-conjugative plasmids using *Bacillus subtilis* where YmdB act as a component required for the formation of nanotube and molecular exchange between cells (Dubey et al. 2016).

15.7 Conclusions

Bacteria in biofilm exhibit increased degree of resistance as compared to planktonic state. Other than intrinsic mechanisms of bacteria, ECM and QS play a major role in promoting AMR in biofilms. Increased HGT rates in biofilm are responsible for dissemination of ARGs in environment. Resistance toward β-lactam antibiotics seems to more frequently emerged and disseminated with in bacterial population both in planktonic and biofilms. Environmental biofilms are expected further provides opportunity to microbial community to greater gene exchange and spread AMR.

Understanding the mechanisms that cause recalcitrance will undoubtedly help to guide therapy methods for biofilm infections. These should be supplemented by diagnostic procedures for biofilm infections quickly and determining in vivo biological composition of biofilms. Furthermore, the availability of a panel of compounds that can prevent and dispersed biofilms will aid in the selection of effective treatment options for biofilm-based infections.

Acknowledgments The research was supported by Indo-UK joint Collaborative research project Respharm and SELECTAR from Department of biotechnology. The DST–INSPIRE fellowship (IF180419) support from Department of Science and Technology to Ms. Samreen, is also thankfully acknowledged.

References

Abe K, Nomura N, Suzuki S (2020) Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. FEMS Microbiol Ecol 96:fiaa031. https://doi.org/10.1093/femsec/fiaa031

Ahmad I (1993) Studies on plasmid encoded virulence factors in the strains of Escherichia coli of man and animals: compatibility with R-plasmids (Doctoral dissertation, Aligarh Muslim University)

Ahmad I, Aqil F (eds) (2008) New strategies combating bacterial infection. Wiley, New York

Ahmad I, Khan MS, Altaf MM et al (2017) Biofilms: an overview of their significance in plant and soil health. In: Biofilms in plant and soil health. Wiley, Chichester, pp 1–25

Ahmad I, Qais F, Samreen et al (2019) Antibacterial drug discovery: perspective insights. In: Antibacterial drug discovery to combat MDR. Springer, Singapore, pp 1–21

Alav I, Sutton JM, Rahman KM (2018) Role of bacterial efflux pumps in biofilm formation. J Antimicrob Chemother 73:2003–2020. https://doi.org/10.1093/jac/dky042

Aminov RI (2011) Horizontal gene exchange in environmental microbiota. Front Microbiol 2:158. https://doi.org/10.3389/fmicb.2011.00158

Aristizábal-Hoyos AM, Rodríguez EA, Arias L, Jiménez JN (2019) High clonal diversity of multidrug-resistant and extended spectrum beta-lactamase-producing Escherichia coli in a wastewater treatment plant. J Environ Manag 245:37–47. https://doi.org/10.1016/j.jenvman.2019.05.073

Ashbolt NJ, Amézquita A, Backhaus T et al (2013) Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect 121:993–1001. https://doi.org/10.1289/ehp.1206316

Aslam B, Wang W, Arshad MI et al (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645–1658. https://doi.org/10.2147/IDR.S173867

Ayrapetyan M, Williams T, Oliver JD (2018) Relationship between the viable but Nonculturable state and antibiotic Persister cells. J Bacteriol 200. https://doi.org/10.1128/JB.00249-18

Balcázar JL, Subirats J, Borrego CM (2015) The role of biofilms as environmental reservoirs of antibiotic resistance. Front Microbiol 6:1216. https://doi.org/10.3389/fmicb.2015.01216

Bandyopadhyay S, Bhattacharyya D, Samanta I et al (2021) Characterization of multidrug-resistant biofilm-producing Escherichia coli and Klebsiella pneumoniae in healthy cattle and cattle with diarrhea. Microb Drug Resist mdr.2020.0298. https://doi.org/10.1089/mdr.2020.0298

Beaudoin T, Zhang L, Hinz AJ et al (2012) The biofilm-specific antibiotic resistance gene ndvB is important for expression of ethanol oxidation genes in Pseudomonas aeruginosa biofilms. J Bacteriol 194:3128–3136. https://doi.org/10.1128/JB.06178-11

Beauregard PB, Chai Y, Vlamakis H et al (2013) Bacillus subtilis biofilm induction by plant polysaccharides. Proc Natl Acad Sci 110:E1621–E1630. https://doi.org/10.1073/pnas.1218984110

Bello-López JM, Cabrero-Martínez OA, Ibáñez-Cervantes G et al (2019) Horizontal gene transfer and its association with antibiotic resistance in the genus Aeromonas spp. Microorganisms 7:363. https://doi.org/10.3390/microorganisms7090363

Besemer K (2015) Biodiversity, community structure and function of biofilms in stream ecosystems. Res Microbiol 166:774–781. https://doi.org/10.1016/j.resmic.2015.05.006

Bevan ER, Jones AM, Hawkey PM (2017) Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145–2155. https://doi.org/10.1093/jac/dkx146

Bissong MEA, Ateba CN (2020) Genotypic and phenotypic evaluation of biofilm production and antimicrobial resistance in Staphylococcus aureus isolated from Milk, north West Province, South Africa. Antibiotics 9:156. https://doi.org/10.3390/antibiotics9040156

Blesa A, Averhoff B, Berenguer J (2018) Horizontal gene transfer in Thermus spp. Curr Issues Mol Biol 29:23–36. https://doi.org/10.21775/cimb.029.023

Bogino P, Oliva M, Sorroche F, Giordano W (2013) The role of bacterial biofilms and surface components in plant-bacterial associations. Int J Mol Sci 14:15838–15859. https://doi.org/10.3390/ijms140815838

Brown L, Kessler A, Cabezas-Sanchez P et al (2014) Extracellular vesicles produced by the gram-positive bacterium B acillus subtilis are disrupted by the lipopeptide surfactin. Mol Microbiol 93:183–198. https://doi.org/10.1111/mmi.12650

Bürgmann H, Frigon D, Gaze WH et al (2018) Water and sanitation: an essential battlefront in the war on antimicrobial resistance. FEMS Microbiol Ecol 94. https://doi.org/10.1093/femsec/fiy101

Buroni S, Matthijs N, Spadaro F, Van Acker H, Scoffone VC, Pasca MR et al (2014) Differential roles of RND efflux pumps in antimicrobial drug resistance of sessile and planktonic Burkholderia cenocepacia cells. Antimicrob Agents Chemother 58(12):7424–7429

Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54:969–976. https://doi.org/10.1128/AAC.01009-09

Calero-Cáceres W, Ye M, Balcázar JL (2019) Bacteriophages as environmental reservoirs of antibiotic resistance. Trends Microbiol 27:570–577. https://doi.org/10.1016/j.tim.2019.02.008

Carattoli A (2013) Plasmids and the spread of resistance. Int J Med Microbiol 303:298–304. https://doi.org/10.1016/j.ijmm.2013.02.001

Cha J-O, Il YJ, Yoo JS et al (2013) Investigation of biofilm formation and its association with the molecular and clinical characteristics of methicillin-resistant Staphylococcus aureus. Osong Public Heal Res Perspect 4:225–232. https://doi.org/10.1016/j.phrp.2013.09.001

Chen L, Tang Z-Y, Cui S-Y et al (2020) Biofilm production ability, virulence and antimicrobial resistance genes in Staphylococcus aureus from various veterinary hospitals. Pathogens 9:264. https://doi.org/10.3390/pathogens9040264

Cherazard R, Epstein M, Doan T-L et al (2017) Antimicrobial resistant Streptococcus pneumoniae: prevalence, mechanisms, and clinical implications. Am J Ther 24:e361–e369. https://doi.org/10.1097/MJT.0000000000000551

Chiang W-C, Nilsson M, Jensen PØ et al (2013) Extracellular DNA shields against aminoglycosides in Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 57:2352–2361. https://doi.org/10.1128/AAC.00001-13

Ciofu O, Tolker-Nielsen T (2019) Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—how P. aeruginosa can escape antibiotics. Front Microbiol 10:913. https://doi.org/10.3389/fmicb.2019.00913

Colpan A, Johnston B, Porter S et al (2013) Escherichia coli sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among US veterans. Clin Infect Dis 57:1256–1265. https://doi.org/10.1093/cid/cit503

Conlon BP, Rowe SE, Lewis K (2015) Persister cells in biofilm associated infections. In: Biofilm-based healthcare-associated infections, pp 1–9

Correa JE, De Paulis A, Predari S et al (2008) First report of qacG, qacH and qacJ genes in staphylococcus haemolyticus human clinical isolates. J Antimicrob Chemother 62:956–960. https://doi.org/10.1093/jac/dkn327

Costa SS, Viveiros M, Amaral L, Couto I (2013) Multidrug efflux pumps in Staphylococcus aureus: an update. Open Microbiol J 7:59–71. https://doi.org/10.2174/1874285801307010059

Cowley LA, Petersen FC, Junges R et al (2018) Evolution via recombination: cell-to-cell contact facilitates larger recombination events in Streptococcus pneumoniae. PLoS Genet 14:e1007410. https://doi.org/10.1371/journal.pgen.1007410

Cui P, Feng L, Zhang L et al (2020) Antimicrobial resistance, virulence genes, and biofilm formation capacity among enterococcus species from yaks in aba Tibetan autonomous prefecture, China. Front Microbiol 11:01250. https://doi.org/10.3389/fmicb.2020.01250

Dantas G (2017) Networks of exchanging antibiotic resistance between environmental, commensal, and pathogenic microbes. FASEB J 31:404–401

Darphorn TS, Bel K, Koenders-van Sint Anneland BB et al (2021) Antibiotic resistance plasmid composition and architecture in Escherichia coli isolates from meat. Sci Rep 11:2136. https://doi.org/10.1038/s41598-021-81683-w

Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2:114–122. https://doi.org/10.1038/nrd1008

De Groote VN, Verstraeten N, Fauvart M et al (2009) Novel persistence genes in Pseudomonas aeruginosa identified by high-throughput screening. FEMS Microbiol Lett 297:73–79. https://doi.org/10.1111/j.1574-6968.2009.01657.x

Di Cesare A, Eckert EM, Rogora M, Corno G (2017) Rainfall increases the abundance of antibiotic resistance genes within a riverine microbial community. Environ Pollut 226:473–478. https://doi.org/10.1016/j.envpol.2017.04.036

Ding Y, Onodera Y, Lee JC, Hooper DC (2008) NorB, an efflux pump in Staphylococcus aureus strain MW2, contributes to bacterial fitness in abscesses. J Bacteriol 190:7123–7129. https://doi.org/10.1128/JB.00655-08

Domingues S, Nielsen KM (2017) Membrane vesicles and horizontal gene transfer in prokaryotes. Curr Opin Microbiol 38:16–21

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890. https://doi.org/10.3201/eid0809.020063

Dorward DW, Garon CF, Judd RC (1989) Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. J Bacteriol 171:2499–2505. https://doi.org/10.1128/jb.171.5.2499-2505.1989

Dragoš A, Kovács ÁT (2017) The peculiar functions of the bacterial extracellular matrix. Trends Microbiol 25:257–266. https://doi.org/10.1016/j.tim.2016.12.010

Dubey GP, Ben-Yehuda S (2011) Intercellular nanotubes mediate bacterial communication. Cell 144:590–600. https://doi.org/10.1016/j.cell.2011.01.015

Dubey GP, Malli Mohan GB, Dubrovsky A et al (2016) Architecture and characteristics of bacterial nanotubes. Dev Cell 36:453–461. https://doi.org/10.1016/j.devcel.2016.01.013

Eckert EM, Di Cesare A, Coci M, Corno G (2018) Persistence of antibiotic resistance genes in large subalpine lakes: the role of anthropogenic pollution and ecological interactions. Hydrobiologia 824:93–108. https://doi.org/10.1007/s10750-017-3480-0

Emami S, Nikokar I, Ghasemi Y et al (2015) Antibiotic resistance pattern and distribution of pslA gene among biofilm producing Pseudomonas aeruginosa isolated from waste water of a burn center. Jundishapur J Microbiol 8:e23669. https://doi.org/10.5812/jjm.23669

Ferri M, Ranucci E, Romagnoli P, Giaccone V (2017) Antimicrobial resistance: a global emerging threat to public health systems. Crit Rev Food Sci Nutr 57:2857–2876. https://doi.org/10.1080/10408398.2015.1077192

Fey PD (2010) Modality of bacterial growth presents unique targets: how do we treat biofilm-mediated infections? Curr Opin Microbiol 13:610–615. https://doi.org/10.1016/j.mib.2010.09.007

Fritts RK, McCully AL, McKinlay JB (2021) Extracellular metabolism sets the table for microbial cross-feeding. Microbiol Mol Biol Rev 85. https://doi.org/10.1128/MMBR.00135-20

Gay N, Belmonte O, Collard J-M et al (2017) Review of antibiotic resistance in the Indian Ocean commission: a human and animal health issue. Front Public Heal 5:162. https://doi.org/10.3389/fpubh.2017.00162

Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB (2019) Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. Heliyon 5:e02192. https://doi.org/10.1016/j.heliyon.2019.e02192

Guérin F, Galimand M, Tuambilangana F et al (2014) Overexpression of the novel MATE fluoroquinolone efflux pump FepA in listeria monocytogenes is driven by inactivation of its local repressor FepR. PLoS One 9:e106340. https://doi.org/10.1371/journal.pone.0106340

Guo X, Yang Y, Lu D et al (2018) Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze estuary. Water Res 129:277–286. https://doi.org/10.1016/j.watres.2017.11.029

Hall CW, Mah T-F (2017) Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 41:276–301. https://doi.org/10.1093/femsre/fux010

Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2:95–108. https://doi.org/10.1038/nrmicro821

Harnvoravongchai P, Pipatthana M, Chankhamhaengdecha S, Janvilisri T (2017) Insights into drug resistance mechanisms in Clostridium difficile. Essays Biochem 61:81–88. https://doi.org/10.1042/EBC20160062

Harrison JJ, Wade WD, Akierman S et al (2009) The chromosomal toxin gene yafQ is a determinant of multidrug tolerance for Escherichia coli growing in a biofilm. Antimicrob Agents Chemother 53:2253–2258. https://doi.org/10.1128/AAC.00043-09

Hasegawa H, Suzuki E, Maeda S (2018) Horizontal plasmid transfer by transformation in Escherichia coli: environmental factors and possible mechanisms. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.02365

Høiby N, Ciofu O, Johansen HK et al (2011) The clinical impact of bacterial biofilms. Int J Oral Sci 3:55–65. https://doi.org/10.4248/IJOS11026

Hurtig J, Chiu DT, Önfelt B (2010) Intercellular nanotubes: insights from imaging studies and beyond. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2:260–276. https://doi.org/10.1002/wnan.80

Huyan J, Tian Z, Zhang Y et al (2020) Dynamics of class 1 integrons in aerobic biofilm reactors spiked with antibiotics. Environ Int 140:105816. https://doi.org/10.1016/j.envint.2020.105816

Islam B, Khan SN, Haque I et al (2008) Novel anti-adherence activity of mulberry leaves: inhibition of Streptococcus mutans biofilm by 1-deoxynojirimycin isolated from Morus alba. J Antimicrob Chemother 62:751–757. https://doi.org/10.1093/jac/dkn253

Jamal M, Ahmad W, Andleeb S et al (2018) Bacterial biofilm and associated infections. J Chinese Med Assoc 81:7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Jan AT (2017) Outer membrane vesicles (OMVs) of gram-negative bacteria: a perspective update. Front Microbiol 8:1053. https://doi.org/10.3389/fmicb.2017.01053

Jeannot K, Elsen S, Köhler T et al (2008) Resistance and virulence of Pseudomonas aeruginosa clinical strains overproducing the MexCD-OprJ efflux pump. Antimicrob Agents Chemother 52:2455–2462. https://doi.org/10.1128/AAC.01107-07

Johnson L, Horsman SR, Charron-Mazenod L et al (2013) Extracellular DNA-induced antimicrobial peptide resistance in Salmonella enterica serovar Typhimurium. BMC Microbiol 13:115. https://doi.org/10.1186/1471-2180-13-115

Jorge P, Lourenço A, Pereira MO (2012) New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 28:1033–1061. https://doi.org/https://doi.org/10.1080/08927014.2012.728210

Jorge P, Magalhães AP, Grainha T et al (2019) Antimicrobial resistance three ways: healthcare crisis, major concepts and the relevance of biofilms. FEMS Microbiol Ecol 95:fiz115. https://doi.org/10.1093/femsec/fiz115

Kaplan JB (2010) Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res 89:205–218. https://doi.org/10.1177/0022034509359403

Karkman A, Do TT, Walsh F, Virta MPJ (2018) Antibiotic-resistance genes in waste water. Trends Microbiol 26:220–228. https://doi.org/10.1016/j.tim.2017.09.005

Karkman A, Pärnänen K, Larsson DGJ (2019) Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nat Commun 10:80. https://doi.org/10.1038/s41467-018-07992-3

Kasari V, Mets T, Tenson T, Kaldalu N (2013) Transcriptional cross-activation between toxin-antitoxin systems of Escherichia coli. BMC Microbiol 13:45. https://doi.org/10.1186/1471-2180-13-45

Khan FA (2020) Carbapenemase-producing Enterobacteriaceae in wastewater-associated aquatic environments (Doctoral dissertation, Örebro University)

Kidd TJ, Canton R, Ekkelenkamp M et al (2018) Defining antimicrobial resistance in cystic fibrosis. J Cyst Fibros 17:696–704. https://doi.org/10.1016/j.jcf.2018.08.014

Kim J-S, Wood TK (2016) Persistent persister misperceptions. Front Microbiol 7:2134. https://doi.org/10.3389/fmicb.2016.02134

Kour D, Rana KL, Kaur T, et al (2020) Microbial biofilms: functional annotation and potential applications in agriculture and allied sectors. In: New and future developments in microbial biotechnology and bioengineering: microbial biofilms. Elsevier, pp 283–301

ter Kuile BH, Kraupner N, Brul S (2016) The risk of low concentrations of antibiotics in agriculture for resistance in human health care. FEMS Microbiol Lett 363:fnw210. https://doi.org/10.1093/femsle/fnw210

Kumar A, Mayo M, Trunck LA et al (2008) Expressiostrainsn of resistance-nodulation-cell-division efflux pumps in commonly used Burkholderia pseudomallei and clinical isolates from northern Australia. Trans R Soc Trop Med Hyg 102:S145–S151. https://doi.org/10.1016/S0035-9203(08)70032-4

Laganà P, Votano L, Caruso G et al (2018) Bacterial isolates from the Arctic region (Pasvik River, Norway): assessment of biofilm production and antibiotic susceptibility profiles. Environ Sci Pollut Res 25:1089–1102. https://doi.org/10.1007/s11356-017-0485-1

Laganenka L, Sander T, Lagonenko A et al (2019) Quorum sensing and metabolic state of the host control Lysogeny-lysis switch of bacteriophage T1. MBio 10:e01884. https://doi.org/10.1128/mBio.01884-19

Lambert M-L, Suetens C, Savey A et al (2011) Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect Dis 11:30–38. https://doi.org/10.1016/S1473-3099(10)70258-9

Landis R (2019) Hybridized polymeric nano-assemblies: key insights into addressing MDR infections (Doctoral dissertation)

Laxminarayan R, Chaudhury RR (2016) Antibiotic resistance in India: drivers and opportunities for action. PLoS Med 13:e1001974. https://doi.org/10.1371/journal.pmed.1001974

Li B, Yan T (2021) Next generation sequencing reveals limitation of qPCR methods in quantifying emerging antibiotic resistance genes (ARGs) in the environment. Appl Microbiol Biotechnol 105:2925–2936. https://doi.org/10.1007/s00253-021-11202-4

Lopez D, Vlamakis H, Kolter R (2010) Biofilms. Cold Spring Harb Perspect Biol 2:a000398. https://doi.org/10.1101/cshperspect.a000398

Ma Y, Wang C, Li Y et al (2020) Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. Adv Sci 7:1901872. https://doi.org/10.1002/advs.201901872

Maal-Bared R, Bartlett KH, Bowie WR, Hall ER (2013) Phenotypic antibiotic resistance of Escherichia coli and E. coli O157 isolated from water, sediment and biofilms in an agricultural watershed in British Columbia. Sci Total Environ 443:315–323. https://doi.org/10.1016/j.scitotenv.2012.10.106

Magana M, Sereti C, Ioannidis A et al (2018) Options and limitations in clinical investigation of bacterial biofilms. Clin Microbiol Rev 31:e00084. https://doi.org/10.1128/CMR.00084-16

Maheshwari M, Abulreesh HH, Khan MS et al (2017) Horizontal gene transfer in soil and the rhizosphere: impact on ecological fitness of bacteria. In: Agriculturally important microbes for sustainable agriculture. Springer, Singapore, pp 111–130

Marks LR, Reddinger RM, Hakansson AP (2012) High levels of genetic recombination during nasopharyngeal carriage and biofilm formation in Streptococcus pneumoniae. MBio 3:e00200. https://doi.org/10.1128/mBio.00200-12

Marti E, Huerta B, Rodríguez-Mozaz S et al (2014) Characterization of ciprofloxacin-resistant isolates from a wastewater treatment plant and its receiving river. Water Res 61:67–76. https://doi.org/10.1016/j.watres.2014.05.006

McDougald D, Rice SA, Barraud N et al (2012) Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat Rev Microbiol 10:39–50. https://doi.org/10.1038/nrmicro2695

Michels J, Stippkugel A, Lenz M et al (2018) Rapid aggregation of biofilm-covered microplastics with marine biogenic particles. Proc R Soc B Biol Sci 285:20181203. https://doi.org/10.1098/rspb.2018.1203

Morinaga K, Yamamoto T, Nomura N, Toyofuku M (2018) Paracoccus denitrificans can utilize various long-chain N -acyl homoserine lactones and sequester them in membrane vesicles. Environ Microbiol Rep 10:651–654. https://doi.org/10.1111/1758-2229.12674

Mukherjee S, Bassler BL (2019) Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol 17:371–382. https://doi.org/10.1038/s41579-019-0186-5

Murphy CN, Clegg S (2012) Klebsiella pneumoniae and type 3 fimbriae: nosocomial infection, regulation and biofilm formation. Future Microbiol 7:991–1002. https://doi.org/10.2217/fmb.12.74

Nesse LL, Simm R (2018) Biofilm: a hotspot for emerging bacterial genotypes. Adv Appl Microbiol 103:223–246

Nonaka L, Yamamoto T, Maruyama F et al (2018) Interplay of a non-conjugative integrative element and a conjugative plasmid in the spread of antibiotic resistance via suicidal plasmid transfer from an aquaculture vibrio isolate. PLoS One 13:e0198613. https://doi.org/10.1371/journal.pone.0198613

O'Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations

O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79. https://doi.org/10.1146/annurev.micro.54.1.49

Oberbeckmann S, Loeder MGJ, Gerdts G, Osborn AM (2014) Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in northern European waters. FEMS Microbiol Ecol 90:478–492. https://doi.org/10.1111/1574-6941.12409

Okshevsky M, Meyer RL (2015) The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. Crit Rev Microbiol 41:341–352. https://doi.org/10.3109/1040841X.2013.841639

Olivares J, Alvarez-Ortega C, Linares JF et al (2012) Overproduction of the multidrug efflux pump MexEF-OprN does not impair Pseudomonas aeruginosa fitness in competition tests, but produces specific changes in bacterial regulatory networks. Environ Microbiol 14:1968–1981. https://doi.org/10.1111/j.1462-2920.2012.02727.x

Olsen I (2015) Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis 34:877–886. https://doi.org/10.1007/s10096-015-2323-z

