Microbial Pathogenesis and Antimicrobial Drug Resistance

6

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Contents

Abstract

Antimicrobial drug resistance has become a serious threat and it caused the death of 700,000 individuals in 2016. Gram-negative bacteria such as Acinetobacter, Pseudomonas, Enterobacter spp. *Enterococcus faecium*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* are insensitive to antibiotics. *E. faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.* are called as "ESKAPE" group of pathogens which have multidrug resistance property. Multidrug-resistant (MDR) bacteria are involved in

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B. Siddhardha et al. (eds.), *Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery*,
https://doi.org/10.1007/978-981-15-1695-5_6

increased mortality and produce economic burden in the world. Bacteria escape the toxic action of antibiotics by increasing the efflux of antibiotics, inactivation of antibiotics through chemical modifications and modification of antibiotic targets. Bacteria use a genetic mechanism to avoid antibiotic effect through mutations in the gene(s) which are associated with antibiotic action and acquisition of resistance genes through horizontal gene transfer (HGT). The antibiotic resistance property in bacteria occurred through horizontal gene transfer such as transformation, transduction, and conjugation of plasmids or transposons, and mutations in the existing genes. Efflux pumps contribute antibiotic resistance at three levels such as intrinsic, acquired, and phenotypic. The human microbiome is considered as a reservoir of antibiotic resistance genes. Development of antibiotic resistance should be considered as an adaptive response in Darwinian's principles of evolution. Therefore, understanding the molecular mechanisms and evolution of multidrug-resistant bacteria are need to be studied.

Keywords
Antibiotics · Multiple drug resistance · Gram-positive and gram-negative bacteria · Efflux pumps · Antibiotic resistance genes

6.1 Introduction

Antimicrobial drug resistance is a phenomenon by which bacteria, parasites, and viruses modify themselves to bypass the action of antibiotics, antiviral, and antiparasitic drugs. Due to antimicrobial drug resistance, medical treatments such as surgery, organ transplants, chemotherapy, and diabetes management became a serious threat (WHO. Antimicrobial resistance. www.who.int/mediacentre/factsheets/fs194/en/. Accessed February 26, 2018). 700,000 deaths due to antimicrobial resistance were reported in 2016 and this number may increase to ten million annual deaths by 2050 (Bello and Dingle 2018). Antimicrobial resistance (AMR) has become a major global concern observed in gram-negative bacteria such as *Acinetobacter*, *Pseudomonas*, and *Enterobacter spp*. *Enterococcus faecium*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Pham et al. 2019). *Acinetobacter baumannii*, *Campylobacter jejuni*, *Clostridium difficile*, *Enterobacter spp*. *Enterococcus faecium*, *E. faecalis*, *Escherichia coli*, *Haemophilus influenzae*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella spp*. *S. aureus*, *S. epidermidis*, and *Streptococcus pneumoniae* are commonly found in hospitals and develop resistance to variety of antibiotics. *Mycobacterium tuberculosis* is also extremely drug-resistant (Davies and Davies 2010). *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp. are called as "ESKAPE" group of pathogens which have multidrug resistance property (Rice

Table 6.1 List of key antibiotics used in treatment of infections due to gram-negative bacteria

Name of antibiotics	Class of antibiotics	Applied in clinical condition
Amoxycillin, ampicillin	Penicillin	Respiratory tractinfections
Imipenem, meropenem, doripenem ertapenem	Carbapenems	Infections of gram-negative bacteria
Doxycycline, minocycline	Tetracyclines	Minor infections in respiratory tract
Gentamicin	Aminoglycosides	Endocarditis
Norfloxacin, ciprofloxacin, Moxifloxacin	Quinolones	Infections in urinary tract
Polymyxin B, colistin	Polymyxins	Treatment of multi-resistant gram-negative infections
Chloramphenicol	Phenicols	Bacterial meningitis
Erythromycin	Macrolides	Treatment of minor infections Due to gram-positive bacteria