Ory J, Bricheux G, Robin F et al (2019) Biofilms in hospital effluents as a potential crossroads for carbapenemase-encoding strains. Sci Total Environ 657:7–15. https://doi.org/10.1016/j.scitotenv.2018.11.427

Papenfort K, Bassler BL (2016) Quorum sensing signal–response systems in gram-negative bacteria. Nat Rev Microbiol 14:576–588. https://doi.org/10.1038/nrmicro.2016.89

Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol 13:27–33. https://doi.org/10.1016/j.tim.2004.11.007

Peterson E, Kaur P (2018) Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol 9:2928. https://doi.org/10.3389/fmicb.2018.02928

Petrovich M, Chu B, Wright D et al (2018) Antibiotic resistance genes show enhanced mobilization through suspended growth and biofilm-based wastewater treatment processes. FEMS Microbiol Ecol 94:fiy041. https://doi.org/10.1093/femsec/fiy041

Poole K (2011) Pseudomonas aeruginosa: resistance to the max. Front Microbiol 2:65. https://doi.org/10.3389/fmicb.2011.00065

Powell LC, Abdulkarim M, Stokniene J et al (2021) Quantifying the effects of antibiotic treatment on the extracellular polymer network of antimicrobial resistant and sensitive biofilms using multiple particle tracking. NPJ Biofilms Microbiomes 7:13. https://doi.org/10.1038/s41522-020-00172-6

Qiao M, Ying G-G, Singer AC, Zhu Y-G (2018) Review of antibiotic resistance in China and its environment. Environ Int 110:160–172. https://doi.org/10.1016/j.envint.2017.10.016

Rampioni G, Pillai CR, Longo F et al (2017) Effect of efflux pump inhibition on Pseudomonas aeruginosa transcriptome and virulence. Sci Rep 7:11392. https://doi.org/10.1038/s41598-017-11892-9

Rawson TM, Moore LSP, Gilchrist MJ, Holmes AH (2016) Antimicrobial stewardship: are we failing in cross-specialty clinical engagement? J Antimicrob Chemother 71:554–559. https://doi.org/10.1093/jac/dkv337

Reygaert WC (2018) An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 4:482–501. https://doi.org/10.3934/microbiol.2018.3.482

Roberto AA, Van Gray JB, Engohang-Ndong J, Leff LG (2019) Distribution and co-occurrence of antibiotic and metal resistance genes in biofilms of an anthropogenically impacted stream. Sci Total Environ 688:437–449. https://doi.org/10.1016/j.scitotenv.2019.06.053

Roca I, Akova M, Baquero F et al (2015) The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 6:22–29. https://doi.org/10.1016/j.nmni.2015.02.007

Rumbo C, Fernández-Moreira E, Merino M, Poza M, Mendez JA, Soares NC et al (2011) Horizontal transfer of the OXA-24 carbapenemase gene via outer membrane vesicles: a new mechanism of dissemination of carbapenem resistance genes in Acinetobacter baumannii. Antimicrob Agents Chemother 55(7):3084–3090

San Millan A (2018) Evolution of plasmid-mediated antibiotic resistance in the clinical context. Trends Microbiol 26:978–985. https://doi.org/10.1016/j.tim.2018.06.007

Schrijver R, Stijntjes M, Rodríguez-Baño J et al (2018) Review of antimicrobial resistance surveillance programmes in livestock and meat in EU with focus on humans. Clin Microbiol Infect 24:577–590. https://doi.org/10.1016/j.cmi.2017.09.013

Secor PR, Sweere JM, Michaels LA et al (2015) Filamentous bacteriophage promote biofilm assembly and function. Cell Host Microbe 18:549–559. https://doi.org/10.1016/j.chom.2015.10.013

Shitut S, Ahsendorf T, Pande S et al (2019) Nanotube-mediated cross-feeding couples the metabolism of interacting bacterial cells. Environ Microbiol 21:1306–1320. https://doi.org/10.1111/1462-2920.14539

Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209. https://doi.org/10.1146/annurev.micro.56.012302.160705

Sugimoto Y, Suzuki S, Nonaka L et al (2017) The novel mef (C)– mph (G) macrolide resistance genes are conveyed in the environment on various vectors. J Glob Antimicrob Resist 10:47–53. https://doi.org/10.1016/j.jgar.2017.03.015

Sun J, Deng Z, Yan A (2014) Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. Biochem Biophys Res Commun 453:254–267. https://doi.org/10.1016/j.bbrc.2014.05.090

Tacconelli E, Carrara E, Savoldi A et al (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18:318–327. https://doi.org/10.1016/S1473-3099(17)30753-3

Tang M, Wei X, Wan X et al (2020) The role and relationship with efflux pump of biofilm formation in Klebsiella pneumoniae. Microb Pathog 147:104244. https://doi.org/10.1016/j.micpath.2020.104244

Tao W, Zhang X-X, Zhao F et al (2016) High levels of antibiotic resistance genes and their correlations with bacterial community and mobile genetic elements in pharmaceutical wastewater treatment bioreactors. PLoS One 11:e0156854. https://doi.org/10.1371/journal.pone.0156854

Tashiro Y, Hasegawa Y, Shintani M et al (2017) Interaction of bacterial membrane vesicles with specific species and their potential for delivery to target cells. Front Microbiol 8:571. https://doi.org/10.3389/fmicb.2017.00571

Toyofuku M, Nomura N, Eberl L (2019) Types and origins of bacterial membrane vesicles. Nat Rev Microbiol 17:13–24. https://doi.org/10.1038/s41579-018-0112-2

Trappetti C, Potter AJ, Paton AW et al (2011) LuxS mediates iron-dependent biofilm formation, competence, and fratricide in Streptococcus pneumoniae. Infect Immun 79:4550–4558. https://doi.org/10.1128/IAI.05644-11

Tseng BS, Zhang W, Harrison JJ et al (2013) The extracellular matrix protects Pseudomonas aeruginosa biofilms by limiting the penetration of tobramycin. Environ Microbiol 15:2865. https://doi.org/10.1111/1462-2920.12155

Uruén C, Chopo-Escuin G, Tommassen J et al (2020) Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics 10:3. https://doi.org/10.3390/antibiotics10010003

Valle J, Latasa C, Gil C et al (2012) Bap, a biofilm matrix protein of Staphylococcus aureus prevents cellular internalization through binding to GP96 host receptor. PLoS Pathog 8:e1002843. https://doi.org/10.1371/journal.ppat.1002843

Van Acker H, Coenye T (2016) The role of efflux and physiological adaptation in biofilm tolerance and resistance. J Biol Chem 291:12565–12572. https://doi.org/10.1074/jbc.R115.707257

Van Houdt R, Michiels CW (2010) Biofilm formation and the food industry, a focus on the bacterial outer surface. J Appl Microbiol 109:1117–1131. https://doi.org/10.1111/j.1365-2672.2010.04756.x

Velmourougane K, Prasanna R, Saxena AK (2017) Agriculturally important microbial biofilms: present status and future prospects. J Basic Microbiol 57:548–573. https://doi.org/10.1002/jobm.201700046

Verchère A, Dezi M, Adrien V et al (2015) In vitro transport activity of the fully assembled MexAB-OprM efflux pump from Pseudomonas aeruginosa. Nat Commun 6:6890. https://doi.org/10.1038/ncomms7890

Wang X, Wood TK (2011) Toxin-antitoxin systems influence biofilm and Persister cell formation and the general stress response. Appl Environ Microbiol 77:5577–5583. https://doi.org/10.1128/AEM.05068-11

Wang S, Liu X, Liu H et al (2015) The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm skeleton in P seudomonas aeruginosa. Environ Microbiol Rep 7:330–340. https://doi.org/10.1111/1758-2229.12252

Wang Y, Venter H, Ma S (2016a) Efflux pump inhibitors: a novel approach to combat efflux-mediated drug resistance in bacteria. Curr Drug Targets 17:702–719

Wang Y, Yi L, Wang Y et al (2016b) Isolation, phylogenetic group, drug resistance, biofilm formation, and adherence genes of Escherichia coli from poultry in Central China. Poult Sci 95:2895–2901. https://doi.org/10.3382/ps/pew252

Wang H, Wang H, Liang L et al (2018) Prevalence, genetic characterization and biofilm formation in vitro of staphylococcus aureus isolated from raw chicken meat at retail level in Nanjing, China. Food Control 86:11–18. https://doi.org/10.1016/j.foodcont.2017.10.028

Wilton M, Charron-Mazenod L, Moore R, Lewenza S (2016) Extracellular DNA acidifies biofilms and induces aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 60:544–553. https://doi.org/10.1128/AAC.01650-15

Wimpenny P, Gass J (2000) Interviewing in phenomenology and grounded theory: is there a difference? J Adv Nurs 31:1485–1492. https://doi.org/10.1046/j.1365-2648.2000.01431.x

von Wintersdorff CJH, Penders J, van Niekerk JM et al (2016) Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.00173

Wright GD (2016) Antibiotic adjuvants: rescuing antibiotics from resistance. Trends Microbiol 24:862–871. https://doi.org/10.1016/j.tim.2016.06.009

Wu M-C, Lin T-L, Hsieh P-F et al (2011) Isolation of genes involved in biofilm formation of a Klebsiella pneumoniae strain causing pyogenic liver abscess. PLoS One 6:e23500. https://doi.org/10.1371/journal.pone.0023500

Wu H, Moser C, Wang H-Z et al (2015) Strategies for combating bacterial biofilm infections. Int J Oral Sci 7:1–7. https://doi.org/10.1038/ijos.2014.65

Yelin I, Kishony R (2018) Antibiotic resistance. Cell 172:1136–1136.e1. https://doi.org/10.1016/j.cell.2018.02.018

Yin W, Wang Y, Liu L, He J (2019) Biofilms: the microbial "protective clothing" in extreme environments. Int J Mol Sci 20:3423. https://doi.org/10.3390/ijms20143423

Yonezawa H, Osaki T, Hanawa T et al (2013) Impact of helicobacter pylori biofilm formation on clarithromycin susceptibility and generation of resistance mutations. PLoS One 8:e73301. https://doi.org/10.1371/journal.pone.0073301

Zhang L, Mah T-F (2008) Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J Bacteriol 190:4447–4452. https://doi.org/10.1128/JB.01655-07

Zhou T-C, Zhong J-J (2015) Production of validamycin a from hemicellulose hydrolysate by Streptomyces hygroscopicus 5008. Bioresour Technol 175:160–166. https://doi.org/10.1016/j.biortech.2014.10.051

Zhou L, Tan Y, Kang M et al (2017) Preliminary epidemiology of human infections with highly pathogenic avian influenza a(H7N9) virus, China, 2017. Emerg Infect Dis 23:1355–1359. https://doi.org/10.3201/eid2308.170640

Part V

From Basic to Newer Therapeutic Options

β-Lactamase Inhibitor Combinations Targeting Antibiotic Resistance in Gram-Negative Bacteria

16

Nabeela Farhat and Asad U. Khan

Abstract

Antibiotic resistance induced by β-lactamase is posing a significant problem to the pharmaceutical industry. For the last 30 years, only a few inhibitors of β-lactamase were released in the market. The first-generation β-lactamase inhibitors include sulbactam, tazobactam, and clavulanic acid which are derivatives of β-lactams. They mainly function by serine β-lactamases (SBL) inactivation. The next generation of inhibitors of β-lactamase (such as vaborbactam and avibactam) are non-β-lactam derivatives with a broad inhibition range (including KPC, a major class A carbapenemase). Current inhibitors, on the other hand, are resistant to all clinically significant class B β-lactamases and certain class D β-lactamases. This chapter gives information on current research activities aimed at discovering and developing new β-lactamase inhibitors, as well as a summary of recent β lactam/lactamase inhibitor combinations approved by FDA.

Keywords

β-Lactams · Inhibitors · Multiresistant bacteria · Combination therapy

16.1 Introduction

The antibiotic discovery has succeeded in curing diseases which were earlier deadly, resulting in increased life expectancy, but now it has less potential because of the emergence and spread of multiresistant bacteria worldwide (Gilbert 2018; Sommer

N. Farhat · A. U. Khan (✉)
Medical Microbiology and Molecular Biology Lab, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_16

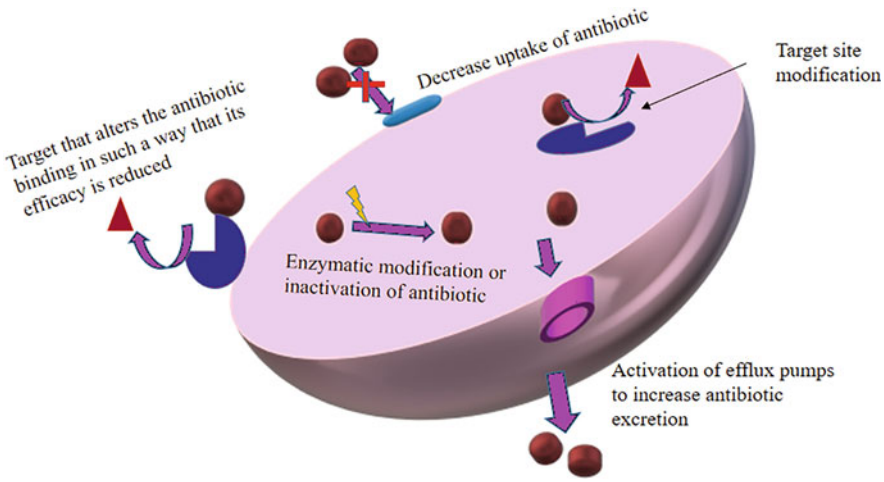


Fig. 16.1 Bacterial resistance mechanism (1) cellular target modification; (2) alteration in the permeability of the outer membrane; (3) activation of efflux pump; and (4) inactive forms of drug by enzymatic degradation

et al. 2017; Brown and Wright 2016; Bengtsson-Palme et al. 2018; Satyanarayana 2018; McKenna 2013; Martinez 2014; Levin-Reisman et al. 2017; Vikesland et al. 2019; Thayer 2016). The bacteria possess innate ability to resist treatment and transmit genetic material to other bacteria, making it drug resistant (D'Costa et al. 2011). Now resistant bacterial infections are very common, and some of the bacteria have become multidrug resistant, in such cases very few or no treatment options are present. Current condition has been reached because of (1) the excessive and inappropriate usage of antibiotics in different field over the years like medicine, the veterinary and agriculture. These drugs are used even for nonbacterial infections treatment; (2) lack of development of new antibiotics due to not getting adequate profit; and (3) absence of new and inventive strategies of new antibiotics development (Wright 2007; Theuretzbacher 2016).

In current situation, the most effective strategy for multidrug-resistant bacterial infections treatment is the combination therapy. This type of combination therapy has two components an antibiotic and resistance mechanism inhibitor, restoring the effectiveness of antibiotic (Brown 2015; Douafer et al. 2019; Chakradhar 2016; Gill et al. 2015; Kalan and Wright 2011; Farha and Brown 2013;Bush 2015; Worthington and Melander 2013; Wright 2016; Melander and Melander 2017; Schillaci et al. 2017; Tyers and Wright 2019; Docquier et al. 2018; Tooke et al. 2019; Laws et al. 2019). Benefit with the use of inhibitors are: (1) it repurposes the drugs clinically available which were safe and effective, and (2) it avoids efforts, challenges, and expenses of new therapeutic targets validation and identification (Tyers and Wright 2019).

The bacterial resistance mechanisms as shown in Fig. 16.1 are (1) cellular target modification altering binding of antibiotic resulting in reduced efficacy; (2) changes

in the terminal moieties results in alteration of the outer membrane permeability which in turns decreased uptake; (3) increase antibiotic ejection due to activation of efflux pump; and (4) generation of inactive forms of drug by bacterial enzymes degradation (Khameneh et al. 2016).

The classification of inhibitors for overcoming bacterial resistance mechanisms are:

(a) Inhibitors of efflux pump;
(b) Permeabilizers of outer membrane; and
(c) Inhibitors of β-lactamase.

This chapter emphasizes on the inhibitors of β-lactamase, which overcome predominant source of drug resistance in Gram-negative bacteria (that is β-lactam antibiotic inactivation due to the production of β-lactamases). β-Lactam antibiotics safety, efficacy and broad-spectrum activity makes it the most commonly prescribed antibiotic in the clinical setting. The four main classes of β-lactams containing four-membered azetidinone ring structure are penicillins, cephalosporins, carbapenems and monobactams. Their mechanism of action is also similar involving transpeptidases inactivation. These transpeptidases are essential for the synthesis of cell wall terminal step. These transpeptidases are included in penicillin-binding proteins (PBPs), β-lactam acylate active site serine residue, which is irreversible resulting in inactivation of β-lactam antibiotics due to the production of β-lactamases.

Active site of β-lactamases contains either one divalent zinc atom (MBLs) or a serine residue (SBL). Fig. 16.2 shows the active sites for SHV beta-lactamase and NDM-1 metallo-beta-lactamase. Arrival of 'penicillinase-stable' penicillins shifts importance towards β-lactamases encoded by both plasmid and chromosome in Gram-negative bacteria. Presently, over 2770 different, naturally occurring β-lactamases have been reported (Levin-Reisman et al. 2017).

16.2 β-Lactamase Nomenclature

Both the functional approach (Bush-Jacoby-Medeiros) and the structural approach (Ambler) (Ambler 1980) are used for the β-lactamase classification. Therefore, the focus of this review is β-lactamases description based on the Ambler classification.

Class A (serine carbapenemases). Class A includes common β-lactamases like SHV and TEM that inactivate penicillins and narrow spectrum cephalosporins. Many of them, in addition to CTX-M family, are effective against extended-spectrum β-lactams, which is the reason for them being referred as extended-spectrum β-lactamases (ESBLs). Carbapenemases like KPC, IMI and SME (Drawz et al. 2010; Bush 2018) are also included as class A.

Class B metallo-β-lactamases. Metalloenzyme active site contains zinc ions, and so, class B β-lactamases are also considered as metalloenzymes. Unlike class A, C or

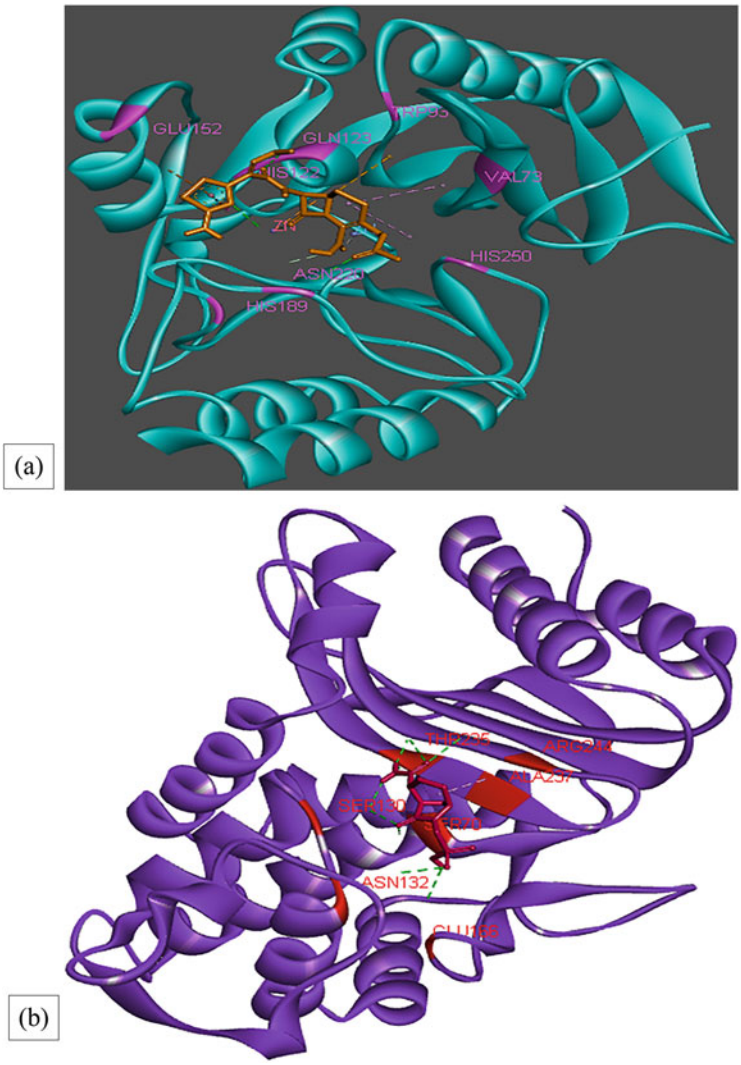


Fig. 16.2 Active site of β-lactamases: (**a**) NDM (metallo-β-lactamases (MBLs)), (**b**) SHV (serine β-lactamases)

D that hydrolyses β-lactams by serine nucleophile action, metallo-β-lactamases serve as nucleophile by water molecule present in the active site. By coordinating with zinc, water molecule is activated, hydrolysing the β-lactam ring making antibiotic ineffective. Except for monobactams, all classes of β-lactams are hydrolysed by class B metallo-β-lactamases (MBLs). Most clinically important MBLs possessing carbapenemase activity are VIM and IMP along with rapidly emerging NDM (Wang and Chou 2013; Shakil et al. 2011; Johnson and Woodford 2013; Wang et al. 1999).

Class C serine cephalosporinases. ACT, CMY and DHA are also some representations of class C (AmpC β-lactamases), and Gram-negative bacteria yielding class C enzymes are frequently resistant to certain cephalosporins and penicillins.

Class D serine oxacillinases. Class D comprises OXA, few are cephalosporin, carbapenem and penicillin resistant. However, occurrence of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* producing OXA is of great alarm (Potron et al. 2015).

16.3 β-Lactamase Inhibitors

Among the most prevalent resistance mechanisms in Gram-negative bacteria is the production of β-lactamases (Neu 1990; Prabaker and Weinstein 2011; Harris et al. 2015). In the mid-1970s, for the identification of a potent inhibitor of the enzyme TEM-1 β-lactamase, many pharmaceutical companies began many research (Eisenstein et al. 1977; Medeiros and O'Brien 1975).

The selection of β-lactam which has pharmacokinetic properties similar to the inhibitor is the utmost important consideration in developing a BL–BLI combination. Combinations of β-lactam with BLI introduced into clinical practice are clavulanic acid–amoxicillin (oral bioavailable agent); sulbactam–ampicillin (parenterally administered for the therapy of intra-abdominal infections and urinary tract infections (UTIs)); clavulanic acid–ticarcillin (for parenteral delivery covering infections caused by Gram-negative bacteria) and piperacillin–tazobactam (cover staphylococcal infections, Gram-negative and pseudomonal infections including soft tissue and lower respiratory tract infections and intra-abdominal infections among inpatients) (Charbonneau 1994).

Optimal dosing of a BL–BLI combination is patient specific. It is dependent on the pathogen, β-lactamase involved, β-lactamase transcription, the microbial load at the infection site, the engagement of other mechanisms of resistance, the effectiveness of the inhibitor and β-lactam, the pharmacokinetics/biodistribution of the β-lactamase inhibitor and β-lactam (FDA 2014).

Some of the β-lactamase inhibitors are summarized in Table 16.1 along with their chemical class, trade names and clinical developmental stages.

16.4 β-Lactamase Inhibitors: Mechanistic Considerations

An effective strategy for fighting resistance mediated by β-lactamase is using agents that bind to the active site (commonly β-lactams). This can be accomplished in two ways. The first method is to develop substrates that can bind the enzyme with great affinity reversibly and/or irreversibly, forming unfavourable steric interactions as the acyl-enzyme forms acyl-enzymes and accept catalytically inept conformations that are hydrolysed poorly, such as carbapenems, extended-spectrum cephalosporins or monobactams. The other technique is the creation of mechanism-based or

Table 16.1 Inhibitors in clinical development phases

Inhibitor	Chemical class	Clinical development stage	Trade name
Clavulanic acid	Clavam	Approved in combination with amoxicillin Approved in combination with ticarcillin	Augmentin Timentin
Sulbactam	Penicillanic acid sulfone	Approved in combination with ampicillin	Unasyn
Tazobactam	Penicillanic acid sulfone	Approved in combination with piperacillin Approved in combination with cefepime Approved ceftolozane	Zosyn/ Tazocin – Zerbaxa
Enmetazobactam	Penicillanic acid sulfone	Phase II in combination with cefepime	
Avibactam	DBO	Approved in combination with ceftazidime Phase III in combination with aztreonam Phase II in combination with ceftaroline fosamil	Avycaz/ Zavicefta
Relebactam	DBO	Phase III in combination with imipenem	
Nacubactam	DBO	Phase I in combination with meropenem	
Zidebactam	DBO	Phase I in combination with cefepime	
ETX2514	DBO	Phase II in combination with sulbactam	
Vaborbactam	Boronic acid	Approved in combination with meropenem Phase I in combination with biapenem	Vabomere
VNRX-5133	Boronic acid	Phase I in combination with cefepime	
ANT431	Pyridine-2-carboxylic acid	Preclinical in combination with meropenem	
AAI101	Penam sulfone	Phase II in combination with cefepime	

irreversible ‘suicide inhibitors’, which can irreversibly inactivate the β-lactamase by causing secondary chemical events in the enzyme active site (Bush 1988). Inhibitors of class A, such as clavulanic acid, sulbactam and tazobactam, are examples. They are characterized by first-order rate constants for inhibition (kinact, the inactivation rate attained at ‘infinite’ concentration of inhibitor) and KI values (the inhibitor concentration results in an inactivation rate that is half the value of kinact) and KI values (the inhibitor concentration yields an inactivation rate that is half the value of kinact). Bush (1986) and Copeland (2005) are two examples of this. The IC50 (50%

inhibitory concentration) is the inhibitor concentration necessary to reduce enzyme activity by 50% as compared to unregulated velocity. The IC50 value reveals the inhibitor's affinity or the kcat/kinact ratio, although these values are not always the same. An inhibitor can have low 'affinity' and acylate the enzyme slowly, but still have a low IC50 due to very low deacylation rates.

16.5 Recent BL/BLI Combinations Approved by FDA

16.5.1 Vabomere

Vaborbactam (RPX7009) contains a cyclic boronate pharmacophore and is the first β-lactamase inhibitor which is approved by FDA (Lee et al. 2018; Jorgensen and Rybak 2018; Farrell et al. 2013; Cho et al. 2018). Vaborbactam forms a covalent adduct with CTX-M-15 and AmpC catalytic serine residue, and it is confirmed by X-ray crystallography studies. Vaborbactam inhibits a variety of β-lactamases with sub-μM IC50 values (Class A/C enzymes) (Hecker et al. 2015). And over 300 clinical strains of *Enterobacteriaceae* (greater part of which contained the KPC genes) were used to test the vaborbactam and meropenem combination. A fixed activity of meropenem was potentiated by vaborbactam (8 μg/mL) by a minimum of 64-fold, giving rise to MIC50 of $\leq$0.06 and MIC90 of 1 μg/mL, respectively (Castanheira et al. 2016). Lomovskaya and co-workers used clone *E. coli* strains which produce Ambler β-lactamases of all four classes to check vaborbactam's capability of potentiating various antibiotics (Lomovskaya et al. 2017). Most of the Ambler class A and C β-lactamase-producing strains are susceptible to meropenem. Addition of ceftazidime and aztreonam fully characterizes inhibitory activity spectral range of vaborbactam combinations. Their findings disclose that when 4 μg/mL vaborbactam is combined with aztreonam, meropenem or ceftazidime, it has a wide range of synergistic activity against *Escherichia coli*, producing β-lactamases of Ambler class A including KPC, SME, etc., and class C including DHA, MIR, FOX, etc. Meropenem-vaborbactam demonstrated promising in vitro activity that resulted in clinical studies indicating its effectiveness, safety and reduced side effects (Griffith et al. 2016; Kaye et al. 2018). Vaborbactam-meropenem (Vabomere) was FDA authorized in 2017 for UTIs and is promoted as injectables by Melinta therapeutics with 1:1 ratio of meropenem and vaborbactam (vabomere, www.Vabomere.com). Presently, new combinations of vaborbactam and antibiotic are under clinical assessment.

16.5.2 Avycaz

The combination of avibactam/ceftazidime is marketed as Avycaz. It received FDA approval in 2015 for the treatment of problematic intra-abdominal infection (cIAI) and complex urinary tract infection (cUTI). Ehmann and colleagues discovered that avibactam (formerly known as NXL104) has a mechanism based on TEM-1

covalent inhibition with slow regeneration of the inhibitor using a range of biophysical techniques. Testing avibactam against *P. aeruginosa* PAO1 AmpC, *Enterobacter cloacae* P99 AmpC, class A (TEM-1, KPC-2, CTX-M-15) and class D (OXA-48, OXA-10) revealed that avibactam inhibits enzymes through acylation followed by delayed release of inhibitor by cyclization (Ehmann et al. 2013); however, in KPC-2, inhibition mechanism of avibactam was found to be different and recyclization competes with desulphation following further degradation steps (Ehmann et al. 2013). A number of in vitro studies of avibactam activity in combo with carbapenems, cephalosporins and monobactams against both Gram-positive and Gram-negative pathogens are reported. *P. aeruginosa* clinical isolates tested reduced the MIC90 of ceftazidime in combination with avibactam (4 μg/mL) to a greater extent than clavulanic acid and tazobactam. Another study reported ceftazidime–avibactam showed poor potency against *A. baumannii* strains producing PER-1, OXA-51 and OXA-58, whereas favourable effectiveness was detected against CTX-M-15 or OXA-48 producing *Klebsiella pneumoniae* strains and CTX-M-15 producing *E. coli* strains (Aktaş et al. 2012).

16.5.3 Zerbaxa

In 2014, Zerbaxa was FDA approved and recognized as β-lactamase inhibitor for the BL/BLI combination of antibiotic ceftolozane and tazobactam. Ceftolozane was discovered by the efforts of medicinal chemistry, aiming to develop cephalosporin-enhanced effectiveness against *P. aeruginosa* AmpC strains (Toda et al. 2008; Murano et al. 2008; Takeda et al. 2007). The inhibition activity of tazobactam is maximum against CTX-M, TEM, SHV enzymes (Drawz et al. 2010). Tazobactam in combination with ceftolozane strongly increases the ceftolozane spectrum activity against AmpC-hyperproducing and ESBL-producing Gram-negative bacteria. KPC producing strains were not susceptible to the combination (Livermore et al. 2010) Farrel and colleagues reported that ceftolozane–tazobactam (TOL-TAZ) demonstrated potent activity, after testing 7071 *Enterobacteriaceae* isolated from hospitals in the United States. The combination of ceftolozane–tazobactam was also found to be effective against ESBL phenotype producing *E. coli* isolates, as well as 1971 tested *P. aeruginosa* isolates and 2435 *P. aeruginosa* strains in Canadian hospitals (Walkty et al. 2013). About 605 anaerobic isolates were obtained by Snydman and colleagues. They reported that TOL-TAZ has a great activity against *Bacteroides* spp. (particularly *Bacteroides fragilis*), exceptional activity against *Prevotella* spp. and *Fusobacterium* spp., and very low activity against *Clostridium* spp., according to the researchers (Snydman et al. 2014). TOL-TAZ with metronidazole was tested in patients with severe intra-abdominal infections in the ASPECT-cIAI phase III clinical trial (cIAI) (Solomkin et al. 2015). The mix is effective against *Enterobacteriaceae* infections that produce CTX-M-type ESBLs. Another phase III clinical trial, ASPECT-cUTI, was done to test the efficacy of TOL-TAZ with levofloxacin in the treatment of cUTI, including pyelonephritis. TOL-TAZ was found to be more potent than levofloxacin, with just

minor side effects (Wagenlehner et al. 2015). Merck makes Zerbaxa, a powder for injection that contains a 2:1 blend of ceftolozane and tazobactam.

16.6 Ongoing Developments of SBL Inhibitors

Many SBLI clinical trials have been active for the past 10 years, and these can be categorized into β-lactams and non-β-lactams. Clavulanic acid, sulbactam and tazobactam are examples of β-lactam inhibitors. The phase II clinical trial for the tazobactam structural analogue AAI101 was successfully completed (EudraCT Number: 2016-005161-31). Diazabicyclooctanes (defined by avibactam) and cyclic boronates (defined by vaborbactam) have been added to the list.

16.6.1 β-Lactams

AAI101 is being tested in clinical studies in combination with cefepime (EudraCT Number: 2016-005161-31). The outcomes of MIC screening shows synergy for the combination of cefepime and AAI101 at various concentration against carbapenem-resistance *E. coli* and *K. pneumoniae* strains. Pattanaik and co-workers found penicillin sulfone inhibitor LN-1-255 inactivates SBLs like SHV-1 and SHV-2 (class A) and increases the activity of ceftazidime against TEM, SHV, CTX-M and Sme-1 enzymes producing strains (Pattanaik et al. 2009). Crystallographic evidence for SHV-1 confirms that LN-1-255 acylates the enzyme before rearrangement to a bicyclic indolizine adduct (Pattanaik et al. 2009). It also has potential efficacy against several OXA enzymes and has been shown to lower carbapenem MICs in OXA-producing *E. coli, K. pneumoniae* and *A. baumannii* strains (Drawz et al. 2010; Vallejo et al. 2016; Vázquez-Ucha et al. 2017).

16.6.2 Diazabicyclooctanes

Relebactam. Zhanel and co-workers reported that the analogue of diazabicyclooctane (DBO) relebactam has β-lactamase inhibition activity like an SBL inhibitor avibactam. Relebactam is effective against class B and class D OXA-type enzymes, but not against Ambler class A β-lactamases like KPC carbapenemase and class C (Zhanel et al. 2018). Susceptibility screenings with relebactam (4 μg/mL) and imipenem show that this inhibition scale is well shown against Gram-negative clinical isolates (Lapuebla et al. 2015). According to the Gram-negative isolates from the US and European hospitals, *A. baumannii* and other metallo-beta-lactamases or OXA-type enzymes produced pose a challenge for imipenem-relebactam use (Lob et al. 2017; Karlowsky et al. 2017). Phase III clinical evaluation of relebactam combination with imipenem–cilastatin is presently in progress (ClinicalTrials.gov identifier: NCT03293485).

Zidebactam. PBP inhibitors include the acyl hydrazide DBO, a zidebactam analogue of the DBO family. The zidebactam and cefepime combination efficiently inhibits the PAO1 *P. aeruginosa* strain and its knockouts with faulty porins (Karlowsky et al. 2017). The increased activity of zidebactam–aztreonam combination with selected β-lactams against VIM-1/VIM-2 *P. aeruginosa* producing clones was also intriguing (Karlowsky et al. 2017). Likewise, zidebactam (8 μg/mL) has been shown to lower cefepime and sulbactam MIC against *A. baumannii* which produce OXA-23. PBP (but not β-lactamase) is inhibited by zidebactam, which improves antibiotic efficacy. At this time, two phase I clinical trials assessing zidebactam's safety, tolerability and pharmacokinetics have already been finished, with a third study presently attempting to recruit patients.

Nacubactam. CTX-M, TEM, KPC-2 (class A), AmpC, CMY-2 (class C) and PBP2 are all inhibited by nacubactam (OP0595), an aminoethoxy substituted analogue of avibactam. It weakly inhibits OXA enzymes and does not show any activity against IMP-1 (Morinaka et al. 2015). However, when tested alone, nacubactam is found to have antibacterial activity (Morinaka et al. 2016, 2017; Livermore et al. 2015). In comparison to ceftazidime–avibactam, nacubactam significantly increased the activity of aztreonam, cefepime, biapenem and piperacillin against *Enterobacteriaceae* producing carbapenemases (KPC, OXA-48 and MBLs) in a concentration-dependent manner (Livermore et al. 2015).

ETX2514, a reversible DBO inhibitor, was explicitly designed for class D, class A and class C β-lactamases inhibition (Shapiro et al. 2017; Durand-Réville et al. 2017). ETX2514 and OXA-24 molecular modelling shows tighter covalent binding at the active site as compared to avibactam (Durand-Réville et al. 2017). *Acinetobacter* spp. producing OXA β-lactamases are targeted by using ETX2514. ETX2514 is used in combination with sulbactam, making it more potent as sulbactam has exceptional characteristic feature of binding to important PBPs in *H. influenza*, *N. gonorrhoeae* and *Acinetobacter* spp., and exhibits better activity against multidrug-resistant *A. baumannii* (Durand-Réville et al. 2017; Higgins et al. 2004). Sulbactam–ETX2514 has completed a phase II clinical trial for safety and efficacy in cUTIs as a narrow-spectrum agent targeting *Acinetobacter* spp.

16.6.3 Boronates

When the nucleophilic serine of β-lactamases attacks β-lactam ring, it generates a tetrahedral intermediate that looks like boronate-based β-lactamase inhibitors (Ke et al. 2011). As a result, these BLIs are known as boronic acid transition-state inhibitors (BATSIs) (Trippier and McGuigan 2010). Various boronates, such as acyclic boronic acids, are examined for SBL inhibition (Powers et al. 2014; Nguyen et al. 2016; Bouza et al. 2018) or cyclic boronate analogues (Werner et al. 2017; Brem et al. 2016a, b; Cahill et al. 2017). Acyclic boronic acids show inhibition of class B VIM-2 enzyme as well as some SBLs (Bonomo et al. 2017). Cyclic boronates also show sub-μM IC50 values for both SBLs and MBLs (Reddy et al. 2014, 2016; Burns et al. 2014a, b, 2016). Brem and co-workers after screening a

number of cyclic boronates found a number of SBL inhibitors which have potency against MBLs, specially VIM-2 and NDM-1 (Brem et al. 2016a, b). X-ray crystallographic investigations on BcII, OXA-10, PBP-5 and VIM-2 have revealed that the cyclic boronate comes in contact with the critical residues of β-lactamase and coordinates with metallo beta-lactamases Zn^{2+}, reflecting the high energy transition state intermediates (Brem et al. 2016a, b).

Vaborbactam. The first BLI developed from boronic acid is vaborbactam (formerly known as RPX7009). It has activity against other class A and class C β-lactamases but was specifically designed to inhibit KPC carbapenemases and functions as a competitive inhibitor by forming a reversible covalent bond with the targeted β-lactamase (Burns et al. 2016). Recent approval to use of Meropenem–vaborbactam passed phase III clinical trials for UTIs (Burns et al. 2014a, b; Kaye et al. 2018).

VRNX-5133. A new boronic acid BLI is in experimental development. It is used against NDM (class B MBLs) in CRE and *P. aeruginosa* (Mushtaq et al. 2018). CTX-M-15-VNRX-5133 and VIM-2-VNRX-5133 combination with X-ray crystal structure showed that the inhibitor was bound covalently to CTX-M-15 (catalytic serine residue) and the boron hydroxyl groups interacted with the conserved Asn233 of VIM-2 and Zn1 (Docquier et al. 2018). Both in vitro and in vivo activity, the combination of cefepime–VNRX-5133 has shown good results, against ESBL-producers and *P. aeruginosa* and carbapenemase-producing *Enterobacteriaceae* (Donnelly et al. 2018; Weiss et al. 2018; Georgiou et al. 2018).

16.6.4 Other Inhibitors

Entmetazobactam. It was earlier known as AAI101 and is a methylated penicillanic acid sulfone BLI that shows activity against many serine β-lactamases, particularly ESBLs. Enmetazobactam cefepime combination showed good in vitro activity against *E. coli* strains producing clinically relevant β-lactamases and *Enterobacteriaceae* producing ESBL clinical isolates (Papp-Wallace et al. 2017; Huband et al. 2018) According to one study, the combination of cefepime–enmetazobactam was eight times more potent than piperacillin–tazobactam. It also showed good in vivo efficacy by reducing neutropenic mouse bacterial infection >0.5 log10 CFUs among 12 out of 20 carbapenemase-producing *E. coli* and *K. pneumoniae* isolates (Crandon and Nicolau 2015). In cUTIs, enmetazobactam has successfully completed a phase II clinical trial.

ANT431, a novel pyridine-2-carboxylic acid ANT431 was specially designed for MBL inhibition, and in a biochemical assay, the inhibitory activity against purified NDM-1 and VIM-2 was sub-micromolar. It is currently in preclinical development (Everett et al. 2018). ANT431 meropenem combination shows good activity against many NDM-1 producing *Enterobacteriaceae* clinical strains. It shows low potency against other variants producing MBL (Everett et al. 2018).

16.7 Ongoing Development of MBL Inhibitors Based on Zinc Chelation

As serine β-lactamases and metallo-β-lactamases, the classification of β-lactamases is based on catalytic activities. MBL active site contains zinc ion(s) which is stabilized by aspartate residues, cysteine and histidine. The zinc ion responsible for hydrolysis of the β-lactams is also attached to an active molecule of water. The subclasses of MBLs are further subdivided into B1, B2 and B3, containing two zinc ions are class B1 and B3 and B2 contains just one (Somboro et al. 2018; Wang and Chou 2013). Class B1 includes NDM, IMP and VIM enzymes which have a low affinity for monobactams and so are the most clinically relevant MBLs as they deactivate a wide range of β-lactams (King and Strynadka 2013). Gram-negative bacteria caused infection's treatment, MBLs poses a serious test due to their rapid dissemination and carbapenemase activity. Designing and developing broad-spectrum MBL inhibitors become a big challenge for this family because of the high active site (Drawz et al. 2010; Wang et al. 1999; Fast and Sutton 2013; Ju et al. 2018). There are currently no specific MBL inhibitors in clinical use.

Klingler and co-workers reported thiorphan (the active metabolite of the antidiarrheal racecadotril) inhibits low-μM IC50 producing VIM-1, NDM-1 and IMP-7 and increases the imipenem activity against MBL-producers. An anti-hypertensive drug named Captopril (FDA-approved) inhibits NDM-1 (IC50 = 7.9 μM) (Klingler et al. 2015). As reported by Brem and colleagues, the D-captopril MBL inhibition as opposed to its other stereoisomers is greater when valued against NDM-1, BCII, SPM-1,VIM-2, and IMP-1, X-ray crystallography further supported these findings (Brem et al. 2016a, b).

In MBL catalytic activity, Zn^{2+} plays an important role, and a number of chelating agents are reported as inhibitor of this enzyme class resulting in bringing back the efficiency of β-lactam antibiotics against pathogens producing MBL. King and co-workers reported aspergillomarasmine A (AMA) as inhibitor of NDM-1 (IC50 = 4.0 μM) and VIM-2 (IC50 = 9.6 μM) after screening fungal extract collection for meropenem synergistic activity using a phenotypic assay (King et al. 2014). AMA is shown to reduce the MIC of meropenem against NDM and VIM producing Gram-negative bacteria (5 to $\leq$2 μg/mL). It also shows encouraging results in vivo (King et al. 2014). Presently multiple chemical (Liao et al. 2016; Koteva et al. 2016; Albu et al. 2016; Zhang et al. 2017a, b) and chemoenzymatic (Fu et al. 2018) methodologies have been developed for the synthetization of AMA and its closely associated analogues, and it was reported that the AMA diastereomers also had comparable actions against VIM-2 and NDM-1 (Koteva et al. 2016). Metal-chelators described as MBL inhibitors are 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and their analogues. Against NDM, IMP or VIM producing Gram-negative strains, these have an ability to potentiate carbapenems (Somboro et al. 2014; Zhang et al. 2018). Likewise, the *N*,*N*,*N*′,*N*′-tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) shows synergy with β-lactam antibiotics acting as MBL inhibitor (Azumah et al. 2016), in vitro it is explained as nonhemolytic and nonhazardous to mammalian

cells, but for clinical application, there is a risk because of lack in target specificity. Some sulphonamides possess anti-MBL activity reported by researchers at Merck. They have also assessed numerous patent compounds such as 2-tetrazolylbenzenesulfonamides (Giacobbe et al. 2018; Mandal et al. 2016; Bennett et al. 2016) as effective inhibitors of VIM-1, IMP-1 and NDM-1. Furthermore, the discovery by screening approaches of MBL inhibitors, other compounds also possess MBL inhibitory activity such as the β-lactam antibiotic cefaclor (Thomas et al. 2014), 3-formylchromone (Christopeit et al. 2016), ebselen (Chiou et al. 2015), many hydrazones (Brindisi et al. 2016), phosphonic acids (Zhang et al. 2017a, b), oxoisoindolines (Li et al. 2017), diphenylpyrroles (McGeary et al. 2017) and bismuth complexes (Wang et al. 2018).

Antabio Inc. reports a discovery of ANT431 (small sulphonamide molecule) which evolved from 2-picolinic acid (47) (Everett et al. 2018). It showed strong inhibition of VIM-2 (Ki = 0.19 μM) and NDM-1 (Ki = 0.29 μM) and potentiate meropenem activity against the BL21 *E. coli* producing these enzymes. ANT431 was tested against 94 clinical isolates of *Enterobacteriaceae* family that produce MBL, and 72% isolates were resensitized to meropenem when used at 30 μg/mL. It is also reported to show good in vivo efficacy of NDM-1 producing *E. coli* infection in a mouse model (Everett et al. 2018).

16.8 Conclusion and Outlook

In Gram-negative bacteria, the most common causes for β-lactam resistance are ESBLs, serine carbapenemases, hyperproduced AmpC cephalosporinases and MBLs For the counteraction of many organisms which produces serine β-lactamases, the new BLIs like DBO and boronic acid are helpful. The compounds of the advanced generation DBO analogues are successful in achieving broad-spectrum SBL inhibitors, and D OXA enzymes and PBPs are clinically covered important class. The analogues of cyclic boronate could lead to broad-spectrum β-lactamase inhibitors due to its structural similarity to the common transition state of SBL- and MBL-mediated β-lactam hydrolysis. Designing inhibitors with Site-specificity (as for metal chelators) and physiological stability (i.e. for thiol-based inhibitors), inhibition of class B MBLs is a great current challenge. MBL inhibitors like ANT431 are still in some preclinical trial, but it is not required by them to go on for a complete development for phase II or III clinical protocol. For the upcoming times, we can believe for the emergence of other unique β-lactamases together with the current MBLs which will have intrinsic resistance towards the new inhibitors. So, the cyclic boronate taniborbactam (boron-based compounds), against a wide range of serine-β-lactamases, when shared with cefepime proved to have a broad-spectrum of activity, as well as relevant metallo-β-lactamases, representing the current hope.