2008). These bacteria developed multidrug resistance property through inactivation of drug, modification of drug target site, and enhancement of efflux of drug (Santajit and Indrawattana 2016). Biofilm formation by these bacteria inhibits the action of antibiotics against these bacteria (Lewis 2007). Antibiotics (Table 6.1) inhibit the growth of gram-negative bacteria by crossing the cell envelope. Bacteria escape the toxic action of antibiotics by increasing the efflux of antibiotics, inactivation of antibiotics through chemical modifications and modification of antibiotic targets (Kohanski et al. 2010). The outer membrane (OM) of gram-negative bacteria (GNB) prevents the movement of amphipathic drugs whereas an inner membrane of GNB inhibits the transport of hydrophilic drugs (Masi et al. 2017). 6-Deoxynybomycin amine is effective against multidrug-resistant gram-negative bacteria such as *E. coli*, *K. pneumoniae*, and *A. baumannii* (Richter et al. 2017). Antimicrobial peptide, Lassomycin from *Lentsea kentuckyensis* inhibits ATP-dependent protease complex ClpP1P2C1 protease in *Mycobacterium tuberculosis*. Teixobactin which is the product of *Eleftheria terrae* prevents the biosynthesis of cell wall in gram-positive bacteria (Pham et al. 2019). *P. aeruginosa* developed antibiotic resistance during long antibiotic treatment of cystic fibrosis patients (Horrevorts et al. 1990). Twenty-eight genomic islands encoding antibiotic resistance property have been reported in *A. baumannii* (Barbe et al. 2004). More than 20,000 potential resistance genes are discovered in available bacterial genome sequences (Liu and Pop 2009). Extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and *Clostridium difficile* are considered as a serious threat within hospitals (Pamer 2016). Discovery of antibiotics and its resistance property against bacteria are thrust area of research in academic and pharmaceutical world. In this chapter, antibacterial drug resistance mechanisms in relevant bacterial pathogens are emphasized.

6.2 Demographic Distribution of Antimicrobial Drug Resistance Strains

The incidence of AMR has been highly reported in Asia, Africa, and the Middle East. In India, China, and Pakistan, people consume large number of antibiotics. In Africa, high prevalence of chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline resistant gram-negative bacteria was reported. These strains are susceptible to third-generation cephalosporins and fluoroquinolones. Travel across international borders enhances the exposure of antimicrobial resistance strains. Multidrug-resistant (MDR) *S. pneumoniae* strain spreads from South Africa to Europe whereas drug-resistant gonorrhea spreads from Asia to the Pacific and North America. New Delhi Metallo-beta-lactamase 1 (blaNDM-1) gene dramatically migrated from India and Pakistan to Europe. The colistin-resistant (*mcr-1*) gene was first reported from people in China in 2016 and later on it migrated to the USA. Migration of population is a major source of burden of AMR in Europe and other developed countries. Over burden of population in refugee camps induces transmission of AMR. Carriers of extended-spectrum β-lactamase enzyme (ESBL-E) genes are reported in 1.1 billion population in Southeast Asia, 280 million in Western Pacific region, and 110 million in Africa. Patients hospitalized in Asia, sub-Saharan Africa, and Latin America were more prone to get infected with methicillin-resistant *S. aureus* (MRSA). It has been reported about infection of carbapenem-resistant *A. baumannii* among soldiers in Iraq during gulf war (Semret and Haraoui 2019). Quinolones and cephalosporins resistant strains have been reported in Pakistan in 2016 (Wain et al. 2015). Global Gonococcal Antimicrobial Surveillance Program in participating countries revealed that 97% gonococcal isolates are resistant to ciprofloxacin (Unemo and Shafer 2014). Vancomycin-resistant Enterococci (VRE) are reported in the Europe (van den Bogaard et al. 1997). Quinolone-resistant *Salmonella enterica* are reported in Denmark and Taiwan (Molbak et al. 2002). Hospital wastewater and wastewater treatment plants which are contaminated by antibiotic contribute to the development of bacterial resistance (Johnning et al. 2013) (Table 6.2). Infections of carbapenem-resistant bacterial strains such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were reported in blood of 40 patients at Tata Medical Center, Kolkata, India. Out of 40 patients, 21 patients had hematologic malignancy and 19 patients had solid tumor (Exner et al. 2017). *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* which are positive for ESBL are reported in south-eastern Asian countries such as Thailand, Singapore, Malaysia, Vietnam, Indonesia, Philippines, Laos, Cambodia, Myanmar, and Brunei. The percentage of carbapenem-resistant (CR) *A. baumannii* and *P. aeruginosa* was 76% and 23%, respectively (Suwantarat and Carroll 2016). The pathogens responsible for nosocomial infections such as *E. coli*, *E. faecalis*, *E. faecium*, *Enterococci*, *S. aureus*, and *C. difficile* resistant to antibiotics such as cephalosporins, penicillins, and carbapenems were reported in Germany in 2011 with frequency of 18, 13.2, 13.1, and 8.1%, respectively (Behnke et al. 2013).