References

Aktaş Z, Kayacan C, Oncul O (2012) Int J Antimicrob Agents 39:86–89
Albu SA, Koteva K, King AM, Al-Karmi S, Wright GD, Capretta A (2016) Angew Chem Int Ed 55: 13259–13262
Ambler RP (1980) Philos Trans R Soc Lond Ser B Biol Sci 289:321–331
Azumah R, Dutta J, Somboro AM, Ramtahal M, Chonco L, Parboosing R, Bester LA, Kruger HG, Naicker T, Essack SY, Govender T (2016) J Appl Microbiol 120:860–867
Bengtsson-Palme J, Kristiansson E, Larsson DGJ (2018) Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev 42:fux053
Bennett J, Jiang A, Pasternak S, Dong X, Gu J, Scott D, Tang H, Zhao Z, Huang Y, Hunter D, Yang D, Zhang Z, Fu J, Bai Y, Zheng Z, Zhang X, Young K, Xiao L (2016) WO Patent, 2016206101A1
Bonomo R, Prati F, Caselli E, Romagnoli C (2017) US Patent, 20170065626A1
Marshall BM, Ochieng DJ, Levy SB (2009) Commensals: underappreciated reservoirs of resistance. Microbe 4:231–238
Bouza HC, Swanson KA, Smolen AL, Vandine MA, Taracila C, Romagnoli E, Caselli F, Prati RA, Bonomo RA, Powers WBJ (2018) Structure-Based Analysis of Boronic Acids as Inhibitors of Acinetobacter-Derived Cephalosporinase-7, a Unique Class C β-Lactamase. ACS Infect Dis 4:325–336
Brem J, Cain R, Cahill S, McDonough MA, Clifton IJ, Jiménez-Castellanos JC, Avison MB, Spencer J, Fishwick CWG, Schofield CJ (2016a) Structural basis of metallo-β-lactamase, serine-β-lactamase and penicillin-binding protein inhibition by cyclic boronates. Nat Commun 7:12406
Brem J, Van Berkel SS, Zollman D, Lee SY, Gileadi O, McHugh PJ, Walsh TR, McDonough MA, Schofield CJ (2016b) Structural Basis of Metallo-β-Lactamase Inhibition by Captopril Stereoisomers. Antimicrob Agents Chemother 60:142–150
Brindisi M, Brogi S, Giovani S, Gemma S, Lamponi S, De Luca F, Novellino E, Campiani G, Docquier JD, Butini S (2016) Targeting clinically-relevant metallo-β-lactamases: from high-throughput docking to broad-spectrum inhibitors. J Enzyme Inhib Med Chem 31:98–109
Brown D (2015) Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? Nat Rev Drug Discov 14:821–832
Brown ED, Wright GD (2016) Antibacterial drug discovery in the resistance era. Nature 529:336–343
Burns J, Daigle D, Liu B, Mcgarry D, Pevear DC, Lee Trout RE (2014a) WO Patent, 2014151958A1
Burns J, Daigle D, Liu B, McGarry D, Pevear DC, Lee Trout RE, Jackson RW (2014b) US Patent, US20140194386A1
Burns J, Daigle D, Liu B, Jackson RW, Hamrick J, McGarry D, Pevear DC, Lee Trout RE (2016) US Patent, 20160264598A1
Bush K (1986) Evaluation of enzyme inhibition data in screening for new drugs. Drugs Exp Clin Res 12:565–576
Bush K (1988) β-Lactamase inhibitors from laboratory to clinic. Clin Microbiol Rev 1:109–123
Bush K (2015) Investigational agents for the treatment of Gram negative bacterial infections: a reality check. ACS Infect Dis 1:509–511
Bush K (2018) Past and present perspectives on β-lactamases. Antimicrob Agents Chemother 62: e01076–e01018
Cahill ST, Cain R, Wang DY, Lohans CT, Wareham DW, Oswin HP, Mohammed J, Spencer J, Fishwick CWG, McDonough MA, Schofield CJ, Brema J (2017) Antimicrob Agents Chemother 61:e02260–e02216
Castanheira M, Rhomberg PR, Flamm RK, Jones RN (2016) Antimicrob Agents Chemother 60: 5454–5458

Chakradhar S (2016) Whats old is new: reconfiguring known antibiotics to fight drug resistance. Nat Med 22:1197–1198
Charbonneau P (1994) Review of piperacillin/tazobactam in the treatment of bacteremic infections and summary of clinical efficacy. Intensive Care Med 20(suppl 3):S43–S48
Chiou J, Wan S, Chan K-F, So P-K, He D, Chan EW, Chan T, Wong K, Tao J, Chen S (2015) Chem Commun 51:9543–9546
Cho JC, Zmarlicka MT, Shaeer KM, Pardo J (2018) Ann Pharmacother 52:769–779
Christopeit T, Albert A, Leiros HKS (2016) Bioorg Med Chem 24:2947–2953
Copeland RA (2005) Evaluation of enzyme inhibitors in drug discovery. Wiley, New York
Crandon JL, Nicolau DP (2015) In vivo activities of simulated human doses of cefepime and cefepimeAAI101 against multidrug-resistant gram-negative Enterobacteriaceae. Antimicrob Agents Chemother 59:2688–2694
D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD (2011) Antibiotic resistance is ancient. Nature 477:457–461
Docquier J-D et al (2018) Structural basis for serine and metallo-β-lactamase inhibition by VNRX-5133, a new β-lactamase inhibitor (BLI) in clinical development [abstract O0603]. Presented at the 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid, Spain
Donnelly R et al (2018) In vitro activity of cefepime alone and in combination with the broad-spectrum β-lactamase inhibitor VNRX-5133 against ESBL and carbapenamases harbouring Enterobacteriaceae and pseudomonas spp [abstract P1539]. Presented at the 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid, Spain
Douafer H, Andrieu V, Phanstiel O, Brunel JM (2019) Antibiotic adjuvants: make antibiotics great again! J Med Chem 62:8665–8681
Drawz SM, Bethel CR, Doppalapudi VR, Sheri A, Pagadala SRR, Hujer AM, Skalweit MJ, Anderson VE, Chen SG, Buynak JD, Bonomo RA (2010) Antimicrob Agents Chemother 54: 1414–1424
Durand-Réville TF et al (2017) ETX2514 is a broad-spectrum β-lactamase inhibitor for the treatment of drug-resistant gram-negative bacteria including Acinetobacter baumannii. Nat Microbiol 2:17104
Ehmann DE, Jahić H, Ross PL, Gu RF, Hu J, Durand-Réville TF, Lahiri S, Thresher J, Livchak S, Gao N, Palmer T, Walkup GK, Fisher SL (2013) J Biol Chem 288:27960–27971
Eisenstein BI, Sox T, Biswas G, Blackman E, Sparling PF (1977) Conjugal transfer of the gonococcal penicillinase plasmid. Science 195:998–1000
Everett M et al (2018) Discovery of a novel metallo-β lactamase inhibitor that potentiates meropenem activity against carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 62:e00074–e00018
Farha MA, Brown ED (2013) Discovery of antibiotic adjuvants. Nat Biotechnol 31:120–122
Farrell DJ, Flamm RK, Sader HS, Jones RN (2013) Antimicrob Agents Chemother 57:6305–6310
Fast W, Sutton LD (2013) Biochim Biophys Acta Proteins Proteomics 1834:1648–1659
FDA (2014) Ceftolozane-tazobactam. Center for Drug Evaluation and Research. Approval package for application number 206829Orig1s000. FDA, Silver Spring
Fu J, Zhang M, Saifuddin G, Cruiming PG, Tepper, Poelarends GJ (2018) Nat Catal 1:186–191
Georgiou P-C et al (2018) Pharmacodynamics of the novel broad-spectrum β-lactamase inhibitor VNRX-5133 in combination with cefepime in neutropenic female CD-1 mice with experimental pneumonia [abstract O0575]. Presented at the 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid, Spain
Giacobbe DR, Bassetti M, De Rosa FG, Del Bono V, Grossi PA, Menichetti F, Pea F, Rossolini GM, Tumbarello M, Viale P, Viscoli C (2018) Expert Rev Anti-Infect Ther 16:307–320
Gilbert N (2018) Four stories of antibacterial breakthroughs. Nature 555:S5–S7
Gill EE, Franco OL, Hancock REW (2015) Antibiotic adjuvants: diverse strategies for controlling drug-resistant pathogens. Chem Biol Drug Des 85:56–78

Griffith DC, Loutit JS, Morgan EE, Durso S, Dudley MN (2016) Antimicrob Agents Chemother 60: 6326–6332
Harris P, Paterson D, Rogers B (2015) Facing the challenge of multidrug-resistant gram-negative bacilli in Australia. Med J Aust 202:243–247
Hecker SJ, Reddy KR, Totrov M, Hirst GC, Lomovskaya O, Griffith DC, King P, Tsivkovski R, Sun D, Sabet M, Tarazi Z, Clifton MC, Atkins K, Raymond A, Potts KT, Abendroth J, Boyer SH, Loutit JS, Morgan EE, Durso S, Dudley MN (2015) J Med Chem 58:3682–3692
Higgins PG, Wisplinghoff H, Stefanik D, Seifert H (2004) In vitro activities of the β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam alone or in combination with β-lactams against epidemiologically characterized multidrug-resistant Acinetobacter baumannii strains. Antimicrob Agents Chemother 48:1586–1592
Huband MD et al (2018) In vitro activity of a novel extended-spectrum β-lactamase inhibitor, AAI101, in combination with cefepime against Enterobacteriaceae isolates collected during 2016 [abstract Friday-601]. Presented at the 2018 American Society for Microbiology (ASM) Microbe conference, Atlanta
Johnson P, Woodford N (2013) J Med Microbiol 62:499–513
Jorgensen SCJ, Rybak MJ (2018) Pharmacotherapy 38:444–461
Ju L-C, Cheng Z, Fast W, Bonomo RA, Crowder MW (2018) Trends Pharmacol Sci 39:635–647
Kalan L, Wright GD (2011) Antibiotic adjuvants: multicomponent anti-infective strategies. Expert Rev Mol Med 13:e5
Karlowsky JA, Lob SH, Kazmierczak KM, Hawser SP, Magnet S, Young K, Moya B, Barcelo IM, Bhagwat S, Patel M, Bou G, Papp-Wallace KM, Bonomo RA, Oliver A (2017) Antimicrob Agents Chemother 61:e02529–e02516
Kaye KS et al (2018) Effect of meropenem-vaborbactam versus piperacillin-tazobactam on clinical cure or improvement and microbial eradication in complicated urinary tract infection: the TANGO I randomized clinical trial. JAMA 319:788–799
Ke W, Sampson JM, Ori C, Prati F, Drawz SM, Bethel CR, Bonomo RA, Van Den Akker F (2011) Antimicrob Agents Chemother 55:174–183
Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS (2016) Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microb Pathog 95:32–42
King T, Strynadka NCJ (2013) Future Med Chem 5:1243–1263
King M, Reid-Yu SA, Wang W, King DT, De Pascale G, Strynadka NC, Walsh TR, Coombes BK, Wright GD (2014) Nature 510:503–506
Klingler M, Wichelhaus TA, Frank D, Cuesta-Bernal J, El-Delik J, Müller HF, Sjuts H, Göttig S, Koenigs A, Pos KM, Pogoryelov D, Proschak E (2015) J Med Chem 58:3626–3630
Koteva K, King AM, Capretta A, Wright GD (2016) Angew Chemie Int Ed 55:2210–2212
Lapuebla M, Abdallah O, Olafisoye C, Cortes C, Urban C, Landman D, Quale J (2015) Antimicrob Agents Chemother 59:5029–5031
Laws M, Shaaban A, Rahman KM (2019) Antibiotic resistance breakers: current approaches and future directions. FEMS Microb Rev 43:490–516
Lee YR, Baker NT, Eur J (2018) Clin Microbiol Infect Dis 37:1411–1419
Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N, Balaban NQ (2017) Antibiotic tolerance facilitates the evolution of resistance. Science 355:826–830
Li G-B, Abboud MI, Brem J, Someya H, Lohans CT, Yang S-Y, Spencer J, Wareham DW, McDonough MA, Schofield CJ (2017) Chem Sci 8:928–937
Liao S, Yang J, Wang J, Zhang B, Hong FW, Lei X (2016) Angew Chemie Int Ed 55:4291–4295
Livermore DM, Mushtaq S, Ge Y (2010) J Antimicrob Chemother 65:1972–1974
Livermore M, Mushtaq S, Warner M, Woodford N (2015) J Antimicrob Chemother 70:3032–3041
Lob SH, Hackel MA, Kazmierczak KM, Young K, Motyl MR, Karlowsky JA, Sahm DF (2017) Antimicrob Agents Chemother 61:e02209–e02216
Lomovskaya O, Sun D, Rubio-Aparicio D, Nelson K, Tsivkovski R, Griffith DC, Dudley MN (2017) Antimicrob Agents Chemother 61:e01443–e01417

Mandal M, Tang H, Xiao L, Su J, Li G, Yang S-W, Pan W, Tang H, DeJesus R, Hicks J, Lombardo M, Chu H, Hagmann W, Pasternak A, Gu X, Jiang J, Dong S, Ding F-X, London C, Biswas D, Young K, Hunter DN, Zhao Z, Yang D (2016) US Patent, 20160333021A1

Martinez JL (2014) General principles of antibiotic resistance in bacteria. Drug Discov Today Technol 11:33–39

McGeary RP, Tan DTC, Selleck C, Monteiro Pedroso M, Sidjabat HE, Schenk G (2017) Eur J Med Chem 137:351–364

McKenna M (2013) Antibiotic resistance: the last resort. Nature 499:394–396

Medeiros AA, O'Brien TF (1975) Ampicillin-resistant Haemophilus influenzae type B possessing a TEM-type β-lactamase but little permeability barrier to ampicillin. Lancet 1:716–719

Melander RJ, Melander C (2017) The challenge of overcoming antibiotic resistance: an adjuvant approach? ACS Infect Dis 3:559–563

Morinaka Y, Tsutsumi M, Yamada K, Suzuki T, Watanabe T, Abe T, Furuuchi S, Inamura Y, Sakamaki N, Mitsuhashi TI, Livermore DM (2015) J Antimicrob Chemother 70:2779–2786

Morinaka Y, Tsutsumi K, Yamada Y, Takayama S, Sakakibara T, Takata T, Abe T, Furuuchi S, Inamura Y, Sakamaki NT, Ida T (2016) Antimicrob Agents Chemother 60:3001–3006

Morinaka Y, Tsutsumi K, Yamada Y, Takayama S, Sakakibara T, Takata T, Abe T, Furuuchi S, Inamura Y, Sakamaki NT, Ida T (2017) J Antibiot 70:246–250

Murano K, Yamanaka T, Toda A, Ohki H, Okuda S, Kawabata K, Hatano K, Takeda S, Akamatsu H, Itoh K, Misumi K, Inoue S, Takagi T (2008) Bioorg Med Chem 16:2261–2275

Mushtaq S, Vickers A, Woodford N, Livermore DM (2018) Potentiation of cefepime by the boronate VNRX-5133 versus gram-negative bacteria with known β-lactamases [abstract P1536]. Presented at the 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid, Spain

Neu HC (1990) β-Lactamases β-lactamase inhibitors, and skin and skin-structure infections. J Am Acad Dermatol 22:896–904

Nguyen NQ, Krishnan NP, Rojas LJ, Prati F, Caselli E, Romagnoli C, Bonomo RA, Van Den Akker F (2016) Antimicrob Agents Chemother 60:1760–1766

Papp-Wallace KM et al (2017) AAI101, a novel β-lactamase inhibitor: microbiological and enzymatic profiling. Open Forum Infect Dis 4:S375

Pattanaik P, Bethel CR, Hujer AM, Hujer KM, Distler AM, Taracila M, Anderson VE, Fritsche TR, Jones RN, Pagadala SRR, Van Den Akker F, Buynak JD, Bonomo RA (2009) J Biol Chem 284: 945–953

Potron A, Poirel L, Nordmann P (2015) Int J Antimicrob Agents 45:568–585

Powers RA, Swanson HC, Taracila MA, Florek NW, Romagnoli C, Caselli E, Prati F, Bonomo RA, Wallar BJ (2014) Biochemistry 53:7670–7679

Prabaker K, Weinstein RA (2011) Trends in antimicrobial resistance in intensive care units in the United States. Curr Opin Crit Care 17:472–479

Reddy R, Glinka T, Totrov M, Hecker S (2014) WO Patent, 2014107536A1

Reddy RK, Glinka T, Totrov M, Hecker S, Rodny O (2016) WO Patent, 2016003929A1

Satyanarayana M (2018) The hunt for new antibiotics grows harder as resistance builds. Chem Eng News 96:16–18

Schillaci D, Spanô V, Parrino B, Carbone A, Montalbano A, Barraja P, Diana P, Cirrincione G, Cascioferro S (2017) Pharmaceutical approaches to target antibiotic resistance mechanisms. J Med Chem 60:8268–8297

Shakil S, Azhar EI, Tabrez S, Kamal MA, Jabir NR, Abuzenadah AM, Damanhouri GA, Alam Q (2011) J Chemother 23:263–265

Shapiro AB et al (2017) Reversibility of covalent, broad-spectrum serine β-lactamase inhibition by the diazabicyclooctenone ETX2514. ACS Infect Dis 3:833–844

Snydman DR, McDermott LA, Jacobus NV (2014) Antimicrob Agents Chemother 58:1218–1223

Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I, Steenbergen J, Yoon M, Collins S, Yuan G, Barie PS, Eckmann C (2015) Clin Infect Dis 60:1462–1471

Somboro M, Tiwari D, Bester LA, Parboosing R, Chonco L, Kruger HG, Arvidsson PI, Govender T, Naicker T, Essack SY (2014) J Antimicrob Chemother 70:1594–1596
Somboro M, Osei Sekyere J, Amoako DG, Essack SY, Bester LA (2018) Appl Environ Microbiol AEM00698-18
Sommer MOA, Munck C, Toft-Kehler RV, Andersson DI (2017) Prediction of antibiotic resistance: time for a new preclinical paradigm? Nat Rev Microbiol 15:689–696
Takeda S, Nakai T, Wakai Y, Ikeda F, Hatano K (2007) Antimicrob Agents Chemother 51:826–830
Thayer AM (2016) Antibiotics: will the bugs always win? Chem Eng News 94:36–43
Theuretzbacher U (2016) Market watch: antibacterial innovation in European SMEs. Nat Rev Drug Discov 15:812–813
Thomas PW, Cammarata M, Brodbelt JS, Fast W (2014) Chembiochem 15:2541–2548
Toda H, Ohki T, Yamanaka K, Murano S, Okuda K, Kawabata K, Hatano K, Matsuda K, Misumi K, Itoh KS, Inoue S (2008) Bioorg Med Chem Lett 18:4849–4852
Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J (2019) β-Lactamases and β lactamase inhibitors in the 21st century. J Mol Biol 431:3472–3500
Trippier PC, McGuigan C (2010) MedChemComm 1:183–198
Tyers M, Wright GD (2019) Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol 17:141–155
Vabomere product sheet. http://www.vabomere.com/media/pdf/P-VAB-US00050_R01_MVAB_HCP_OrderSheet_Web.pdf
Vallejo JA, Martínez-Guitián M, Vázquez-Ucha JC, González-Bello C, Poza M, Buynak JD, Bethel CR, Bonomo RA, Bou G, Beceiro A (2016) J Antimicrob Chemother 71:2171–2180
Vázquez-Ucha JC, Maneiro M, Martínez-Guitián M, Buynak J, Bethel CR, Bonomo RA, Bou G, Poza M, González-Bello C, Beceiro A (2017) Antimicrob Agents Chemother 61:e01172–e01117
Vikesland P, Garner E, Gupta S, Kang S, Maile-Moskowitz A, Zhu N (2019) Differential drivers of antimicrobial resistance across the world. Acc Chem Res 52:916–924
Wagenlehner FM, Umeh O, Steenbergen J, Yuan G, Darouiche RO (2015) Lancet 385:1949–1956
Walkty A, Karlowsky JA, Adam H, Baxter M, Lagacé-Wiens P, Hoban DJ, Zhanel GG (2013) Antimicrob Agents Chemother 57:5707–5709
Wang J-F, Chou K-C (2013) Curr Top Med Chem 13:1242–1253
Wang Z, Fast W, Valentine AM, Benkovic SJ (1999) Curr Opin Chem Biol 3:614–622
Wang R, Lai TP, Gao P, Zhang H, Ho PL, Woo PCY, Ma G, Kao RYT, Li H, Sun H (2018) Nat Commun 9:439
Weiss WJ et al (2018) Efficacy of cefepime/VNRX-5133, a novel β-lactamase inhibitor, against cephalosporin resistant, ESBL-producing K. pneumoniae in a murine lung-infection model [abstract O0600]. Presented at the 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid, Spain
Werner JP, Mitchell JM, Taracila MA, Bonomo RA, Powers RA (2017) Protein Sci 26:515–526
Worthington RJ, Melander C (2013) Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol 31:177–184
Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 5:175–186
Wright GD (2016) Antibiotic adjuvants: rescuing antibiotics from resistance. Trends Microbiol 24:862–871
Zhanel GG, Lawrence CK, Adam H, Schweizer F, Zelenitsky S, Zhanel M, Lagacé-Wiens PRS, Walkty A, Denisuik A, Golden A, Gin AS, Hoban DJ, Lynch JP, Karlowsky JA (2018) Drugs 78:65–98
Zhang YL, Zhang YJ, Wang WM, Yang KW (2017a) Phosphorus, sulfur silicon. Relat Elements 192:14–18
Zhang S, Wang Y, Bai Q, Guo JZ, Lei X (2017b) J Org Chem 82:13643–13648
Zhang MM, Wang SC, Huang SMX, Cui DY, Bo YL, Bai PY, Hua YG, Xiao CL, Qin S (2018) Bioorg Med Chem Lett 28:214–221

Current Reserve Drugs

17

Irfan Ahmad Khan

Abstract

The incidence of resistance development against antimicrobial agents is increasing in Gram-negative bacteria (GNB) worldwide. This has resulted into great threat in managing infections acquired from hospital. Early intervention with appropriate antibiotic reduces the emergence of drug resistance. As per several reports of the World Health Organization (WHO), antimicrobial resistance affects both health and economy. Newer agents are the need of hour to tackle the multi-drug resistant (MDR), extensively drug resistant (XDR), and pan drug resistant (PDR) pathogens, having acquired newer mechanisms of resistance. Clinical outcomes are grave due to decreasing options therapeutically. Novel agents have shown potential for carbapenem-resistant *Enterobacterales* (CRE) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) but lesser for carbapenem-resistant *Acinetobacter baumannii* (CRAB). The action of newer β-lactam/β-lactamase inhibitors (BL-BLI) against CRE depends on the variety of carbapenemases providing resistance. Newer agents like ceftazidime/avibactam, meropenem/vaborbactam, ceftolozane/tazobactam, eravacycline, plazomicin, etc. are approved for treating resistant bacteria. Few pathogens have demonstrated some resistance against these newer agents too. Therefore, these agents should be used with caution. The management of infections by MDR-GNB is very complex. It requires expertise and updated knowledge.

Keywords

Gram-negative bacteria · Multi-drug resistance · β-lactam/β-lactamase inhibitors · Enterobacterales · Pseudomonas

I. A. Khan (✉)
Department of Pharmacology, J.N.M.C., AMU, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_17

17.1 Introduction

The incidence of resistance development against antimicrobial agents is increasing in Gram-negative bacteria (GNB) worldwide. This has resulted into great threat in managing infections acquired from hospital. As per several reports of the World Health Organization (WHO), antimicrobial resistance affects both health and economy (World Health Organization 2011). Newer agents are the need of hour to tackle the multi-drug resistant (MDR), extensively drug resistant (XDR), and pan drug resistant (PDR) pathogens, having acquired newer mechanisms of resistance. Carbapenem resistance, along with all beta-lactams, due to production of carbapenemases has led to the generation of XDR pathogens. Enterobacteriaceae members such as *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* are the important pathogens having carbapenemases. The drug resistant ones are known as carbapenem-resistant *Enterobacterales* (CRE), carbapenem-resistant *P. aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB) (Hawkey et al. 2018). Clinical outcomes are grave due to decreasing options therapeutically. Novel agents have shown potential against CRE and CRPA but lesser against CRAB. The action of newer β-lactam/β-lactamase inhibitors (BL-BLI) against CRE depends on the variety of carbapenemases providing resistance. Few pathogens have demonstrated some resistance against these newer agents too. Therefore, these agents should be used with caution. The management of infections by MDR-GNB is very complex. It requires expertise and updated knowledge (Karaiskos et al. 2019).

17.1.1 Colistin

It is administered systemically. It is a prodrug. It exerts bactericidal action due to its detergent-like action on the outer membrane of GNB. In the past few years, they were frequently used for the treatment of CRE, CRAB, and CRPA. They are one of the first-line therapies for CRAB infections. The already available novel agents are preferred over them for CRE and CRPA due to nephrotoxicity and suboptimal concentrations, mainly in lung.

As per the INFORM studies, in vitro efficacy of ceftazidime+avibactam was best against *Enterobacteriaceae* isolates as compared to colistin (Kazmierczak et al. 2018). In clinical practice, a loading dose along with daily maintenance doses of colistin is administered. But a high CrCL reduces colistin levels as more of colistin is cleared by the kidneys. So, there is a need of combinations or higher dosing.

In CRE, colistin+meropenem has been found to be beneficial in relation to survival. Colistin single use is a treatment of choice in lower risk blood stream infections (BSIs) and non-bacteremic infections of abdomen and urinary tract. Presently there is lack of clinical evidence to suggest that monotherapy is better than combination therapy in managing CRPA. Therefore, due to poor pharmacokinetics colistin is often used in combination for CRAB treatment. Due to high rates of

clinical failures, it is necessary to search for newer agents to combat CRAB infections effectively.

17.1.2 Fosfomycin

It is bactericidal. It inhibits the beginning step in synthesis of peptidoglycan. It interferes with the generation of UDP *N*-acetylmuramic acid, a precursor of peptidoglycan. It has high concentrations in serum as well as urine which is above the MIC of susceptible organisms. It is administered intravenously. Hypokalaemia and sodium overload are the important adverse effect. It is often administered in combinations for the therapy of drug-resistant GNB due to the development of resistance. Along with resistance development, there is lack of high-quality evidence of efficacy and difficulties of unobstructed fosfomycin supply (Vardakas et al. 2016).

In previous in vitro studies, fosfomycin was found active against *Enterococcus faecium*, *Staphylococcus aureus,* extended-spectrum beta-lactamases synthesizing *K. pneumoniae and E. coli* and less against CRKP. In ICU, fosfomycin is used against CR infections. Fosfomycin is active against CRE infections, mainly those producing metallo-β-lactamases. For CRPA infections, fosfomycin MIC is high, and better agents are available. Fosfomycin is highly active in the urinary tract. Its combination with aminoglycosides has high efficacy, and fosfomycin decreases aminoglycoside toxicity. Studies have demonstrated its synergy with carbapenems (Vardakas et al. 2016).

17.1.3 Aminoglycosides

Aminoglycosides act by inhibiting 30S ribosomal subunit of the bacteria. They are often used in case of polymyxin resistance to treat various CR-GNB. Two limiting factors associated with the use of aminoglycosides (gentamicin, amikacin, tobramycin) for the MDR-GNB infections treatment are: (a) nephrotoxicity and lesser lung concentrations and (b) higher resistance chances due to 16S rRNA methyltransferase expression (Doi et al. 2016).

Aminoglycosides are often part of combination regimens and used as monotherapy only in UTI. Treating other compartments of the body is poor due to PK/PD drawbacks associated with it. For VAP, aerosolized aminoglycosides overcome the PK/PD drawbacks of IV administration (Almaghrabi et al. 2014).

Less nephrotoxicity develops when once-daily doses of aminoglycosides are given as a short course (5–7 days). The high doses of the drug is well tolerated despite associated risk of nephrotoxicity. Therefore, therapeutic monitoring of drug is needed to maintain levels in therapeutic range. As shown in Table 17.1, gentamicin is administered once daily. High doses are used for treating severe CRE infections. The synergy between aminoglycosides and carbapenems was demonstrated beneficial in CRE. Further clinical evidence is needed (Almaghrabi et al. 2014).

Table 17.1 Antimicrobial agents (intravenous) against drug-resistant Gram-negative pathogens

Drugs	Dose	Dose adjustment based on CrCl
Colistin	• Loading dose: 9 MIU as an infusion for 30 min to 1 h • Maintenance dose: 4.5 MIU every 12 hourly after 12 h	Daily dose divided by two: ≥90: 10.9 MIU 80–90: 10.3 MIU 60–70: 8.35 MIU 70–80: 9.00 MIU 50–60: 7.40 MIU 40–50: 6.65 MIU 30–40: 5.90 MIU 20–30: 5.30 MIU 10–20: 4.85 MIU 05–10: 4.4 MIU Haemodialysis: 3.95 MIU and extra dose of 1.2–1.6 MIU (post haemodialysis)
Fosfomycin	6–8 g every 8 h	40: Reduced to 70% 30: Reduced to 60% 20: Reduced to 40% 10: Reduced to 20% Haemodialysis: 2 g every 48 h (post haemodialysis)
Gentamicin	5 mg/kg/day (7 mg/kg/day when critically ill)	>80: 5 mg/kg/day 60–80: 4 mg/kg/day 40–60: 3.5 mg/kg/day 30–40: 2.5 mg/kg/day 20–30: 4 mg/kg every 48 h 10–20: 3 mg/kg every 48 h 0–10: 2 mg/kg every 72 h Haemodialysis: 2 mg/kg every 72 h (post haemodialysis)
Tigecycline	Loading dose: 200 mg, maintenance dose: 100 mg	No dose adjustment
Meropenem	2 g every 8 hourly	≥50: 2 g every 8 h 30–49: 1 g every 8 h 10–29: 1 g every 12 h <10: 1 g/day Haemodialysis: 1 g/day (post haemodialysis)
Ertapenem	1 g/day	30–90: No dose adjustment <30: 0.5 g/day Haemodialysis: 0.5 g/day (post haemodialysis)

Modified from Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Frontiers in Public Health June 7

17.1.4 Tigecycline

It is a glycylcycline antimicrobial agents administered intravenously. After binding to 30S subunit of ribosome, it blocks interaction between aminoacyl-tRNA and ribosomal A site and inhibits protein synthesis. Tigecycline has been used as a

rescue treatment against CRE and CRAB but not CRPA. In combinations, it has been used for severe CRE and CRAB infections (Betts et al. 2014). It has to be used cautiously for VAP as increased mortality when compared to other regimens has been reported. The higher dose therapy (HDT) (200 mg as loading dose and then 100 mg twice a day) of tigecycline is used in pneumonia to attain PK/PD targets. A reduction in fibrinogen levels has been seen with higher dosage of tigecycline. With HDT, there is higher occurrence of nausea, diarrhoea, and vomiting (Ramirez et al. 2013).

Combination of tigecycline with other agents is essential due to the above reasons. There is higher mortality, especially in critically ill ones, when administered as monotherapy. Colistin-tigecycline combination in CRE associated BSI was found better than colistin only in terms of mortality. It is useful against *A. baumannii*. Reports on synergy with newer agents need further analysis to get newer combinations (Pournaras et al. 2011).

17.1.5 Carbapenem Containing Combinations (CCC) and Double Carbapenem Combination (DCC)

Carbapenems in infections caused by carbapenemases producing pathogens were lifesaving drugs. They are active against KPC producers. This is due to achieving sufficient carbapenem concentrations and also synergistic effects. Along with other active agents, higher doses and extended infusions of carbapenems have been used (mainly for meropenem). Treating patients such as in septic shock, these combinations were found very benificial. Meropenem administration requires therapeutic drug monitoring. Newly developed antibiotics having activity against *Klebsiella pneumoniae* carbapenemase (KPC), replaced carbapenems.

Double carbapenem combination (DCC) is a rescue therapy against XDR and PDR CRE (Poirel et al. 2016). Ertapenem due to high affinity to KPCs acts as a 'suicide inhibitor'. Due to this, the other carbapenem remains active and intact. The commonest combination is ertapenem (1 g every 24 h IV, infused in half hour) + meropenem (2 g every 8 h, infused in 3 h). DCC (with or without colistin) is administered in infections due to resistant pathogens with serine-carbapenemases. Further RCTs need to be done to assess the role of DCC in resistant pathogens (Bulik and Nicolau 2011).

17.2 Newer Antibiotics

17.2.1 Ceftazidime–Avibactam (CAZ/AVI)

Avibactam is a newer BLI and a non-β-lactam. The combination inhibits most β-lactamases, CRE, and some CRPA strains (as they have metallo-β-lactamases like VIM-2). FDA and EMA have approved CAZ/AVI for cUTIs, cIAIs, hospital acquired, and ventilator-associated pneumonia (HAP/VAP), and aerobic

Table 17.2 Newer antimicrobial agents (intravenous) against drug-resistant Gram-negative pathogens

Drug	Dose	Dose adjustment based on CrCl
Ceftazidime/ avibactam	2.5 g 8 hourly (infusion in 2 h)	>50: 2.5 g every 8 h 31–50: 1.25 g every 8 h 10–30: 0.94 g every 12 h <10: 0.94 g every 48 h Haemodialysis: 0.94 g every 48 h (post haemodialysis)
Ceftolozane/ Tazobactam	Dose in pneumonia: 3 g 8 hourly (infusion in 1 h)	>50: 3 g 8 hourly 30–50: 1.5 g 8 hourly 15–29: 750 mg 8 hourly <15: No data
	For other indications: 1.5 g (1 g/ 0.5 g) 8 hourly (infusion in 1 h)	>50: 1.5 g 8 hourly 30–50: 750 mg 8 hourly 15–29: 375 mg 8 hourly <15: 750 mg loading dose then 150 mg 8 hourly Haemodialysis: 750 mg as loading dose then 150 mg 8 hourly (post haemodialysis)
Meropenem/ Vaborbactam	4 g (2 g each) 8 hourly (infusion in 3 h)	>50: 4 g 8 hourly 30–49: 2 g 8 hourly 15–29: 2 g 12 hourly <15: 1 g 12 hourly Haemodialysis: 1 g 12 hourly (post haemodialysis)
Plazomicin	15 mg/kg/day (infusion in 30 min)	≥60: 15 mg/kg/day 30–60: 10 mg/kg/day 15–29: 10 mg/kg 48 hourly
Eravacycline	1 mg/kg 12 hourly (infusion in 1 h)	No dose adjustment

Modified from: Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Frontiers in Public Health June 7

Gram-negative pathogen-associated infections. The combination has lower activity against CRAB due to susceptibility of avibactam by OXA-type carbapenemases present in *A. Baumannii*. Addition of metronidazole along with the combination is needed against anaerobic Gram-negative bacteria in intra-abdominal infections. It is not active against Gram-positive cocci (Karaiskos et al. 2019).

Newer resistance to CAZ/AVI is due to KPC expression with changes in omega-loop. This information has encouraged to search for newer agent that will overcome this resistance. Therefore, experts suggest combination treatments (with aminoglycoside, colistin, tigecycline) to avoid the resistance emergence.

PK/PD studies for both CAZ/AVI demonstrate linear pharmacokinetics, human protein binding 10% and 8%, respectively, and renal excretion. Both penetrate equally into ELF (Karaiskos et al. 2019). Dose modification as per CrCl is shown in Table 17.2.

Table 17.3 Conditions where monotherapy is used as definitive treatment

Antibiotic's profile • No toxic effect previously • Adequate PK/PD profile • Resistance development chances are minimal	Patient profile • Local infection • Without septic shock • Improving after therapy • No severe comorbid conditions
Healthcare facility profile • No uncontrolled infection source • No co-infection chances	Pathogen's profile • TDM available • ADR management available

Modified from: Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Frontiers in Public Health June 7

RECLAIM trials I and II showed CAZ/AVI + metronidazole is non-inferior to meropenem for treating cIAIs patients (Qin et al. 2017). RECAPTURE I and II studies confirmed its non-inferiority to doripenem in cUTIs patients. It has mild adverse events such as headache, hypersensitivity reactions, constipation, and diarrhoea (Wagenlehner et al. 2016).

It is the first marketed product active in KPC and OXA producing pathogens. Data are very encouraging in terms of efficacy and safety. Monotherapy is definitive treatment for infections which are non-life-threatening. The monotherapy is used as definitive treatment of infections in conditions shown in Table 17.3. The algorithm for treatment of CRE is shown in Table 17.4. CAZ/AVI has a vital role in patients with risk of MDR infections and should be reserved for treating infections caused by pathogens producing KPC- or OXA-48 (Table 17.4).

17.2.2 Ceftolozane–Tazobactam (CLZ/TAZ)

This is a combo containing oxyimino–cephalosporin and BLI, tazobactam. It has high activity against CRPA by attaching to penicillin-binding proteins. Common resistance mechanisms in *P. aeruginosa* do not affect ceftolozane. Several mutations are required for developing resistance against CLZ/TAZ. Tazobactam confers stability against most of the ESBLs. It is not active against CRE, staphylococci, enterococci, and very less against anaerobes. It is used in managing cIAI and cUTI (Solomkin et al. 2015; Wagenlehner et al. 2015). CLZ/TAZ is also useful for respiratory infections and VAP. CLZ/TAZ dose is adjusted as per the creatinine clearance.

The major use of CLZ/TAZ is in therapy of infections caused by *P. aeruginosa* (Table 17.5). For patients with risk for MDR, CLZ/TAZ is used along with the second agent as empirical regimen to get adequate coverage. CLZ/TAZ is the drug of choice in infections due to CRPA without carbapenemase production (Karaiskos et al. 2019).

Table 17.4 Treatment of CRE

Algorithm for treatment of CRE				
Empiric treatment		Definitive treatment		
Risk factors • Prior infection or colonisation by CRE mainly producing KPC or OXA-48 OR • Local epidemiology PLUS ANY • Carbapenems use with/without colistin • ICU or long hospital stay • Septic shock • Multiple comorbidities		Microbiology results • Identify • Testing susceptibility • Resistance mechanism detection • MIC determination		
Combination regimen (double/ triple)		Monotherapy or combination regimen		
CAZ/AVI- Or MER/VAB-based combination	Colistin-based combination	Monotherapy (as per Table 17.3)	CAZ/AVI- or MER/VAB-based combination	Colistin-based combination
Gentamicin/ other aminoglycoside Fosfomycin Colistin Tigecycline	Carbapenem Gentamicin/ other aminoglycoside Fosfomycin Tigecycline	CAZ/AVI MER/VAB Colistin Aminoglycoside	Gentamicin/ other aminoglycoside Fosfomycin Colistin Tigecycline	Carbapenem Gentamicin/ other aminoglycoside Fosfomycin Tigecycline

Modified from:-Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Frontiers in Public Health June 7

17.2.3 Meropenem–Vaborbactam (MER/VAB)

Vaborbactam, a cyclic pharmacophore of boronic acid and non-BL-BLI without antibacterial activity. It is active against KPCs due to high affinity for serine proteases. It is effective against class A ESBLs, class A carbapenemases, and class C cephalosporinases. The combination has no add-on benefit as compared to meropenem alone against *P. Aeruginosa* and *Acinetobacter* spp.

MER/VAB is the first available carbapenem/BLI. The common adverse reactions are headache, diarrhoea and infusion site complications. With very high dose, mild lethargy is seen.

MER/VAB is active against all Gram-negative pathogens. MER/VAB is approved for cUTIs. The dosage is 4 g every 8 hourly with urinary excretion. Other indications are cIAI, HAP, and VAP where treatment options are limited. The major advantage of the combination is the lower resistance development potential (Hackel et al. 2017).

Table 17.5 Treatment of MDR *P. aeruginosa*

Algorithm for the treatment of multi-drug-resistant *Pseudomonas aeruginosa*				
Empiric treatment		Empiric treatment		
Risk factors • Comorbidities • Prior infection or colonisation by CRPA • Prior therapy with antipseudomonal beta lactam (<3 months)		Microbiology results • Identify • Testing susceptibility • MIC determination		
Combination regimen (double/triple)		Monotherapy or combination regimen		
First antipseudomonal agent	Companion antipseudomonal agent	Monotherapy (as per Table 17.3)	Beta-lactam-based combination	Beta-lactam-resistant isolates
CLZ/TAZ CAZ/AVI MER MER/VAB Pipercillin/tazobactam	Gentamicin/other aminoglycoside Colistin Fosfomycin	CLZ/TAZ MER MER/VAB Pipercillin/tazobactam CAZ/AVI Colistin Aminoglycoside	CLZ/TAZ MER MER/VAB Pipercillin/tazobactam CAZ/AVI Plus one from aminoglycoside Fosfomycin	DCC: Gentamicin/other aminoglycoside Colistin Fosfomycin

Modified from: Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. Frontiers in Public Health June 7

17.2.4 Plazomicin

It is a semisynthetic parenteral aminoglycoside and inhibiting synthesis of protein. It is beneficial against drug-resistant *Enterobacteriaceae* as not destroyed by aminoglycoside modifying enzymes. It has dose-dependent bactericidal effect. The *t*1/2 is 4 h and dose is 15 mg/kg/day (Zhanel et al. 2012).

It is approved for patients above 18 years with cUTIs caused by microorganisms like *P. mirabilis*, *E. coli, K. pneumoniae,* and *Enterobacter cloacae*. Nephrotoxicity, ototoxicity, neuromuscular blockade and foetal toxicity are known adverse events.

Its high lung penetration makes it useful for the treatment of VAP specially when monotherapy is not suitable. It is a perfect companion to new BL-BLI. In the initial empiric regimen, it can be used in place of colistin in infections of lung due to poor PK profile of colistin (Tables 17.4 and 17.5).

17.2.5 Eravacycline

It is a new synthetic fluorocycline. It is similar to tigecycline in action, molecular structure, and spectrum like Gram-negative bacilli and Gram-positive. The extra benefit over tigecycline is its effect against both Gram-positive cocci and -negative bacilli, good oral bioavailability with lesser drug interactions (Bassetti and Righi 2014).

In comparison to tigecycline, it is administered once-daily, higher serum concentrations, and better tolerability. It is used in therapy of MDR bacteria-related pneumonia due to high ELF concentrations. It has been reported to be the most potent agent in comparison to beta-lactams, tetracyclines, aminoglycosides, colistin, etc. It is approved for cIAIs. Eravacycline has demonstrated to be active against CRAB (Livermore et al. 2016) (Table 17.6).

17.3 Integration of Antibiotics in Clinical Practice

The newer BL/BLI combinations, like avibactam–vaborbactam, are best available agents for treating CRE. These agents are effective against pathogens producing AmpC, ESBL, KPC-2, and KPC-3 while avibactam only inhibits OXA-48. CAZ/AVI is active against OXA-48 enzymes, and its antipseudomonal action is beneficial in patients with mixed infections. MER/VAB in comparison to CAZ/AVI has lesser chances for resistance among KPC producers. Meropenem provides

Table 17.6 Treatment of CRAB

Drugs to be used	• Administer polymyxin (colistin)as main agent • Combination of older and newer agent (eravacycline) • Concomitant addition of inhalational polymyxin/aminoglycoside

Modified from: Bassetti M, Peghin M, Vena A, Giacobbe DR (2019) Treatment of Infections Due to MDR Gram-Negative Bacteria. Frontiers in Public Health April 6

excellent coverage against anaerobes and therefore useful in intra-abdominal infections.

The epidemiological information of infections due to CRE is important in every country. OXA-48 is more prevalent in Belgium, France, and Spain while NDM-1 in countries such as India. In empiric therapy of CRE infections, epidemiological profile is also taken into consideration. In KPC producing pathogens, either CAZ/AVI or MER/VAB is indicated. But in MBLs producing pathogens, colistin combination must be considered. Table 17.4 shows an algorithm for the treatment of CRE. Table 17.3 enlists prerequisites for selecting monotherapy for treating MDR pathogens.

In MDR *P. aeruginosa*, combination therapy is ideal in empirical treatment. The meta-analysis data has shown no benefit of combination therapy in definite treatment. But clinicians do not recommend monotherapy for those with risk for mortality and septic shock. The CLZ/TAZ provides a potent backbone by expanding beta-lactam activity in CR strains. Colistin, fosfomycin, and plazomicin acts as adjuvants (Table 17.5).

Colistin or tigecycline is only currently available option against CRAB (Table 17.5). The novel eravacycline holds promise, but more studies are needed (Livermore et al. 2016). Other novel agents such as cefiderocol are expected to have high cure rates against CRAB.

The novel combinations cannot be thought as cure for all crisis in treatment of drug-resistant GNB. Colistin is also important against CRPA and CRAB. CAZ/AVI and MER/VAB work as backbones in treating CRE and when used as definitive monotherapy. CLZ/TAZ is an ideal for CRPA as well as monotherapy. Plazomicin can reduce colistin usage, in both empiric and definitive therapy. Fosfomycin is used as an add-on antibiotic for *P. aeruginosa* and CRE infections with no cross-resistance and very less toxicity. We should be more vigilant for the emergence of resistance. Finally, it is necessary to minimize the usage of newer agents in empiric therapy and microbiologic documentation of infections should be focused (Wright et al. 2017).