Table 6.2 Sources of antibiotic resistance bacteria

Antibiotic resistance bacterial species	Common types of antibiotic resistance	Source	Types of infection
Streptococcus pneumonia	Penicillin, macrolides, cephalosporins, tetracyclines	Childcare facilities	Pneumonia
Streptococcus pyogenes	Macrolides, tetracyclines	Schools	Pharyngitis
Staphylococcus aureus	Methicillin, cephalosporins, macrolides	Hospitals, soldiers	Pneumonia, sepsis
Enterococcus spp.	Ampicillin, vancomycin, aminoglycosides	Hospitals	Urinary tract
Neisseria gonorrhoeae	Penicillin, cephalosporins, quinolones	Sex workers	Inflammatory disease
Salmonella spp.	Cephalosporins, quinolones, tetracyclines	Poultry	Diarrhea
Campylobacter jejuni	Erythromycin, quinolones	Poultry	Gastroenteritis
Escherichia coli	Trimethoprim, sulphonamides, quinolones	Childcare facilities	Urinary tract, diarrhea

6.3 Molecular Mechanism of Antibiotic Resistance

Bacteria use genetic mechanism to avoid antibiotic effect through mutations in gene(s) which are associated with antibiotic action and acquisition of resistance genes through horizontal gene transfer (HGT). The antibiotic resistance property in bacteria occurred through horizontal gene transfer such as transformation, transduction, and conjugation of plasmids or transposons, and mutations in the existing genes (Giedraitienė et al. 2011). The antimicrobial resistance occurred through the molecular mechanisms such as modifications of the antibiotic target site that reduces the affinity for the antibiotics, reduced uptake of antibiotic, activation of efflux pumps and alterations of metabolic pathways (Munita and Arias 2016). Conjugation of mobile genetic elements (MGEs) such as plasmids and transposons occurs at high frequency in the gastrointestinal tract of humans in patients undergoing antibiotic treatment in the hospital (Manson et al. 2010). Other antimicrobial resistance genes are integrons which are site-specific recombination systems. Integrons are responsible for the incorporation of new genes into bacterial chromosomes (Thomas and Nielsen 2005). Fluoroquinolone (FQ) resistance developed in the bacteria through mutations in DNA gyrase and topoisomerase IV and overexpression of efflux pumps. Enzymes which are involved in chemical alterations of antibiotics developed antibiotic resistance in both gram-negative and gram-positive bacteria. They catalyzed acetylation (aminoglycosides, chloramphenicol, and streptogramins), phosphorylation (aminoglycosides, chloramphenicol), and adenylation (aminoglycosides, lincosamides) (Munita and Arias 2016). Aminoglycoside modifying enzymes (AMEs) are involved in covalent modification in hydroxyl or amino groups of the aminoglycoside molecule. Aminoglycoside acetyltransferases are reported in *Providencia stuartii*, *E. faecium*, and *S. marcescens* (Ramirez and Tolmasky 2010). Phosphotransferase (APH) is responsible to altered kanamycin and streptomycin in gram-positive and gram-negative bacteria. Acetyltransferase

(ACC) in *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* effects aminoglycosides including amikacin and gentamicin. High-level gentamicin and vancomycin resistance are detected in enterococci and methicillin-resistant in *S. aureus* (Ramirez and Tolmasky 2010; Hollenbeck and Rice 2012). Chloramphenicol acetyltransferases (CATs) are involved in chemical modification of chloramphenicol which inhibits protein synthesis by binding peptidyl-transfer center of the 50S ribosomal subunit. High-level resistance type A cat gene and low-level resistance type B cat gene are reported in both gram-positive and gram-negative bacteria (Schwarz et al. 2004).

Efflux of antibiotics into bacterial cell before it reaches into the target site is due to overexpression of transmembrane multidrug efflux pumps (Alcalde-Rico et al. 2016). In *P. aeruginosa*, mutation in porins results in the development of resistance against antibiotics such as carbapenems by reducing the permeability of the cell wall. In *E. coli*, transmembrane proton gradient efflux pumps expel multiple antibiotics. In gram-positive bacteria, the outer membrane inhibits transport of hydrophobic drugs (Shaikh et al. 2015). In gram-positive and gram-negative bacteria, tetracycline efflux pumps (*TetA*) protein is normally not expressed but TetR repressor protein is expressed. Tetracycline binds and inactivates TetR that drives activation of TetA which induces the efflux of antibiotic. Penicillin-resistant gene (MecA) in *Streptococcus pneumoniae*; vancomycin-resistant gene (*vanA*) in *S. aureus*; *Enterococcus* and sulfonamide-resistant gene in *S. pneumoniae*, *S. pyogenes*, *Neisseria* spp., and *E. coli* (Pelgrift and Friedman 2013) were reported. Genes encoding aminoglycoside-modifying enzymes in plasmids or transposons altered the binding affinity for 30S ribosomal subunit by modifying OH or NH_2 groups on aminoglycosides. Mutations of β-lactamase genes (Table 6.3) enhance the resistance property of bacteria against β-lactam antibiotics such as cephalosporins (Shaikh et al. 2015). *S. aureus* and *Neisseria meningitidis* developed resistance against sulfonamide by secreting para-aminobenzoic acid which binds with the active site of bacterial dihydropteroate synthetase (Yun et al. 2012). Streptomycetes produce a variety of β-lactamases (Ogawara et al. 1999). *S. pneumoniae* acquired resistance property by alterations of penicillin-binding proteins (PBPs) which reduced the binding affinity of drug (Furuya and Lowy 2006). Healthcare-associated methicillin-resistant *S. aureus* MRSA (HA-MRSA) and community-associated methicillin resistant *S. aureus* (CA-MRSA) carried mobile chromosomal element which is known as staphylococcal chromosomal cassette (SCC). SCC

Table 6.3 Key antibiotic resistance mechanism involved in gram-positive pathogens

Gene(s) involved	Antibiotic	Resistance mechanisms in pathogens
blaZ	β-Lactams (penicillins)	Penicillins are hydrolyzed by plasmid-encoded β-lactamase
mecA	β-Lactams	Low affinity of PBP2a
23S rRNA genes, L3/L4 ribosomal proteins	Linezolid	Mutations in 23S rRNA genes
mprF, dlt, *vraRS*, *yycFG*, *pgsA*, *cls*	Daptomycin	Repulsion of the antibiotic due to increased positive charge of cell envelope

carries the methicillin-resistance gene (mec). SCCmec element from groups I–III are reported in HA-MRSA whereas SCCmec type IV and SCCmec type V are reported in CA-MRSA. Among them, SCCmec type IV is more mobile. CA-MRSA is more genetically diverse as compared to HA-MRSA (Daum et al. 2002; Hiramatsu et al. 2002).

6.4 Role of Bacterial Outer Membrane in Influx and Efflux of Antibiotic

Gram-negative bacteria acquired antibiotic resistance due to cell envelope which consists of an outer membrane (OM) and inner membrane (IM). Periplasmic space separates OM and IM (Zgurskaya et al. 2015). The most abundant OM proteins such as OmpF and OmpC are reported in *E. coli* (Baslé et al. 2006). The permeability of the OM in gram-negative bacteria becomes lower to antibiotics. Multidrug efflux pumps belonging to the ABC (ATP-binding cassette family), MF (the major facilitator superfamily), SMR (small multidrug resistance family), MATE (multidrug and toxic compound extrusion family), PACE and RND (resistance-nodulation-cell division) super families are reported in bacterial genomes. Reduced numbers of porins in the OM and modular tripartite efflux pumps (ABC, MFS, or RND) in IM provide resistance against antibiotics (Nikaido and Pagès 2012). The ABC exporters have two categories such as homodimeric and heterodimeric. The heterodimeric ABC exporters do not support ATP hydrolysis. The ABC exporter MacB is responsible for the development of antibiotic resistance in *E. coli* and other gram-negative bacteria. The MATE transporters are classified into NorM, DinF (DNA-damage inducible protein F), and eukaryotic subfamilies on the basis of their amino acid sequence homology. MATE transporters efflux of polyaromatic and cationic drugs through transmembrane H+ and/or Na+ gradients. RND family such as AcrB, MexB, MtrD, and CmeB are reported in *E. coli*, *P. aeruginosa*, *N. gonorrhoeae*, and *C. jejuni*, respectively. *A. chlorhexidine* efflux protein (AceI) of PACE family developed resistance against chlorhexidine, acriflavine, proflavine, and benzalkonium. Two-component systems (TCSs) such as CpxAR in Enterobacteriaceae, AdeSR in *A. baumannii*, and AmgRS in *P. aeruginosa* control the expression of multidrug resistance efflux pumps. *A. baumannii* acquired mutations in adeSR and overexpression of the RND pump AdeAB. AdeSR is responsible for the expression of the tripartite pump system such as ade-ABC and biofilm formation. TCSs also regulate the expression of efflux pumps such as BaeSR and AdeAB and AdeIJK RND in *A. baumannii*. In *E. coli*, five out of 15 TCSs regulates the expression of drug efflux pump genes. Mutations in tetracycline repressor protein (TetR) family of transcriptional repressors (emrR, acrR, and mtrR) enhance the overexpression of efflux pumps (Du et al. 2018). Efflux pumps such as mefA and mefE are responsible to efflux erythromycin and are mainly reported in *S. pyogenes* and *S. pneumonia*. Transposon Tn1207 is located in MefA (Ross et al. 1990). RND pumps developed resistance to tetracyclines, chloramphenicol, some β-lactams, novobiocin, fusidic acid, and fluoroquinolones (Munita and Arias 2016).