References

Almaghrabi R, Clancy CJ, Doi Y, Hao B, Chen L, Shields RK et al (2014) Carbapenem-resistant Klebsiella pneumoniae strains exhibit diversity in aminoglycoside modifying enzymes, which exert differing effects on plazomicin and other agents. Antimicrob Agents Chemother 58:4443–4451

Bassetti M, Righi E (2014) Eravacycline for the treatment of intra- abdominal infections. Expert Opin Investig Drugs 23:1575–1584

Bassetti M, Peghin M, Vena A, Giacobbe DR (2019) Treatment of infections due to MDR gram-negative bacteria. Front Med (Lausanne) 16(6):74

Betts JW, Phee LM, Hornsey M, Woodford M, Wareham DW (2014) In vitro and in vivo activities of tigecycline-colistin combination therapies against carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 58:3541–3546

Bulik CC, Nicolau DP (2011) Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother 55:3002–3004

Doi Y, Wachino JI, Arakawa Y (2016) Aminoglycoside resistance:-the emergence of acquired 16S ribosomal RNA methyltransferases. Infect Dis Clin N Am 30:523–537

Hackel MA, Lomovskaya O, Dudley MN, Karlowsky JA, Sahm DF (2017) In vitro activity of meropenem-vaborbactam against clinical isolates of KPC-positive Enterobacteriaceae. Antimicrob Agents Chemother 62:e01904–e01917

Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA (2018) Treatment of infections caused by multidrug-resistant gGram-negative bacteria:-report of the British Society for Antimicrobial Chemotherapy/ Healthcare Infection Society/British Infection Association Joint Eorking Party. J Antimicrob Chemother 73(3):iii2–iii78

Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G (2019) The "old" and the "new" antibiotics for MDR gram-negative pathogens: for whom, when, and how. Front Public Health 7:151

Kazmierczak KM, de Jonge BLM, Stone GG, Sahm DF (2018) In vitro activity of ceftazidime/avibactam against isolates of Enterobacteriaceae collected in European countries: INFORM global surveillance 2012-15. J Antimicrob Chemother 73:2782–2788

Livermore DM, Mushtaq S, Warner M, Woodford N (2016) In vitro activity of eravacycline against carbapenem-resistant Enterobacteriaceae and Acinetobacter baumannii. Antimicrob Agents Chemother 60:3840–3844

Poirel L, Kieffer N, Nordmann P (2016) In vitro evaluation of dual carbapenem combinations against carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 71:156–161

Pournaras S, Vrioni G, Neou E, Dendrinos J, Dimitroulia E, Poulou A et al (2011) Activity of tigecycline alone and in combination with colistin and meropenem against Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae strains by time-kill assay. Int J Antimicrob Agents 37:244–247

Qin X, Tran BG, Kim MJ, Wang L, Nguyen DA, Chen Q et al (2017) A randomised, double-blind, phase 3 study comparing the efficacy and safety of ceftazidime/avibactam plus metronidazole versus meropenem for complicated intra-abdominal infections in hospitalised adults in Asia. Int J Antimicrob Agents 49:579–588

Ramirez J, Dartois N, Gandjini H, Yan JL, Korth-Bradley J, McGovern PC (2013) Randomized phase 2 trial to evaluate the clinical efficacy of two high-dosage tigecycline regimens versus imipenem-cilastatin for treatment of hospital-acquired pneumonia. Antimicrob Agents Chemother 57:1756–1762

Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I, Steenbergen J et al (2015) Ceftolozane/Tazobactam plus metronidazole for complicated intra-abdominal infections in an era of multidrug resistance:-results from a randomized, double-blind, phase 3 trial (ASPECT-cIAI). Clin Infect Dis 60:1462–1471

Vardakas KZ, Legakis NJ, Triarides N, Falagas ME (2016) Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. Int J Antimicrob Agents 47:269–285

Wagenlehner FM, Umeh O, Steenbergen J, Yuan G, Darouiche RO (2015) Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis:-a randomised, double-blind, phase 3 trial (ASPECT-cUTI). Lancet 385:1949–1956

Wagenlehner FM, Sobel JD, Newell P, Armstrong J, Huang X, Stone GG et al (2016) Ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections, including acute pyelonephritis:-RECAPTURE, a phase 3 randomized trial program. Clin Infect Dis 63:754–762

World Health Organization (WHO) (2011) Antimicrobial resistance:-no action today, no cure tomorrow. WHO Press, Geneva. https://www.who.int/world-health-day/2011/en

Wright H, Bonomo RA, Paterson DL (2017) New agents for the treatment of infections with gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect 23:704–712

Zhanel GG, Lawson CD, Zelenitsky S, Findlay B, Schweizer F, Adam H (2012) Comparison of the next-generation aminoglycoside plazomicin to gentamicin, tobramycin, and amikacin. Expert Rev Anti Infect Ther 10:459–473. https://doi.org/10.1586/eri.12.25

Newer Antibiotic Agents in Pipeline

18

Irfan Ahmad Khan

Abstract

Multi-drug resistance (MDR) and extensive-drug resistance (XDR) have become common, making care of many patients difficult. The rise in resistance mediated through MDR and XDR organisms has led to serious implications which have been great challenge to manage. It is a global health emergency. There is a critical need to develop newer medications to tackle this disaster. As a result, there has been a pressing need to create new antimicrobial agents for the treatment of Gram-negative pathogens. The discovery and development of newer, potent, and safer antibiotics is not an easy task. It involves a huge economic burden. This chapter lists the antimicrobial agents, β-lactamase/β-lactam inhibitor (BL-BLI) combinations, newer carbapenems, and newer topoisomerase inhibitors. Agents which are in Phase 2 trials have also been included. Substitutes to antimicrobials agents such as bacteriophages, DCAP, peptidic benzimidazoles, odilorhabdins, quorum sensing inhibitors, metal-based antibacterial agents are being tried in resistant cases. There has been surge in newer agents but there is still a large gap in the pipeline of the development of new antimicrobials with MDR pathogens.

Keywords

Antibiotics in pipeline · Drug resistance · Newer carbapenems · Newer topoisomerase inhibitors · Agents in phase 2 trials

I. A. Khan (✉)
Department of Pharmacology, J.N.M.C., AMU, Aligarh, Uttar Pradesh, India

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_18

18.1 Introduction

Multi-drug resistance (MDR) and extensive-drug resistance (XDR) have become common, making care of many patients difficult. The rise in resistance mediated through MDR and XDR organisms has led to serious implications which have been great challenge to manage. It is a global health emergency. There is a critical need to develop newer medications to tackle this disaster. As a result, there has been a pressing need to create new antimicrobial agents for the treatment of Gram-negative pathogens (Breijyeh et al. 2018).

18.2 Antibiotic Agents in Pipeline for MDR-GNB (Butler and Paterson 2020)

18.2.1 Omadacycline

Omadacycline belongs to aminomethylcycline subclass of tetracycline. It has been FDA approved recently for use in patients of community acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSI). It acts similarly as tetracyclines by inhibiting bacterial protein synthesis. It kills tetracycline-resistant pathogens (efflux and ribosomal protection) (Gallagher 2019).

18.2.2 Cefiderocol

Cefiderocol is a siderophore cephalosporin that was only recently created. Its catechol end attaches to ferric iron and crosses bacterial outer membrane using iron transporters (Ito et al. 2016). Hence it acts by destroying cell wall synthesis. During an acute infection, these transporters are increased. It is effective against all carbapenemases and stable during hydrolysis by all β-lactamases, including KPC-producing *Enterobacteriaceae*, MBL-producing *Pseudomonas aeruginosa*, OXA-type beta-lactamase-producing *Acinetobacter baumannii*, and *S. maltophilia*. It is one of the most promising therapeutic solutions for pathogens such as carbapenem-resistant Enterobacterales (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB) in the future (Ghazi et al. 2018).

It was administered at 2 g IV infused over an hour every 8 hourly. It is mainly discharged in urine and hence requires dose modification as per CrCl (Saisho et al. 2018). No significant drug interaction has been reported (Katsube et al. 2018). Portsmouth et al. (2018) in a Phase II RCT demonstrated cefiderocol noninferior in contrast with IMI for managing cUTI patients. In a Phase 3 preliminary trial, the adequacy of cefiderocol for treatment of CRE diseases (counting HABP, VABP, cUTI, and blood stream infections (BSI)) was assessed. In the therapy of nosocomial pneumonia, cefiderocol + linezolid was compared to meropenem + linezolid.

18.2.3 Imipenem Plus Relebactam

Imipenem in addition to relebactam (MK7655) is a form of present carbapenem with a novel powerful non-β-lactam, bicyclic diazabicyclooctan with action against class A and C beta-lactamase but not against class D and MBL carbapenemases. Relebactam keeps up imipenem and cilastatin activity against KPC producers and *P. aeruginosa*. Presently, its combination with imipenem and cilastatin is being studied against Gram-negative microorganisms like *E. coli*, *P. aeruginosa*, *K. pneumoniae,* and others. It is mainly excreted in urine and Phase I trial has shown that PK of the individual agents is not affected significantly when given in combination.

In two Phase 2 trials, its efficacy and safety in patients with cIAI or cUTI have been studied. Here imipenem (500 mg 6 hourly) + relebactam at 125 mg and 250 mg 6 hourly was found similar to imipenem alone (Lucasti et al. 2016). The RESTORE-IMI1 study assessed it against colistin/imipenem in cIAI, cUTI or HAP/VAP patients for efficacy and safety (Motsch et al. 2018).

18.2.4 Lefamulin (BC-3781)

Lefamulin acts by hindering protein synthesis by restricting 50S ribosomal subunit. It has speedy and excellent penetration in the lungs epithelial lining. Hence, it has pivotal role in the treatment of infections in the respiratory tract. It also has potential in treating ABSSSI due to rapid distribution into skeletal and fatty tissues. It is active against all aerobic Gram-positive pathogens except *E. faecalis*. It is also active against Gram-negative pathogens (aside from *P. aeruginosa* and *A. baumannii*), some anaerobes, and against atypical pathogens (Jacobsson et al. 2017).

It is currently being studied in the management of ABSSSI and CABP. Phase 2 studies reported comparable cure by lefamulin 100 mg and 150 mg along with vancomycin in ABSSSI patients, whereas LEAP 1, LEAP 2, and Phase 3 studies have shown that lefamulin in treating CABP is noninferior in contrast to moxifloxacin with/without linezolid (Alexander et al. 2018).

18.2.5 Solithromycin

Solithromycin is a fourth-generation macrolide with 67% bioavailability that is not influenced by food. It is degraded by CYP3A4. It is primarily discharged in bile and just 10% in urine. There is no requirement of dose adjustment in hepatic impairment. No effect on QTc has been reported. It has important role in patients of respiratory tract infections because it reaches in higher concentrations in ELF and alveolar macrophages. Due to unique chemical structure, it has high intracellular accumulation. Subsequently, it is active against both extra and intracellular microbes. It has activity against Gram-positive and Gram-negative aerobes, and against Gram-positive anaerobes (Jamieson et al. 2015).

Investigations are being carried out for its role in CABP and urogenital gonorrhea patients. As per SOLITAIRE studies, solithromycin's 5 days treatment is not inferior when compared to moxifloxacin in patients of CABP. First, 800 mg oral or IV dose of solithromycin was given on day 1 and then 400 mg oral daily dose from day 2 to 5 (File et al. 2016; Barrera et al. 2016). In SOLITAIR-J (Phase 3) trial, once 1200 mg oral solithromycin was compared to ceftriaxone + azithromycin in intramuscular dose for efficacy and safety in patients of urogenital gonorrhea.

18.2.6 Sulopenem

Sulopenem is a newer carbapenem available in oral and IV dosage form. It is active against Gram-positive and -negative pathogens and not against CRE, *P. aeruginosa,* or MRSA. It is administered intravenously1000 mg/day or orally 500 mg twice a day alongside oral probenecid 500 mg twice a day (Kosowska-Shick et al. 2009).

It is tried in patients of cIAI and CABP. In a Phase 2 study, its efficacy was compared to ceftriaxone along with amoxy-clav in patients of CABP. A Phase III Sulopenem for Resistant Enterobacteriaceae (SURE) trial is being conducted for comparing it with ertapenem in patients of cIAI. Other indications are UTIs, acute prostatitis, gonococcal urethritis, and pelvic inflammatory disease.

18.2.7 Murepavadin

It (POL7080) is a new class of antibiotics called outer membrane protein targeting antibiotics. It produces its effect through a new mechanism by targeting lipopolysaccharide transport protein D (LptD). LptD has role in synthesis of lipopolysaccharide in the external membrane of *P. aeruginosa*. Hence, it has significant role against *P. aeruginosa*. The volume of distribution is high and $t1/2$ of 2–5 h. It has linear and dose-related pharmacokinetics (Martin-Loeches et al. 2018).

18.2.8 Aztreonam/Avibactam

It has efficacy against MBL-producing microbes and is destroyed by KPCs, ESBL, and class C (AmpC) beta-lactamases. But the combination demonstrated ten times potency against all these strains as compared to aztreonam given singly. But activity is limited against *P. aeruginosa* or *A. baumannii* compared to aztreonam monotherapy. It may be an effective treatment for MBL-producing pathogens related infections (Butler and Paterson 2020).

18.2.9 Ceftobiprole Medocaril

It is a fifth-generation cephalosporin with activity against both Gram-positive and -negative microbes, including MRSA and *Enterococcus* spp. It kills MRSA by inhibiting penicillin binding protein-2. It has activity against *Streptococcus* (including those which are resistant to penicillin and macrolide), *H. influenzae*, and *N. gonorrhoeae*. It has limited activity in *A. baumannii*. It is a prodrug and 500 mg is administered intravenously in 2 h, at every 8 h. It is mainly discharged in urine, and therefore, dose is adjusted according to CrCl.

It is approved in many countries for ABSSSI. It has been demonstrated as noninferior to vancomycin plus ceftazidime in complicated ABSSSI (Noel et al. 2008). Several double-blind RCTs have reported its efficacy in patients of pneumonia. Awad et al. (2014) in a Phase 3 study showed it is noninferior to ceftazidime + linezolid in HABP patients. Currently, Phase 3 studies are being performed to evaluate efficacy in patients with *S. aureus* infections.

18.2.10 Delafloxacin

It is a fluoroquinolone inhibiting MRSA and Gram-positive pathogens. Its diverse structure from other quinolones confers weak acidity (So et al. 2015). Subsequently, it inhibits more infections related to *S. aureus*. It has more potent role in UTI. It is active against Gram-positive, Gram-negative and anaerobic microbes. It is mainly excreted in urine and has 58.8% bioavailability. It is administered intravenously 300 mg or orally 450 mg every 12 h. It does not increase CYP3A, prolong QTc, and dose adjustment for hepatic dysfunction as other fluoroquinolones (Hoover et al. 2017).

It was used in the patients of ABSSSI. Phase 3 trial has shown that it is noninferior in comparison to vancomycin plus aztreonam in the treatment of MRSA. Phase II trial reported similar efficacy vs. tigecycline and linezolid and higher efficacy vs. vancomycin alone. Phase 3 trials evaluated its efficacy in patients of CABP and urogenital gonorrhea.

18.2.11 Cefepime/Zidebactam (FEP–ZID)

Cefepime/zidebactam is a novel mix of a cephalosporin, cefepime and BLI, zidebactam. Zidebactam is a bicyclo-acyl hydrazide. Zidebactam has higher binding for PBP2 and inhibits enzymes of class A and C. Zidebactam not only protects cefepime from β-lactamases but also extends its spectrum of antibacterial activity (Papp-Wallace and Bonomo 2016). It inhibits CRE, *P. aeruginosa*, and *A. baumannii*. FEP–ZID inhibits all three Ambler classes of carbapenemases and *P. aeruginosa*. FEP–ZID inhibits many MDR Gram-negative pathogens (Sader et al. 2017).

18.2.12 Cefepime/Enmetazobactam

Enmetazobactam (earlier known as AAI101) is a new ESBL inhibitor. It is a penicillanic acid sulfone derivative. It has activity against many beta-lactamases, but no add-on benefits occur while treating *Pseudomonas aeruginosa* with cefepime. This combination provides an option against ESBL-producing pathogens which is carbapenem free (Papp-Wallace and Bonomo 2016). It is indicated in cUTIs including acute pyelonephritis, cIAIs, and HABP or VABP. As per the ALLIUM Phase 3 trial, piperacillin/tazobactam was found inferior to it in cUTIs patients. With ESBL-producing bacteria, this combination demonstrated a cure rate of 73.7% as compared to 51.6% in patients who were on piperacillin/tazobactam.

18.2.13 Cefepime/Taniborbactam

Taniborbactam (earlier known as VNRX-5133) is a BLI that inhibits both serine-BL and MBL. It is a newer cyclic boronate. It enhances cefepime's potency while treating *Pseudomonas aeruginosa* and *Enterobacteriaceae* producing beta-lactamases such as KPC, OXA, CTX-M, VIM, and NDM-type beta-lactamases (Papp-Wallace and Bonomo 2016). In an in vitro study, this combination showed more potent activity as compared to ceftazidime/avibactam against various ESBL, AmpC, and MBLs producing urinary Gram-negative bacilli. A Phase 3 RCT is presently under process to evaluate cefepime/taniborbactam vs. meropenem in patients of cUTIs.

18.2.14 Sulbactam/Durlobactam

Durlobactam (earlier known as ETX2514) is a newer diazabicyclooctenone BLI which inhibits class A, C, and D BL. It inhibits Enterobacterales. In a study (Phase 2), it was contrasted to imipenem-cilastatin in cUTI patients, including AP and no significant difference was observed. It has shown very good activity against CRAB as compared to colistin, minocycline, amikacin, and sulbactam alone. Presently, a Phase 3 trial is comparing sulbactam/durlobactam + imipenem/cilastatin vs. colistin + imipenem/cilastatin against *A. baumannii-calcoaceticus* complex infection (Papp-Wallace and Bonomo 2016).

18.2.15 Dalbavancin (Butler and Paterson 2020)

It is a new second-generation semisynthetic lipoglycopeptide anti-microbial. It belongs to the same group as that of vancomycin which is available for patients with MRSA infection. It was developed to improve over the currently available natural glycopeptides like vancomycin and teicoplanin. It is synthesized from a complex glycopeptide, A-40926, which is derived from a new Actinomadura strain.

Similar to other glycopeptides, it exerts killing of bacteria by disrupting cell wall biosynthesis. It stops transpeptidation by binding to D-alanyl-D-alanyl residue on growing peptidoglycan chain. Thus, peptidoglycan elongation and cell wall synthesis are inhibited. It also forms dimers and anchors itself to the lipophilic membrane of microorganisms.

It inhibits Gram-positive microbes including MRSA and MRSE. It is administered once for 2 weeks. It has been approved for intravenous use in treating ABSSSIs caused by MSSA and MRSA.

It is contraindicated for those who are hypersensitive such as prone to skin reactions or anaphylaxis. Caution is to be taken for patients who are hypersensitive to other glycopeptides.

The most well-known adverse reactions reported in Phase II and III trials were nausea, diarrhea, headache, rash, itching. Others are hematologic disorders, bronchospasm, hepatotoxicity, Red Man Syndrome, *C. difficile* colitis, and anaphylactic shock. Ototoxicity is not reported with it. There is no evidence of teratogenicity in animals.

18.2.16 Oritavancin (Butler and Paterson 2020)

It is a semisynthetic glycopeptide. It has activity against serious Gram-positive bacterial infections. Its chemical structure as a lipoglycopeptide is the same as vancomycin. Its 4′-chlorobiphenylmethyl group destroys Gram-positive bacterial cell membrane. It also reduces transpeptidation and transglycosylation.

It is approved for treating ABSSSI. It has demonstrated rapid inhibition of both resistant and susceptible Gram-positive bacteria, including *S. aureus*, MRSA, enterococci, and streptococci. It was more active than metronidazole or vancomycin against *C. difficile* strains. It is useful as a therapy for exposure to *B. anthracis* causing anthrax.

18.3 Newer Carbapenems (Butler and Paterson 2020)

1. Ertapenem
   It is mainly indicated in cIAIs, ABSSSI, cUTIs, acute pelvic infections, and CABP. The net negative charge on it enhances its plasma protein binding (95%), and therefore, it has long half-life. So, administered once a day. *P. aeruginosa*, *Acinetobacter* spp., and *B. cepacia* are less inhibited by it. It has poor activity against ESBL-producing microbes as compared to other carbapenems, but it is approved for treating such infections. It was useful in treating early onset VAP due to ESBL-producing microbes, with clinical and microbiological success rate of 80% and 75%, respectively.
2. Doripenem
   It is a wide range carbapenem administered parenterally. Its molecular structure provides stability against β-lactamase and also resistance against renal DHP-I.

Similar to imipenem or ertapenem, it inhibits Gram-positive cocci. Against Gram-negative, the activity is same as meropenem. But it is not active against MRSA, *E. fecium*, etc. It is very active against ESBL producers. Dose modification is needed when CrCl is <30 ml/min. The most common adverse events with it are nausea and loose stools. The chances of seizures are lower than with others.

3. Biapenem
   It is a parenteral carbapenem. It is presently undergoing Phase II trials. It has higher penetration into respiratory system and body fluids. It has activity against Gram-positive microbes like *S. pneumoniae*, MSSA, and Gram-negative such as *A. baumannii*, ESBL producers, *S. marcescens*, *E. cloacae*, and *C. freundii*. It has been reported to have moderate activity against *P. aeruginosa*. The *T*1/2 is 1 h and administered 300 mg two times a day. Dose is modified as per the CrCl. The adverse events associated with it include nausea, vomiting, diarrhea, and skin eruption.
4. Panipenem/Betamipron
   Panipenem is combined with betamipron because betamipron halts renal excretion of panipenem. It is indicated for LRTI, UTI, obstetric/gynecological, and surgical infections. The recommended dose is 0.5 + 0.5 g two times a day as IV infusion in 30–60 min. It has been reported in three different Phase 3 RCT as efficacious when compared to imipenem+cilastatin in respiratory infections and UTI. It is useful to treat *Enterobacteriaceae* and common pathogens of respiratory tract. But against *H. influenzae*, meropenem is the most active carbapenem. *P. aeruginosa* is resistant to it.
5. Tomopenem
   It is 1-methyl carbapenem. It acts by inhibiting PBP activity and hampers peptidoglycan biosynthesis of cell wall in bacteria. It has lesser chances of resistance development. It is beneficial against MRSA, *P. aeruginosa* (ceftazidime resistant), and microbes producing ESBL.
6. Tebipenem
   Tebipenem pivoxil (earlier known as SPR994) is an oral prodrug. It is activated in plasma and effective against ESBL-producing pathogens. TBPM-PI-HBr is its novel formulation. It has high stability level against DHP-I. Its metabolite is well absorbed into the blood from intestine. It is used to treat UTIs. Various studies reported that tebipenem has more potency than imipenem but equal to that of meropenem against *E. coli, P. mirabilis,* and *K. pneumoniae*. It is not affected by generation of ESBL- and AmpC-BL. Results of a Phase 3 trial (ADAPT-PO) is pending where it was compared with ertapenem intravenous in AP or cUTI patients.
7. Other Newer Carbapenems
   (a) Razupenem (SMP-601) is under Phase 2 trials. It inhibits ESBL-synthesizers, but not carbapenemases. Its utility can be increased by combining it with other drugs. It has add-on effect with amikacin or ciprofloxacin against *B. cepacia* and *S. marcescens*.
   (b) 2-(Thyazol-2-ylthio)-1β-methyl carbapenems group has members such as SM-197436, SM-232721, and SM-232724. They are very effective against

MRSA but insufficient against *E. faecium*. These carbapenems are highly inhibitory for Gram negative microbes such as *H. influenzae*, *B. fragilis,* and *M. catarrhalis*. They are similarly active as imipenem against *E. coli*, *Proteus,* and *K. pneumoniae*. Other indications include hospital acquired bacterial infections by Gram-positive and -negative bacteria mainly MRSA and VRE.

(c) CS-023 (earlier known as RO 4908463) is another new molecule which is lesser effected by DHP-I than imipenem or meropenem. It is highly inhibitory for Gram-positive and -negative microbes. Against MRSA, it seems to be better than imipenem and meropenem. It has lower protein binding.

(d) ME 1036 (CP5609) is a carbapenem administered parenterally. It has an excellent activity against CABP pathogens.

(e) Trinems (earlier known as tribactams): To its carbon 1 and 2, a cyclohexane ring is attached. Sanfetrinem, as a hexatil ester, is administered orally. Sanfetrinem inhibits potent class A β-lactamase producers such as *P. vulgaris* and *K. oxytoca*.

18.3.1 Meropenem/Nacubactam

Nacubactam is a new intravenous BLI which acts by inhibiting serine β-lactamases (class A and C and few class D) and PBP2 in *Enterobacteriaceae*. In placebo-controlled studies, its pharmacokinetics, tolerability, and safety were evaluated in healthy individuals. They were administered once, increasing dose from 50 to 8000 mg, multiple increasing doses from 1000 to 4000 mg every 8 hourly for 7 days, or nacubactam + meropenem 2 g each every 8 hourly for 6 days. Nacubactam exhibited tolerance, with mild to moderate side effect of IV administration and headache. There were no dose-related clinical changes in safety test, or any serious or dose-limiting adverse event reported. Its pharmacokinetics was linear even after once or multiple doses. It was excreted mainly unchanged through kidney. The combination did not alter the pharmacokinetics of the individual drugs significantly. The study results are encouraging, and further clinical development is needed (Mushtaq et al. 2018).

CRE infections (KPC 2 and 3 carbapenemases) are very difficult to treat with present agents. Nacubactam is a bridged diazabicyclooctane BLI (mainly class A and C BL). It has also intrinsic antibiotic and BL "enhancer" activity against *Enterobacteriaceae*. The combination inhibits carbapenem-resistant *K. pneumoniae*. Studies also suggest that it has an alternative pathway of action as compared to avibactam (Papp-Wallace and Bonomo 2016).