6.5 Role of Colistin Resistance Gene in Microbial Pathogenesis

Colistin of *Paenibacillus polymyxa* is an antibiotic. Colistin is widely used in animal husbandry. Colistin-mediated resistance gene (*mcr-1* gene) was found in *S. enterica* and *E. coli*. Colistin resistance genes in plasmids are horizontally transmitted across the bacteria. Positively charged diaminobutyric acid (Dab) residues of colistin primarily interact with the negatively charged phosphate groups of lipid A of lipopolysaccharide (LPS) which is present in outer-membrane (OM) of gram-negative bacteria. Modification of LPS such as addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolamine (PEtn) to lipid A moiety of LPS induces colistin resistance through reducing the negative charge of LPS. Colistin resistance *mcr* genes encode a phosphoethanolamine transferase that induces the binding of PEtn to lipid A. This reduces binding of colistin to LPS. Eight types of mcr genes (mcr-1 to -8) have been reported in different geographical regions such as mcr-2 gene in *E. coli* from pigs and calves of Belgium, mcr-3 in *E. coli* from pigs of China, mcr-4 in *S. enterica serovar Typhimurium* from pigs of Italy, mcr-5 in *S. paratyphi* B dTa+ from poultry of Germany, mcr-6 in Moraxella spp. from pigs of Great Britain, mcr-7 in *Klebsiella pneumoniae* from chickens of China, and mcr-8 in K. pneumonia from pigs and humans of China (Lima et al. 2019). Animals are the primary source of non-typhoidal Salmonella (NTS). 93.8 million illness and 155,000 deaths annually in world due to NTS gastroenteritis have been reported (Majowicz et al. 2010). In *S. enterica*, colistin resistance was developed due to PmrA/PmrB and PhoP/PhoQ two-component regulatory systems that induced the biosynthesis of L-Ara4N and PEtn. Constitutive expression of PmrA/PmrB and PhoP/PhoQ enhanced binding of L-Ara4N and PEtn, respectively, to lipid A (Olaitan et al. 2014).

6.6 Molecular Mechanisms of AMR in Gram-Negative Bacteria

Several multidrug resistance mechanisms are involved in Enterobacteriaceae family of gram-negative bacteria such as enzymatic degradation, modification of target site, overexpression of efflux pumps, and reduction of cell permeability. β-Lactamase enzymes hydrolyze the β-lactam ring of penicillin and cephalosporins to make them inactive. AmpC β-lactamases, extended-spectrum-β-lactamases (ESBLs), and carbapenemase are responsible for the degradation of cephalosporins. Development of fluoroquinolone resistance acquired as a result of modification of enzymes such as DNA gyrase and DNA topoisomerase IV. Antimicrobials are preferred to bind with porins which are present at the outer membrane of gram-negative bacteria. Loss of porins reduces the permeability of cell wall and prevents the entry of antibiotics into the cell. Resistance-nodulation-division (RND) efflux systems ArcAB-TolC of *E. coli* and MexAB-OprM of *P. aeruginosa* are effective against cephalosporins, fluoroquinolones, penicillin, and chloramphenicol. Trimethoprim resistance is developed

due to alteration of target site of trimethoprim (Mukerji et al. 2017; Verraes et al. 2013; Li and Nikaido 2009). Fluoroquinolones are used during urinary tract, respiratory tract, and gastrointestinal infections. Fluoroquinolone resistance is acquired due to mutation in DNA gyrase (*gyrA*, *gyrB*) and DNA topoisomerase IV genes (*parC*, *parE*) (Cavaco et al. 2008). Four types of β-lactamases such as class A serine β-lactamases (ESBLs, penicillinases), class B metallo-β-lactamases, class C AmpC-type-β-lactamases, and class D OXA β-lactamases are reported. Among them, ESBL cephalosporinases (CTX-M type enzymes) are common β-lactamases and these developed resistance against penicillin and cephalosporins (Bush and Jacoby 2010). Different types of carbapenemase enzymes such as class D enzymes (OXA family) of carbapenemases in Enterobacter and *P. aeruginosa*, metallo-β-lactamases (IMP, VIM family) in *Klebsiella* and *Enterobacter* spp., and non-metallo-carbapenemases (SME, IMI/NMC) in *Serratia* and *Enterobacter* spp. are reported (Carattoli 2009). The KPC enzymes in *K. pneumoniae* and *E. cloacae* are responsible for the development of resistance against all β-lactams such as cephalosporins, monobactams, and carbapenems (Livermore and Woodford 2006). Class C β-lactamases developed resistance to all penicillins and cephalosporins. AmpC (cephalosporinase) is most clinically relevant class C enzyme and this has been reported in *E. cloacae*, *E. aerogenes*, *C. freundii*, *S. marcescens*, *Providencia* sp. *Morganella morganii*, and *P. aeruginosa* (Jacoby 2009). Class D β-lactamases hydrolyze oxacillin and many OXA variants such as OXA-11 from *P. aeruginosa*, OXA-23 from *A. baumannii*, and OXA-48 *K. pneumoniae* are able to degrade third-generation cephalosporins (Evans and Amyes 2014).

6.7 Molecular Mechanisms of AMR in Gram-Positive Bacteria

MDR gram-positive organisms such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* (VRE), and drug-resistant *Streptococcus pneumoniae* are serious public threats. (Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. Available at: http://www.cdc.gov/drugresistance/threat-report-2013/index.html. Accessed 9 March 2015). Staphylococcal-type Aβ-lactamase is responsible for hydrolysis of cefazolin. Methicillin resistance depends on gaining of staphylococcal chromosomal cassette mec (SCCmec) which contains mecA. Transpeptidase PBP2a which is encoded by mecA showed low affinity for all β-lactams. Ceftaroline and ceftobiprole resistance results due to substitution mutations such as Y446N and E447K in penicillin-binding site of transpeptidase domain of PBP2a (DeLeo and Chambers 2009; Kelley et al. 2015). Pneumococcal β-lactam resistance results from alterations in native PBPs through recombination with exogenous pbp genes. The resistance property is enhanced in the presence of point mutations in pbp genes (Munita et al. 2015). Mutations in genes encode murein (*murM*), GlcNAc deacetylase (*pdgA*), and glycosyltransferase (*cpoA*) and developed β-lactam resistance in *Pneumococci* (Hakenbeck et al. 2012). Oxazolidinone resistance results

due to mutations in the 23S ribosomal RNA (rRNA) genes, genes encoding L3/L4 ribosomal proteins, and methylation of the 23S rRNA gene (Munita et al. 2015). Daptomycin (DAP) is effective against gram-positive bacteria. DAP resistance in *S. aureus* results due to electrostatic repulsion of the DAP calcium complex from cell surface (Bayer et al. 2013). DAP resistance in *S. aureus* results due to increase in the content of positive charge phospholipid lysyl-phosphatidylglycerol in cell envelop through upregulation of transmembrane protein MprF (Tran et al. 2013).

6.8 Role of Efflux Pumps in Gram-Positive and Gram-Negative Bacteria

Efflux pumps (Tables 6.4 and 6.5) contribute antibiotic resistance at three levels such as intrinsic, acquired, and phenotypic (Fajardo et al. 2008). Acquired resistance can be achieved by horizontal gene transfer and mutations that over expressed chromosomally encoded efflux pumps. Bacteria developed intrinsic resistome towards antibiotics through constitutively lower expression of efflux pumps (Olivares et al. 2013). Phenotypic resistance is defined as inheritable overexpression of an efflux pump in a specific growing condition. In gram-negative bacteria, tripartite complexes are made up of inner-membrane efflux pump, an outer-membrane protein, and a membrane fusion protein. Multi AnTimicrobial Extrusion (MATE) transporters are divided into three subfamilies such as DinF, NorM, and the eukaryotic subfamilies (Lu 2015). The major facilitator superfamily (MFS) consists of importers and exporters (Law et al. 2008). MFS is most predominant in gram-positive bacteria. The *E. coli* EmrAB-TolC efflux pump is one of the examples of MFS. The Resistance Nodulation and cell Division (RND) superfamily of efflux pumps is commonly reported in gram-negative bacteria. These are composed of three different proteins such as active efflux pump, an outer-membrane protein, and a fusion protein. The RND efflux pumps which contributed to intrinsic resistance are AcrAB-TolC in Enterobacteriaceae, MexAB-OprM and MexXY in *P. aeruginosa*, and SmeDEF in *Stenotrophomonas maltophilia* (Hernando-Amado et al. 2016).