18.4 Newer Topoisomerase Inhibitors

1. Gepotidacin (Earlier Known As GSK2140944)
   It is a new type-II topoisomerase inhibitor. It interacts with DNA gyrase (GyrA subunit) and topoisomerase IV (ParC subunit) and thereby inhibits DNA replication of bacteria. In a Phase 3 trial involving patients of cUTIs, it is being compared with nitrofurantoin. But it has lesser activity against Gram-negative microbes. It does not inhibit *C. trachomatis*. Three *N. gonorrhoeae* isolates are reported to have resistance against it (Taneja and Kaur 2016).
2. Zoliflodacin
   It acts by inhibiting type-II topoisomerase (GyrB sububit). The efficacy of this mechanism has been established against ceftriaxone- and ciprofloxacin-resistant *N. gonorrhoeae* and fluoroquinolone-resistant and VRSA isolates. It also inhibits *C. trachomatis*, *C. pneumoniae*, *M. genitalium,* and *M. ureaplasma*. A Phase 2 study demonstrated that oral zoliflodacin was more efficient than ceftriaxone alone in uncomplicated urogenital and rectal gonococcal infections but not pharyngeal infections. A Phase 3 trial is comparing it against ceftriaxone + azithromycin in uncomplicated gonorrhea patients (Kocsis et al. 2016).
3. Finafloxacin
   It is a fluoroquinolone and inhibits bacterial type-2 topoisomerase. It is useful in acute otitis externa caused by *S. aureus* and *P. aeruginosa*. It is very active under acidic environment, where bacteria like *H. pylori* live. It inhibits both Gram-positive and -negative microbes. It has post-antibiotic effect.

   The oral bioavailability is good with *T*1/2 around 10 h. It is available as 0.3% otic suspension. To prevent dizziness, it is gently warmed in hands for 1–2 min before administering.

   On topical application, ear itching and nausea have been reported. On oral or intravenous application, patients have reported GI side effects such as nausea, diarrhea, flatulence. Other ADRs noted are fatigue, musculoskeletal ailments, headaches, and injection site reactions. Rhinitis and nasopharyngitis are also noted (Kocsis et al. 2016).

18.5 Agents in Phase 2 Trials (Taneja and Kaur 2016; Vissichelli and Stevens 2019)

1. BOS228 (earlier known as LYS228) is monobactam administered intravenously. It inhibits both serine and MBL producing microbes. Its Phase 2 trial was done for Gram negative cUTI and cIAI.
2. Benapenem resembles ertapenem structurally and has longer *T*1/2. In Phase 2 trial, it was used intravenously in the treatment of cUTI including pyelonephritis. As per the Phase I trials data supports once a day intravenous administration.
3. Nafithromycin (earlier known as WCK 4873) is an oral ketolide. A Phase 2 trial was done on CABP patients. It inhibits both Gram-positive (*S. aureus* and

S. pneumoniae) and Gram-negative (*H. influenzae*, *M. pneumoniae*, *M. catarrhalis*, *L. pneumophila*, and *Chlamydophila pneumoniae*) bacteria.

4. MGB-BP-3 act by binding to bacterial DNA. In a Phase 2 trial it was administered to patients of *C. difficile*-associated diarrhea (CDAD). Its structure resembles the distamycin, thiazotropsin, and netropsin (lexitropsins: actinomycetes-derived minor groove binders).
5. XF-73 (exeporfinium chloride) is a porphyrin derivative and active against Gram-positive. It is a topically applied. In a Phase 2 trial, its effect was studied in patients at risk of post-operative nasal infections by *S. aureus*. The findings of the trial were found positive.
6. TNP-2092 (CBR 2092) is an amalgam of rifamycin-quinolizinone. In a Phase II trial, it was administered intravenously against Gram-positive ABSSSI infections. In another study, it was studied against infections due to catheter and prosthetic joint. It has high potency against Gram-positive bacteria. This is due to components: rifamycin which is an RNA polymerase inhibitor and quinolone which is a DNA gyrase and topoisomerase IV inhibitor.
7. Auranofin is a gold complex earlier used in treatment of rheumatoid arthritis. It inhibits thioredoxin reductase. It has gained interest for activity against *C. difficile*, *H. pylori*, *MRSA*, *S. pneumoniae,* and *E. faecalis*.
8. MBN-101 (bismuth ethanedithiol, BisEDT) is under Phase 2 trial for orthopedic infection patients. In a Phase 1b/2a trial, it is being used topically in infected diabetic foot. It was used to treat stomach aches and travelers' diarrhea. It is also used in combinations for treating *H. pylori* infections.
9. Afabicin (AFN 1720, Debio 1450) is a prodrug. In a Phase 2 trial, it was used against *S. aureus* bone or joint infection. Its Phase 2 trial for ABSSSI has completed. It mainly inhibits FabI, an enzyme essential for final step of fatty acid chain synthesis in *S. aureus*.
10. OPS-2071 is a quinolone-like agent. In a Phase 2 trial, it was used against enteric infections and *C. difficile*. During another Phase 2 trial, it was evaluated as an adjuvant agent in Crohn's disease.
11. Delpazolid (earlier known as RMX2001, LCB01-0371) is an oxazolidinone which inhibits Gram-positive microbes and TB. In a Phase 2 trial, it is being evaluated orally in the patients of TB.
12. Sutezolid (earlier known as PNU-100480, PF-2341272) is an oxazolidinone which was developed along linezolid. In a Phase 2 trial, it was administered orally to treat TB patients. Recently, a Phase 2 trial has started to evaluate it in combination with moxifloxacin, bedaquiline, and delamanid.
13. DNV-3837 (earlier known as MCB-3837) is a prodrug and hybrid of oxazolidinone-quinolone. In a Phase 2 trial, it was administered IV to treat CDI. It inhibits Gram-positive microbes such as MRSA, *B. anthracis, C. difficile,* and *Francisella tularensis*.
14. Telacebec (earlier known as Q203) is an imidazo [1,2-a] pyridine amide. It is administered orally. In a Phase 2 trial, it was studied in TB patients.
15. Macozinone (PBTZ169) is a benzothiazinone derivative. In a Phase 2 trial, it was studied in the treatment of TB.

16. OPC-167832 is an oral, 3,4-dihyrdo-carbostyril derivative. It has important role in treating pulmonary TB. It inhibits cell wall synthesis.
17. GSK656 (earlier known as GSK3036656) is a boron containing leucyl t-RNA synthetase inhibitor. In a Phase 2 trial, it was evaluated in pulmonary TB patients orally.

18.6 Substitutes to Antimicrobials Agents (Taneja and Kaur 2016; Vissichelli and Stevens 2019)

1. Bacteriophages
   They are bactericidal agents. These viruses infect mainly bacteria. With increasing antimicrobial resistance, there is gain in interest. They inhibit many bacterial functions and are very particular. They do not affect eukaryotes. They can enter and inhibit biofilms.

   A clinical trial studied Biophage-PA (a bacteriophage) against MDR *P. aeruginosa* in chronic otitis. The *P. aeruginosa* number was significantly reduced with its use. No adverse events were reported.

   Two phage mixture (T4-like coliphage or commercial Russian coliphage) in ORS was assessed against *E. coli* in children with acute diarrhea in another study. There were no adverse effects reported. But the phage mixture was not found to have significant effect over the control group in controlling diarrhea.

   In a Phase 1/2 double-blind RCT, PhagoBurn (mixture of 12 anti-*P. aeruginosa* bacteriophages) efficacy and tolerability for treating *P. aeruginosa*-infected burn wounds were studied. It was applied directly into the wounds.

   Various studies have reported synergism between antibiotics and bacteriophages. But the genesis of bacterial resistance to phages is unavoidable.
2. DCAP
   The compound 2-((3-(3,6-dichloro-9H-carbazol-9-yl)-2-hydroxypropyl)-amino)-2-(hydroxy-methyl) propane1,3-diol is an antimicrobial drug. It inhibits Gram-positive and -negative bacteria like *E. coli* and *P. aeruginosa*. DCAP has two ways leading to cell destruction. First, it improves ion movement across membrane and so decreases membrane potential. Second, it hampers cell membrane permeability. It is active against Gram-negative bacteria due to effect on inner membrane.

   It is active in dormant phase and biofilms. They do not affect RBC and destroy mammalian cell only at high concentrations and after 6 h. Synthesis of two analogues of DCAP have been reported. They were active against *B. anthrax* and *F. tularensis*. It showed synergistic effects when given together with ampicillin or kanamycin (targeting cell wall by different mechanism).
3. Odilorhabdins (ODLs)
   Ribosomes are an important target for antimicrobials. But with increasing drug resistance, the efficacy of antibiotics acting on ribosome is reduced. Gram-positive Actinomycetes and Gram-negative Xenrhabdus produce a variety of

metabolites with the help of genes which encode non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs). ODLs are modified peptide agents produced by NRPSs gene of Xenorhabdus nematophila. They are active against Gram-positive and -negative pathogens, including CRE. They bind to bacterial small subunit of ribosomes and inhibit them. They bind to rRNA or tRNA and introduce miscoding during translation process.

4. Peptidic benzimidazoles
   The benzimidazole-containing agents have antibacterial, antiviral, antifungal, anthelmintic, anticancer, and anti-inflammatory activities against various diseases. Benzimidazole-containing agents inhibit peptide deformylase (PDF). PDF inhibits ribosomal synthesis of protein in bacteria, protozoans, and some fungi. Many such agents have shown in vitro activities and hence can be potent newer agents for resistant Gram negative bacteria.

   The anti-microbial activity of these agents was demonstrated against Gram positive microbes (*S. aureus* and *E. faecium*) and Gram negative microbes (*E. coli* and *P. aeruginosa*), *C. albicans* and *C. tropicalis*. There was low to moderate antimicrobial activities. The antioxidant activities were also present.
5. Quorum Sensing (QS) Inhibitors
   Bacterial chemical communication or quorum sensing refers to organized bacterial gene activity to coordinate in process like production of virulence factor, antibiotics damage and biofilm formation. Auto-inducers (AIs) are the molecules which perform intra- and inter-species coordination. The most common QS mediators in Gram positive are oligopeptides while in Gram negative are *N*-acyl homoserine lactones (AHLs). (*S*)-4,5-Dihydroxy-2,3-pentanedione ((*S*)-DPD), is a QS modifier present in both Gram positive and negative bacteria. To activate QS, LsrK is phosphorylated to phosphoryl DPD. So, DPD derivatives act as antimicrobial agents by inhibiting LsrK. Isobutyl-DPD and phenyl-DPD inhibit QS when combined with gentamicin and small molecules. Modifying or inhibiting QS has evolved as an important treatment tool that can stop several bacterial virulence factors like biofilm formation and reduce the bad effect of bacterial infections. QS inhibitors can be combined with other antimicrobials to combat drug resistance.
6. Metal-Based Antibacterial Agents (Taneja and Kaur 2016; Vissichelli and Stevens 2019)
   Metal-based antimicrobials have been of great importance. They have unique modes of action. There are many ongoing research focusing on metal-based drugs. Ruthenium, bismuth, gallium, silver, and copper are the metals used commonly in metal-based antibacterial agents.
   (a) *Ruthenium (Ru):* They are active due to their ability to bind different places in cell like nucleic acids and proteins. Ru complexes exhibits photo-physical properties which can be utilized for knowing cellular accumulation and localization. They too have the pivotal role as antimicrobial agents. They inhibit Gram-negative microbes and some MDR strains due to their damaging effect on microbial cell wall. Studies have tried for the activity of two Ru complexes on Gram-positive (*S. aureus* and MRSA) and -negative (*E. coli*

and *K. pneumoniae*) microbes. The inhibition was reported better than chloramphenicol and ciprofloxacin in these resistant microbes. Hence, they may be considered in antibiotic drug development.

(b) *Gallium (Ga):* Their compounds inhibit microbial iron uptake or iron metabolism by inhibiting iron-dependent enzymes. As they are similar to iron, they incorporate into iron-dependent enzymes. They inhibit Gram-negative such as *K. pneumoniae*, *P. aeruginosa,* and *A. baumannii* and various Gram-positive strains. $Ga(NO_3)_3$ is currently being tested intravenously in a Phase 2 study against *P. aeruginosa* infection in cystic fibrosis patients. Ga-binding proteins in *P. aeruginosa* are the RpoB and RpoC, two subunits of RNA polymerase. They demonstrated that gallium halts transcription.

(c) *Bismuth (Bi):* Bismuth subsalicylate, colloidal bismuth subcitrate, and ranitidine bismuth citrate are used in treating *H. pylori* infections. Bi agents inhibit broad spectrum MBL. Bi showed potent broad-spectrum activity against *E. coli*, *E. faecalis,* and *M. smegmatis*. Bi phosphinates inhibits *E. coli*, and some Gram-positive such as MRSA and VRE.

(d) *Silver (Ag):* Silver, colloidal silver, and silver nitrate are used as wound antiseptics. Silver sulfadiazine is used for burn wounds. Silver nanoparticles inhibits multidrug-resistant *P. aeruginosa, E. coli,* and *P. mirabilis* strains. *N*-Heterocyclic carbene–silver (Ag(I)-NHC) complexes are a new class that inhibits antibiotic resistant bacteria.

(e) *Copper (Cu):* Copper (bis-thiosemicarbazone) (Cu(btsc)) inhibits Gram-positive and -negative microbes such as *N. gonorrhoeae*, *M. tuberculosis*, and *E. coli*. Some studies have synthesized copper complexes with ofloxacin and norfloxacin. These complexes have better binding abilities to DNA.

References

Alexander E, Goldberg L, Das A, Moran GJ, Sandrock C, Gasink LB et al (2018) Oral lefamulin is safe and effective in the treatment of adults with community- acquired bacterial pneumonia (CABP): results of Lefamulin evaluation against pneumonia (LEAP 2) study. Open Forum Infect Dis 5:S761

Awad SS, Rodriguez AH, Chuang Y-C, Marjanek Z, Pareigis AJ, Reis G et al (2014) A phase 3 randomized double-blind comparison of ceftobiprole medocaril versus ceftazidime plus linezolid for the treatment of hospital-acquired pneumonia. Clin Infect Dis 59:51–61

Barrera CM, Mykietiuk A, Metev H, Nitu MF, Karimjee N, Doreski PA et al (2016) Efficacy and safety of Oral solithromycin versus oral moxifloxacin for treatment of community-acquired bacterial pneumonia: a global, double-blind, multicentre, randomised, active-con- trolled, non-inferiority trial (SOLITAIRE-ORAL). Lancet Infect Dis 16:421–430

Breijyeh Z, Jubeh B, Karaman R (2018) Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25:1340. https://doi.org/10.3390/molecules25061340

Butler MS, Paterson DL (2020) Antibiotics in the clinical pipeline in October 2019. J Antibiot 73:329–364

File TM, Rewerska B, Vucinić-Mihailović V, Gonong JRV, Das AF, Keedy K et al (2016) SOLITAIRE-IV: a randomized, double-blind, multicenter study comparing the efficacy and

safety of intravenous-to-oral solithromycin to intravenous-to-oral moxifloxacin for treatment of community-acquired bacterial pneumonia. Clin Infect Dis 63:1007–1016
Gallagher JC (2019) Omadacycline: a modernized tetracycline. Clin Infect Dis 69(S1):S1–S5
Ghazi IM, Monogue ML, Tsuji M, Nicolau DP (2018) Humanized exposures of cefiderocol, a siderophore cephalosporin, display sustained in vivo activity against siderophore-resistant Pseudomonas aeruginosa. Pharmacology 101:278–284
Hoover R, Marbury TC, Preston RA, Quintas M, Lawrence LE, Paulson SK et al (2017) Clinical pharmacology of delafloxacin in patients with hepatic impairment. J Clin Pharmacol 57:328–335
Ito A, Nishikawa T, Matsumoto S, Yoshizawa H, Sato T, Nakamura R et al (2016) Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against Pseudomonas aeruginosa. Antimicrob Agents Chemother 60:7396–7401
Jacobsson S, Paukner S, Golparian D, Jensen JS, Unemo M (2017) In vitro activity of the novel pleuromutilin lefamulin (BC-3781) and effect of efflux pump inactivation on multidrug-resistant and extensively drug-resistant Neisseria gonorrhoeae. Antimicrob Agents Chemother 61: e01497–e01417
Jamieson BD, Ciric S, Fernandes P (2015) Safety and pharmacokinetics of solithromycin in subjects with hepatic impairment. Antimicrob Agents Chemother 59:4379–4386
Katsube T, Miyazaki S, Narukawa Y, Hernandez-Illas M, Wajima T (2018) Drug-drug interaction of cefiderocol, a siderophore cephalosporin, via human drug transporters. Eur J Clin Pharmacol 74:931–938
Kocsis B, Domokos J, Szabo D (2016) Chemical structure and pharmacokinetics of novel quinolone agents represented by avarofloxacin, delafloxacin, finafloxacin, zabofloxacin and nemonoxacin. Ann Clin Microbiol Antimicrob 15:34
Kosowska-Shick K, Ednie LM, McGhee P, Appelbaum PC (2009) Comparative antipneumococcal activities of sulopenem and other drugs. Antimicrob Agents Chemother 53:2239–2247
Lucasti C, Vasile L, Sandesc D, Venskutonis D, McLeroth P, Lala M et al (2016) Phase 2, dose-ranging study of relebactam with imipenem-cilastatin in subjects with complicated intra-abdominal infection. Antimicrob Agents Chemother 60:6234–6243
Martin-Loeches I, Dale GE, Torres A (2018) Murepavadin: a new antibiotic class in the pipeline. Expert Rev Anti-Infect Ther 16:259–268
Motsch J, de Oliveira C, Stus V, Koksal I, Lyulko O, Boucher H et al (2018) RESTORE-IMI 1: a multicenter, randomized, double-blind, comparator-controlled trial comparing the efficacy and safety of imipenem/relebactam versus colistin plus imipenem in patients with imipenem-non-susceptible bacterial infections. In: Presented in 28th European congress of clinical microbiology and infectious diseases (ECCMID), Madrid
Mushtaq S, Vickers A, Woodford N, Haldimann A, Livermore DM (2018) Activity of nacubactam (RG6080/OP0595) combinations against MBL-producing Enterobacteriaceae. J Antimicrob Chemother 74:953–960
Noel GJ, Bush K, Bagchi P, Ianus J, Strauss RS (2008) A randomized, double-blind trial comparing ceftobiprole medocaril with vancomycin plus ceftazidime for the treatment of patients with complicated skin and skin-structure infections. Clin Infect Di 46:647–655
Papp-Wallace KM, Bonomo RA (2016) New β-lactamase inhibitors in the clinic. Infect Dis Clin N Am 30(2):441–464. https://doi.org/10.1016/j.idc.2016.02.007
Portsmouth S, van Veenhuyzen D, Echols R, Machida M, Ferreira JCA, Ariyasu M et al (2018) Cefiderocol versus imipenem-cilastatin for the treatment of complicated urinary tract infections caused by Gram-negative uropathogens: a phase 2, randomised, double-blind, non-inferiority trial. Lancet Infect Dis 18:1319–1328
Sader HS, Rhomberg PR, Flamm RK, Jones RN, Castanheira M (2017) WCK 5222 (cefepime/zidebactam) antimicrobial activity tested against Gram-negative organisms producing clinically relevant beta-lactamases. J Antimicrob Chemother 72:1696–1703

Saisho Y, Katsube T, White S, Fukase H, Shimada J (2018) Pharmacokinetics, safety, and tolerability of cefiderocol, a novel siderophore cephalosporin for gram-negative bacteria, in healthy subjects. Antimicrob Agents Chemother 62:e02163–e02117

So W, Crandon JL, Nicolau DP (2015) Effects of urine matrix and pH on the potency of delafloxacin and ciprofloxacin against urogenic Escherichia coli and Klebsiella pneumoniae. J Urol 194:563–570

Taneja N, Kaur H (2016) Insights into newer antimicrobial agents against gram-negative bacteria. Microbiol insights 9:9

Vissichelli NC, Stevens MP (2019) Antibiotics in the pipeline for treatment of infections due to Gram-negative organisms. Curr Treat Options Infect Dis 11:115–144

Adjuvant Molecules/Compounds in Combating Bacterial Resistance

19

Mohd. Shadab and Mohammad Shahid

Abstract

Historically, one of the major challenges before humans has been the emergence of mutated microbial pathogens. The current covid pandemic is an example of it. But the rise of drug resistance bacteria is another global emergency. In addition, we are not well equipped with the newer antibiotics to cope the surge of MDR, XDR, and PDR pathogens as the potent antibiotics are depleting in healthcare facilities. Though the situation demands a quick action to discover novel antibiotics, some efforts have been done to save the effect of conventional antibiotics. Adjuvants are the chemical compounds which are co-dosed with antibiotics to treat the drug-resistant infections. Adjuvant molecules are found to be affecting and diverting the resistance strategies of bacteria enhancing antibiotics to contend with infections. Uninterestingly, only a few of them are available in commercial therapeutic market. In this chapter, we compiled as much as possible number of adjuvant/antibiotic combinations proved to be effective in this regard. This will help readers in gaining insight on available combinations of adjuvants and antibiotics.

Keywords

Antibiotics · Adjuvants · Adjuvant molecules · Adjuvant compounds · Antibiotics resistance · Bacteria

M. Shadab (✉) · M. Shahid
Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
e-mail: shadab@agu.edu.bh; mohammeds@agu.edu.bh

M. Shahid et al. (eds.), *Beta-Lactam Resistance in Gram-Negative Bacteria*,
https://doi.org/10.1007/978-981-16-9097-6_19

19.1 Introduction

Due to increased uptake of antibiotics, both in the clinics and in community, the bacteria evolved to survive and turned resistant, which we in common language call becoming as "*Superbugs*." They proved to be smart enough to combat the medicinal arsenal ceasing the effects of antibiotics. This evolution has mainly been suggested because of an unregulated/unrecommended use of antibiotics (Raoult and Paul 2016). The more is the use of antibiotics, the more is the outcome of multidrug-resistant (MDR) and extensive-drug-resistant (XDR) pathogens, and the more is the paucity of novel antibiotics. Although we have some last-resort antibiotics like colistin, tigecycline, vancomycin, etc. for usage against the resistant bacteria, these are also losing their effect day by day (Aghapour et al. 2019; Cetinkaya et al. 2000). Thus, the need of newer, novel, and more potent antibiotics/compounds is the demand of the current era (Shahid et al. 2020). Moreover, the ongoing COVID pandemic is posing another threat to exacerbate this problem of antibiotics resistance, mainly due to unwanted use of antibiotics and self-medication (Lucien et al. 2021; Mahoney et al. 2021).

To respond to this threatening situation on antibiotics resistance, the global medical research community has suggested different ways to rescue our conventional antibiotics and to disarm the bacterial resistance against medicinal scaffolds. The alternative treatment strategies suggested through the time lapse includes synergistic combinations of antibiotics, adjuvant compounds (Bernal et al. 2013), phage therapy, monoclonal antibodies, antimicrobial oligonucleotides (Streicher 2021), and herbal antimicrobials (Chedid et al. 2014). Out of these alternatives, we searched online for the research work done in respect of adjuvant/antibiotic combinations. The scientific community has reported several adjuvant compounds to fill the gap of newer antibiotic drug discoveries (Wright 2016).

By simplified definition, an adjuvant is considered as a chemical compound given in combination with antibiotics. To a lesser extent, an adjuvant might show some antimicrobial activity, but when administered with antibiotics, it increases the potential to fight against MDR bacteria (Gonzalez-Bello 2017). The adjuvant molecules either target a specific part of bacteria bypassing its anti-antibiotic activity or enhance the activity of antibiotics against the resistance mechanism of the bacteria. Some adjuvants are also reported to confer the host immune system as a potential to fight with antibiotics (Gonzalez-Bello 2017; Wright 2016).