S. aureus acquired MFS efflux pumps QacA and QacB which reduced susceptibility of *S. aureus* towards antibiotics commonly used in nosocomial infections. (Wassenaar et al. 2015). MFS efflux pumps such as NorA, NorB, NorC, and NorD in *S. aureus* are responsible for the development of resistance against norfloxacin, ciprofloxacin, tetraphenylphosphonium, and cetrimide (Costa et al. 2013). NorD is responsible to develop resistance against norfloxacin, moxifloxacin, delafloxacin, levofloxacin nalidixic acid, ciprofloxacin, tetracycline, polymyxin B, trimethoprim, daptomycin, and triclosan. MdeA which belongs to MFS efflux pump is responsible to efflux norfloxacin and tetraphenylphosphonium (Yamada et al. 2006). Plasmid-borne resistance pumps such as QacG, QacH, QacJ, and Smr are observed in *S. haemolyticus* (Correa et al. 2008). Two MFS efflux pumps such as MdrL and Lde are reported in *Listeria monocytogenes*. Lde provided resistance against fluoroquinolones, whereas overexpression of MdrL provided resistance

Table 6.4 Clinically important efflux pumps present in gram-positive bacteria

Family of efflux pump	Efflux Pump	Organism	Location	Efflux of antibiotics by pump
MFS	QacA	*S. aureus*	Plasmid	Benzalkonium chloride, cetrimide, propamidine, isethionate, diaminodiphenylamine dihydrochloride, pentamidine, chlorhexidine, acriflavine
MFS	QacB	*S. aureus*	Plasmid	Chlorhexidine, benzalkonium chloride, tetraphenylphosphonium, acriflavine
MFS	NorA	*S. aureus*	Chromosome	Norfloxacin, enoxacin, ofloxacin, ciprofloxacin, pentamidine, cetrimide, benzalkonium chloride, tetraphenylphosphonium, bromide, acriflavine
MFS	NorC	*S. aureus*	Chromosome	Norfloxacin, ciprofloxacin, sparfloxacin, gemifloxacin,
MFS	NorD	*S. aureus*	Chromosome	Polymyxin B, nalidixic acid, trimethoprim, daptomycin, tetracycline, norfloxacin, daptomycin
MFS	MefA	*Enterococcus*	Chromosome	Erythromycin
MFS	Tap	*Mycobacterium*	Chromosome	Aminoglycosides, tetracycline, rifampicin, clofazimine, acriflavine
MFS	JefA	*Mycobacterium*	Chromosome	Isoniazid, ethambutol, streptomycin
MATE	MepA	*S. aureus*	Chromosome	Ciprofloxacin, norfloxacin, moxifloxacin, sparfloxacin, tigecycline, pentamidine, cetrimide, benzalkonim chloride, dequalinium tetraphenyl-phosphonium, chlorhexidine, acriflavine
ABC	MsrB & MsrA	*Enterococcus*	Chromosome	Erythromycin
MFS	PmrA	*Streptococcus pneumoniae*	Chromosome	Fluoroquinolones
ABC	PatAB	*Streptococcus pneumoniae*	Chromosome	Fluoroquinolones
MATE	PdrM	*Streptococcus pneumoniae*	Chromosome	Chloramphenicol Erythromycin

against benzalkonium (Godreuil et al. 2003; Romanova et al. 2006). Mutations in gyrA and overexpression of efflux pumps are responsible to develop ofloxacin resistance in *M. tuberculosis* (Sun et al. 2014). MFS efflux pump such as JefA contributed resistance against ethambutol and isoniazid. MFS efflux pump such as Tap is responsible for the development of resistance property against aminoglycosides and tetracyclines (Gupta et al. 2006; Ramon-Garcia et al. 2009). Tap has been detected in *Mycobacterium abscessus*, *M. chelonae*, *M. fortuitum*, *M. mageritense*, *M. peregrinum*, *M. alvei*, and *M. porcinum* (De Groote and Huitt 2006).

Table 6.5 Clinically important RND family efflux pumps present in gram-negative bacteria

Family of efflux pump	Efflux pump	Organism	Efflux of antibiotics by pump
RND	AdeABC	*Acinetobacter baumannii*	Aminoglycosides, cephalosporins, fluoroquinolones, tetracyclines-tigecycline, macrolides, chloramphenicol, and trimethoprim
RND	AdeFGH	*Acinetobacter baumannii*	Fluoroquinolones, tetracyclines-tigecycline, chloramphenicol, lincosamides, sulfonamides and trimethoprim
RND	AdeIJK	*Acinetobacter baumannii*	β-lactams, cephalosporins, fluoroquinolones, tetracyclines-tigecycline, macrolides, lincosamides, novobiocin, rifampicin, cotrimoxazole, trimethoprim, chloramphenicol and fusidic acid
RND	MtrCDE	*Neisseria gonorrhoeae*	Aminoglycosides; penicillin (β- lactams); azithromycin (macrolides); ceftriaxone
RND	MexAB–OprM	*Pseudomonas aeruginosa*	Aminoglycosides; amphenicols; β- lactams (except imipenem); fluoroquinolones; macrolides; novobiocin; sulfonamides; tetracyclines; thiolactomycin; tigecycline; trimethoprim
RND	AcrAB–TolC	*Enterobacteriaceae*	β-lactams; chloramphenicol; erythromycin; fluoroquinolones; novobiocin; tetracycline; linezolid