19.2 How Adjuvants Work in Alleviating Bacterial Resistance

We cannot understand the anti-resistance property of chemical adjuvants unless we discuss about the resistance strategies of multi-drug-resistant bacteria. The research information produced so far depicts different types of resistance mechanisms expressed by bacteria for their very natural Lamarckian evolution. Intrinsically, bacteria do (1) produce different genes which confer them the ability to inhibit the activity of antibiotic molecules, e.g., CTX-M, AmpC, IMP, NDM;

(2) overexpression of efflux pumps, e.g., Nor A, RND, ABC (Soto 2013); (3) production of biofilms and (4) outer membrane permeability barriers; and (5) evolution in signaling and regulatory pathways (Gill et al. 2015; Roy et al. 2018). Systematically, the adjuvant compounds act against one (or more) of the above-mentioned resistance acquiring mechanism(s) of bacteria. Said that, they tend to alleviate the bacterial resistance by one of the following ways (Gill et al. 2015; Gonzalez-Bello 2017; Liu et al. 2019):

(a) By reversing (or inactivating) the resistance activity of the bacterial enzymes.
(b) By permeabilizing the bacterial cell membrane.
(c) By degrading the biofilm, enhancing the accumulation of antibiotics intracellularly.
(d) By inhibiting the efflux pumps.
(e) By modifying the signaling and regulatory pathways.

After the extensive literature search, we tried to compile the published adjuvants along with their antibiotic combinations. Accordingly, these combinations are organized under the respective anti-resistance mechanism(s) in Table 19.1.

19.3 Available Commercial Combinations of Adjuvants

A great effort and investment have been done so far to discover novel antibiotics as well as adjuvants to control the multidrug-resistant infections (Gill et al. 2015). But the published literature highlights that, despite a huge research on adjuvant molecules, only a few of them succeeded to get approval for their therapeutic use. Table 19.2 comprises of commercial products of adjuvant/antibiotic combinations.

19.4 Other Adjuvant Compounds

Several other chemical scaffolds have been tested with different antibiotics to see their effects in combating bacterial resistance. The published findings suggest that some of them can be used as an adjuvant. They either showed synergy with antibiotics, or they help antibiotics to penetrate the bacterial cells (Dobias et al. 2017; Shahzad et al. 2018). Some of the reported adjuvants are enlisted in Table 19.3.

19.5 Conclusion

MDR, XDR, and PDR bacterial infections are one of the current pressing problems. This is because the gap between the clinical arsenal and new antibiotic innovations is increasing. As discussed earlier, the adjuvant molecules when given concurrently with antibiotics, are not only able to fight the drug resistance effectively but also

Table 19.1 List of adjuvants and antibiotic combinations with their respective anti-resistance mechanisms*

Mechanism(s)	Adjuvants	Antibiotics	References
Inhibition of resistant enzymes	Clavulanic acid	Amoxicillin Ticarcillin Meropenem	Bernal et al. (2013), Liu et al. (2019)
	Sulbactam	Amoxicillin Cefoperazone Ampicillin	Bernal et al. (2013), Gill et al. (2015)
	Tazobactam	Piperacillin Ceftolozane	Schuetz et al. (2018), Zhanel et al. (2014)
	Avibactam	Ceftazidime Ceftaroline Aztreonam	Castanheira et al. (2012), Sader et al. (2017, 2021)
	Vaborbactam (RPX7009)	Meropenem Biapenem	Goldstein et al. (2013), Zhanel et al. (2018)
	Zidebactam	Cefepime	Thomson et al. (2019)
	Enmetazobactam	Cefepime	Morrissey et al. (2019)
	Nacubactam	Meropenem	Asempa et al. (2020)
	Durlobactam (ETX2514)	β-Lactams Sulbactam	Seifert et al. (2020)
	Taniborbactam (VNRX-5133)	Cefepime	Hamrick et al. (2020)
	Relebactam (MK-7655)	Imipenem Cilastatin	Zhanel et al. (2018)
	TFDG (Theaflavin-3,3′-digallate)	β-Lactams Cephalothin	Teng et al. (2019)
	Cobaltocenium-containing metallopolymers	Penicillin-G Amoxicillin Ampicillin Cefazolin	Zhang et al. (2014)
	Phthalic acid derivatives	Biapenem Carbapenem	Hiraiwa et al. (2013)
	Succinic acid derivatives	Imipenem	Gill et al. (2015)
	NagZ inhibitor	Ceftazidime	Gill et al. (2015)
	Siderophore monosulfactam BAL30072	Meropenem	
	SA2-13 (Penam sulfones)	Ampicillin	Gill et al. (2015)
	Polyketides: Compounds 1 and 2	Meropenem	Gill et al. (2015)
	Aspergillomarasmine A	Meropenem	Liu et al. (2019)
	FPI-1465	Meropenem Ceftazidime Aztreonam	Gill et al. (2015)

(continued)

Table 19.1 (continued)

Mechanism(s)	Adjuvants	Antibiotics	References
	Copper ions	Carbapenem	Liu et al. (2019)
	PPMOs	Carbapenem Polymyxins	Liu et al. (2019)
	Stigmasterol	Ampicillin	Liu et al. (2019)
	Pterostilbene	Polymyxin B	Liu et al. (2019)
	QPX7728 (Boronate)	Meropenem Ceftazidime Piperacillin Cefepime Ceftolozane Ceftibuten Cefpodoxime Tebipenem	Lomovskaya et al. (2020)
	Tryptamine complexes	Colistin	Barker et al. (2019)
Efflux pumps inhibitors	Phenylalanine-arginine ß-naphthylamide (PAßN)	Quinolones Piperacillin Cefotaxime Ceftazidime Ciprofloxacin	Gill et al. (2015), Liu et al. (2019)
	Peptide nucleic acids (PNAs)	Ciprofloxacin Erythromycin	Gill et al. (2015), Liu et al. (2019)
	SLUPP225/417	Erythromycin	Liu et al. (2019)
	MBX2319/3132/3135	Ciprofloxacin	Liu et al. (2019)
	A22	Novobiocin	Liu et al. (2019)
	Boronic acid derivatives	Ciprofloxacin	Gill et al. (2015)
	Capsaicin	Ciprofloxacin	Gill et al. (2015)
	(*Z*)-*N*-benzylidene-2-(tert-butoxycarbonylamino)-1-(5-iodo-1H-indol-3-yl)ethanamine	Ciprofloxacin	Gill et al. (2015)
	Pyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide analogues	Ciprofloxacin	Gill et al. (2015)
	Flavones & 2-(4-Propoxyphenyl) quinoline derivatives	Ciprofloxacin	Gill et al. (2015)
	3-(Substituted-3,4-dihydronaphthyl)-2-propenoic acid amides	Ciprofloxacin	Gill et al. (2015)
	4-Methyl-*N*-[2-(1-methyl-1H-pyrrol-2-yl)-1H-benzimidazol-5-yl]benzenesulfonamide	Ciprofloxacin	Gill et al. (2015)
	3-{5-[(*Z*)-(3-sec-butyl-2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-furyl}-4-chlorobenzoic acid	Ciprofloxacin	Gill et al. (2015)
	4-({[3-Cyano-6-ethyl-4-(trifluoromethyl)-5,6,7,8-tetrahydroquinolin-2-yl]thio} methyl)benzoic acid	Ciprofloxacin	Gill et al. (2015)

(continued)

Table 19.1 (continued)

Mechanism(s)	Adjuvants	Antibiotics	References
	2-{[3-(Benzyloxy)benzyl]amino}-1-phenylpropan-1-ol	Ciprofloxacin	Gill et al. (2015)
	Organotin (IV) derivatives	Tetracycline	Barbosa et al. (2018)
Bacterial membrane permeabilizers	C_{12}-PRP	Rifampicin Minocycline	Liu et al. (2019)
	OAKs	Rifampicin	Liu et al. (2019)
	SPR741	Rifampicin	Liu et al. (2019)
	B2088	Gatifloxacin Tobramycin	Liu et al. (2019)
	Vanillin	Spectinomycin	Liu et al. (2019)
	Loperamide	Tetracyclines	Bernal et al. (2013), Liu et al. (2019)
	Eugenol (from *Eugenia aromatic*)	Vancomycin	Bernal et al. (2013)
	Phenylpropanoids	Amikacin Ampicillin Ciprofloxacin Erythromycin Vancomycin	Bernal et al. (2013)
	4-Hexylresorcinol	Polymyxin Gentamycin Ciprofloxacin	Nikolaev et al. (2020)
Biofilm inhibitors	D-aminoacids	Ciprofloxacin Tobramycin	Bernal et al. (2013)
	Sophorolipid	Kanamycin Cefotaxime	Lydon et al. (2017)
	Nitric oxide (NO)	Tobramycin	Bernal et al. (2013)
	Triclosan	Tobramycin	Liu et al. (2019)
	Peptide 1018	Ceftazidime Tobramycin Imipenem Ciprofloxacin	Gill et al. (2015)
Inhibitors of signaling and regulatory pathways	Phthalocyanine tetrasulfonic acid	Ciprofloxacin Ampicillin Kanamycin	Liu et al. (2019)
Host defense modulators	LL-37	Multi-classes	Liu et al. (2019)
	hLF1-11(lactoferritin derivative)	Gentamicin	Liu et al. (2019)
	EDC34	Ceftazidime	Gill et al. (2015)

Table is modified from Gill et al. (2015) and Liu et al. (2019)

Table 19.2 Commercially available adjuvant/antibiotic combinations

Trade name	Adjuvants	Antibiotics	References
Augmentin®	Clavulanic acid	Amoxicillin	White et al. (2004)
Timentin®	Clavulanic acid	Ticarcillin	Jacobs et al. (1985)
Elores™	Disodium edetate + Sulbactam	Ceftriaxone	Shahid et al. (2020)
Unasyn®	Sulbactam	Ampicillin	Claussen (1993)
Zosyn®	Tazobactam	Piperacillin	Uji and Hashimoto (2009)
Zerbaxa™	Tazobactam	Ceftolozane	Cluck et al. (2015)
Vabomere™	Vaborbactam	Meropenem	Lee et al. (2019)
Recarbrio®	Relebactam	Imipenem + cilastatin	Ghazi et al. (2020)
Zavicefta®, Avycaz®	Avibactam	Ceftazidime	Mosley II et al. (2016)
Fetroja®	Siderophore	Cephalosporin (cefiderocol)	Dobias et al. (2017)

Table 19.3 List of other adjuvants with their antibiotic combinations

Mechanism	Adjuvants	Antibiotics	References
Others	Siderophore	Cephalosporin	Ellermann and Arthur (2017)
	Vitamin B complex (B1, B2, B12)	Linezolid	Shahzad et al. (2018)
	Vitamin E & K	Multi-classes Piperacillin Imipenem Doripenem Tazobactam	Liu et al. (2019), Shahzad et al. (2018)

proved to be saving our current antibiotic repertoire blessing them with an extended lifespan. Researchers across the world have suggested adjuvants' activities triggering cross mechanisms against different types of bacterial drug resistance when administered with antibiotics. Conclusively, this is sufficient reason why we try to enlist these adjuvant compounds. Hopefully, this will be helpful in further research and the commercial production of new antibiotic adjuvant combinations could be expedited.

References

Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, Yousefi M, Asgharzadeh M, Yousefi B, Kafil HS (2019) Molecular mechanisms related to colistin resistance in Enterobacteriaceae. Infect Drug Resist 12:965–975. https://doi.org/10.2147/IDR.S199844

Asempa TE, Motos A, Abdelraouf K, Bissantz C, Zampaloni C, Nicolau DP (2020) Meropenem-nacubactam activity against AmpC-overproducing and KPC-expressing Pseudomonas

aeruginosa in a neutropenic murine lung infection model. Int J Antimicrob Agents 55(2): 105838. https://doi.org/10.1016/j.ijantimicag.2019.10.019

Barbosa ASL et al (2018) Synthesis and evaluation of the antibiotic and adjuvant antibiotic potential of organotin(IV) derivatives. J Inorg Biochem 180:80–88. https://doi.org/10.1016/j.jinorgbio.2017.12.004

Barker WT, Chandler CE, Melander RJ, Ernst RK, Melander C (2019) Tryptamine derivatives disarm colistin resistance in polymyxin-resistant gram-negative bacteria. Bioorg Med Chem 27(9):1776–1788. https://doi.org/10.1016/j.bmc.2019.03.019

Bernal P, Molina-Santiago C, Daddaoua A, Llamas MA (2013) Antibiotic adjuvants: identification and clinical use. Microb Biotechnol 6(5):445–449. https://doi.org/10.1111/1751-7915.12044

Castanheira M, Sader HS, Farrell DJ, Mendes RE, Jones RN (2012) Activity of ceftaroline-avibactam tested against Gram-negative organism populations, including strains expressing one or more beta-lactamases and methicillin-resistant Staphylococcus aureus carrying various staphylococcal cassette chromosome mec types. Antimicrob Agents Chemother 56(9): 4779–4785. https://doi.org/10.1128/AAC.00817-12

Cetinkaya Y, Falk P, Mayhall CG (2000) Vancomycin-resistant enterococci. Clin Microbiol Rev 13(4):686–707. https://doi.org/10.1128/CMR.13.4.686

Chedid V, Dhalla S, Clarke JO, Roland BC, Dunbar KB, Koh J, Justino E, Tomakin E, Mullin GE (2014) Herbal therapy is equivalent to rifaximin for the treatment of small intestinal bacterial overgrowth. Glob Adv Health Med 3(3):16–24. https://doi.org/10.7453/gahmj.2014.019

Claussen DW (1993) Unasyn (ampicillin sodium/sulbactam sodium). Gastroenterol Nurs 16(1): 36–38. https://doi.org/10.1097/00001610-199308000-00011

Cluck D, Lewis P, Stayer B, Spivey J, Moorman J (2015) Ceftolozane-tazobactam: a new-generation cephalosporin. Am J Health Syst Pharm 72(24):2135–2146. https://doi.org/10.2146/ajhp150049

Dobias J, Denervaud-Tendon V, Poirel L, Nordmann P (2017) Activity of the novel siderophore cephalosporin cefiderocol against multidrug-resistant Gram-negative pathogens. Eur J Clin Microbiol Infect Dis 36(12):2319–2327. https://doi.org/10.1007/s10096-017-3063-z

Ellermann M, Arthur JC (2017) Siderophore-mediated iron acquisition and modulation of host-bacterial interactions. Free Radic Biol Med 105:68–78. https://doi.org/10.1016/j.freeradbiomed.2016.10.489

Ghazi IM, El Nekidy WS, Asay R, Fimognari P, Knarr A, Awad M (2020) Simultaneous administration of imipenem/cilastatin/relebactam with selected intravenous antimicrobials, a stewardship approach. PLoS One 15(5):e0233335. https://doi.org/10.1371/journal.pone.0233335

Gill EE, Franco OL, Hancock RE (2015) Antibiotic adjuvants: diverse strategies for controlling drug-resistant pathogens. Chem Biol Drug Des 85(1):56–78. https://doi.org/10.1111/cbdd.12478

Goldstein EJ, Citron DM, Tyrrell KL, Merriam CV (2013) In vitro activity of Biapenem plus RPX7009, a carbapenem combined with a serine beta-lactamase inhibitor, against anaerobic bacteria. Antimicrob Agents Chemother 57(6):2620–2630. https://doi.org/10.1128/AAC.02418-12

Gonzalez-Bello C (2017) Antibiotic adjuvants - a strategy to unlock bacterial resistance to antibiotics. Bioorg Med Chem Lett 27(18):4221–4228. https://doi.org/10.1016/j.bmcl.2017.08.027

Hamrick JC et al (2020) VNRX-5133 (Taniborbactam), a broad-spectrum inhibitor of serine- and Metallo-beta-lactamases, restores activity of cefepime in Enterobacterales and Pseudomonas aeruginosa. Antimicrob Agents Chemother 64(3):e01963. https://doi.org/10.1128/AAC.01963-19

Hiraiwa Y, Morinaka A, Fukushima T, Kudo T (2013) Metallo-beta-lactamase inhibitory activity of 3-alkyloxy and 3-amino phthalic acid derivatives and their combination effect with carbapenem. Bioorg Med Chem 21(18):5841–5850. https://doi.org/10.1016/j.bmc.2013.07.006

Jacobs RF, Augustine RA, Aronson J, McCarthy RE, Steele RW, Yamauchi T (1985) Timentin therapy for bone, joint, and deep soft tissue infections in children. Am J Med 79(5B):188–191. https://doi.org/10.1016/0002-9343(85)90158-5

Lee Y, Kim J, Trinh S (2019) Meropenem-Vaborbactam (VabomereTM): another option for Carbapenem-resistant Enterobacteriaceae. P T 44(3):110–113

Liu Y, Li R, Xiao X, Wang Z (2019) Antibiotic adjuvants: an alternative approach to overcome multi-drug resistant Gram-negative bacteria. Crit Rev Microbiol 45(3):301–314. https://doi.org/10.1080/1040841X.2019.1599813

Lomovskaya O, Tsivkovski R, Nelson K, Rubio-Aparicio D, Sun D, Totrov M, Dudley MN (2020) Spectrum of beta-lactamase inhibition by the cyclic boronate QPX7728, an ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-beta-lactamases: enhancement of activity of multiple antibiotics against isogenic strains expressing single beta-lactamases. Antimicrob Agents Chemother 64(6):e00212. https://doi.org/10.1128/AAC.00212-20

Lucien MAB et al (2021) Antibiotics and antimicrobial resistance in the COVID-19 era: perspective from resource-limited settings. Int J Infect Dis 104:250–254. https://doi.org/10.1016/j.ijid.2020.12.087

Lydon HL, Baccile N, Callaghan B, Marchant R, Mitchell CA, Banat IM (2017) Adjuvant antibiotic activity of acidic Sophorolipids with potential for facilitating wound healing. Antimicrob Agents Chemother 61(5):e02547. https://doi.org/10.1128/AAC.02547-16

Mahoney AR, Safaee MM, Wuest WM, Furst AL (2021) The silent pandemic: Emergent antibiotic resistances following the global response to SARS-CoV-2. iScience 24(4):102304. https://doi.org/10.1016/j.isci.2021.102304

Morrissey I, Magnet S, Hawser S, Shapiro S, Knechtle P (2019) In vitro activity of Cefepime-Enmetazobactam against gram-negative isolates collected from U.S. and European hospitals during 2014-2015. Antimicrob Agents Chemother 63(7):e00514. https://doi.org/10.1128/AAC.00514-19

Mosley JF II, Smith LL, Parke CK, Brown JA, Wilson AL, Gibbs LV (2016) Ceftazidime-avibactam (Avycaz): for the treatment of complicated intra-abdominal and urinary tract infections. P T 41(8):479–483

Nikolaev YA et al (2020) The use of 4-hexylresorcinol as antibiotic adjuvant. PLoS One 15(9):e0239147. https://doi.org/10.1371/journal.pone.0239147

Raoult D, Paul M (2016) Is there a terrible issue with bacterial resistance: pro-con. Clin Microbiol Infect 22(5):403–404. https://doi.org/10.1016/j.cmi.2016.03.004

Roy R, Tiwari M, Donelli G, Tiwari V (2018) Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action. Virulence 9(1):522–554. https://doi.org/10.1080/21505594.2017.1313372

Sader HS, Castanheira M, Flamm RK (2017) Antimicrobial activity of ceftazidime-avibactam against Gram-negative bacteria isolated from patients hospitalized with pneumonia in U.-S. Medical Centers, 2011 to 2015. Antimicrob Agents Chemother 61(4):e02083. https://doi.org/10.1128/AAC.02083-16

Sader HS, Carvalhaes CG, Arends SJR, Castanheira M, Mendes RE (2021) Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. J Antimicrob Chemother 76(3):659–666. https://doi.org/10.1093/jac/dkaa504

Schuetz AN, Reyes S, Tamma PD (2018) Point-counterpoint: piperacillin-Tazobactam should be used to treat infections with extended-spectrum-beta-lactamase-positive organisms. J Clin Microbiol 56(3):e01917. https://doi.org/10.1128/JCM.01917-17

Seifert H, Muller C, Stefanik D, Higgins PG, Miller A, Kresken M (2020) In vitro activity of sulbactam/durlobactam against global isolates of carbapenem-resistant Acinetobacter baumannii. J Antimicrob Chemother 75(9):2616–2621. https://doi.org/10.1093/jac/dkaa208

Shahid M, Ahmed S, Iqbal Z, Sami H, Singh A (2020) Evaluation of a new combination: ceftriaxone-disodium edetate-sulbactam as a broad-spectrum option for multidrug-resistant bacterial infections. New Zealand Institute of Medical Laboratory Science, New Zealand, pp 22–26

Shahzad S, Ashraf MA, Sajid M, Shahzad A, Rafique A, Mahmood MS (2018) Evaluation of synergistic antimicrobial effect of vitamins (a, B1, B2, B6, B12, C, D, E and K) with antibiotics against resistant bacterial strains. J Glob Antimicrob Resist 13:231–236. https://doi.org/10.1016/j.jgar.2018.01.005

Soto SM (2013) Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virulence 4(3):223–229. https://doi.org/10.4161/viru.23724

Streicher LM (2021) Exploring the future of infectious disease treatment in a post-antibiotic era: a comparative review of alternative therapeutics. J Glob Antimicrob Resist 24:285–295. https://doi.org/10.1016/j.jgar.2020.12.025

Teng Z, Guo Y, Liu X, Zhang J, Niu X, Yu Q, Deng X, Wang J (2019) Theaflavin-3,3 -digallate increases the antibacterial activity of beta-lactam antibiotics by inhibiting metallo-beta-lactamase activity. J Cell Mol Med 23(10):6955–6964. https://doi.org/10.1111/jcmm.14580

Thomson KS, AbdelGhani S, Snyder JW, Thomson GK (2019) Activity of Cefepime-Zidebactam against multidrug-resistant (MDR) Gram-negative pathogens. Antibiotics 8(1):32. https://doi.org/10.3390/antibiotics8010032

Uji T, Hashimoto Y (2009) [Pharmacological properties and clinical efficacies of tazobactam/piperacillin (Zosyn 2.25, Zosyn 4.5), an injectable antibiotic combined with beta-lactamase inhibitor]. Nihon Yakurigaku Zasshi 133(6):351–358. https://doi.org/10.1254/fpj.133.351

White AR, Kaye C, Poupard J, Pypstra R, Woodnutt G, Wynne B (2004) Augmentin (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. J Antimicrob Chemother 53 (suppl 1):i3-20. https://doi.org/10.1093/jac/dkh050

Wright GD (2016) Antibiotic adjuvants: rescuing antibiotics from resistance. Trends Microbiol 24(11):862–871. https://doi.org/10.1016/j.tim.2016.06.009

Zhanel GG et al (2014) Ceftolozane/tazobactam: a novel cephalosporin/beta-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. Drugs 74(1):31–51. https://doi.org/10.1007/s40265-013-0168-2

Zhanel GG et al (2018) Imipenem-Relebactam and Meropenem-Vaborbactam: two novel Carbapenem-beta-lactamase inhibitor combinations. Drugs 78(1):65–98. https://doi.org/10.1007/s40265-017-0851-9

Zhang J, Chen YP, Miller KP, Ganewatta MS, Bam M, Yan Y, Nagarkatti M, Decho AW, Tang C (2014) Antimicrobial metallopolymers and their bioconjugates with conventional antibiotics against multidrug-resistant bacteria. J Am Chem Soc 136(13):4873–4876. https://doi.org/10.1021/ja5011338