AcrAB-TolC contributed antibiotic resistance property of *K. pneumoniae*, *Salmonella*, and *Enterobacter* (Hernando-Amado et al. 2016). MexAB-OprM and MexXY-OprM which belonged to RND family are responsible for the development of intrinsic antibiotic resistance property in *P. aeruginosa* (Morita et al. 2001). Clinically relevant RND efflux systems such as SmeABC, SmeDEF, SmeJK, SmeVWX, and SmeYZ are reported in *S. maltophilia* (Hernando-Amado et al. 2016). SmeJK is involved in the development of resistance against aminoglycosides, tetracyclines, and fluoroquinolones whereas SmeYZ is responsible for the development of intrinsic aminoglycosides resistance (Sanchez 2015). MacABCsm which belongs to ABC efflux pump is responsible for the development of intrinsic resistance to polymyxins, macrolides, and aminoglycosides in *S. maltophilia* (Lin et al. 2014).

6.9 Role of Protection, Modification, and Enzymatic Alteration of Target Site in AMR Development

Tetracycline resistance determinants Tet(M) in Streptococcus spp. and Tet(O) in *C. jejuni* are examples of the target protection mechanism. TetO and TetM bind with the ribosome and replace tetracycline from its binding site through GTP-dependent mechanisms. TetO competes with tetracycline for the binding site at ribosome and

allows to continue protein synthesis (Li et al. 2013). Plasmid-mediated fluoroquinolone resistance gene Qnr is reported in a clinical isolate of K. pneumonia. It competes for the binding site of the DNA gyrase and topoisomerase IV (Aldred et al. 2014). Modifications of target site occur through mutations in the genes encoding the target site, enzymatic alterations of antibiotic binding site such as methyl group addition and replacement of target site (Munita and Arias 2016). Rifamycin inhibits the activity of DNA-dependent RNA polymerase which is composed of α2ββ'σ subunits. Rifamycin binds with the β subunit of the RNA polymerase (rpoB). Amino acid substitutions mutations in the rpoB gene prevent the antibiotic to bind with the rpoB which develops rifampin resistance (Floss and Yu 2005). Genetic alterations in DNA gyrase and topoisomerase IV developed antibiotic resistance. Resistance to oxazolidinones such as linezolid and tedizolid developed due to mutations in genes encoding the domain V of the 23S rRNA in the 50S ribosomal subunit and/or substitutions in the ribosomal proteins L3 (rplC) and L4 (rplD) (Mendes et al. 2014). Resistance to erythromycin is due to mono- or demethylation of an adenine residue at A2058 of the domain V of the 23rRNA of the 50S ribosomal subunit (Weisblum 1995). Resistance to methicillin in *S. aureus* occurs due to gaining of staphylococcal chromosomal cassette mec (SCCmec) (Chambers and Deleo 2009). Vancomycin resistance in enterococci results due to acquiring of van gene clusters which alter D-Ala to D-lactate and D-Ala to D-serine in peptidoglycan for developing high and low resistance, respectively (Arthur 2010).

6.10 Multidrug Resistance *Mycobacterium tuberculosis* in Development of MDR Tuberculosis

As per WHO report, 457,000 multidrug-resistant TB (MDR-TB) cases were reported in 2017; out of which 8.5% cases were considered as extensively drug-resistant TB (XDR-TB) (*Global Tuberculosis Report Geneva*. *Geneva*, *Switzerland*: *World Health Organisation*, *2018*). Mycobacterial cell wall is composed of peptidoglycan (PG), mycolic acid (MA), and arabinogalactan (AG) (Maitra et al. 2019). Drugs for MDR treatment have been classified into following group as World Health Organization (WHO) treatment guidelines: fluoroquinolones (FQ) such as levofloxacin, moxifloxacin, and gatifloxacin; amikacin (AMK), capreomycin (CAP), kanamycin (KAN), and streptomycin (STR); ethionamide (ETH), prothionamide (PTH), cycloserine (CS), terizidone, linezolid (LZD), clofazimine (CFZ); and pyrazinamide (PZA), ethambutol (EMB), high-dose isoniazid (INH), bedaquiline (BDQ), delamanid, (DLM), para-aminosalicylic acid (PAS), imipenem, cilastatin, meropenem, amoxicillin-clavulanate, and thioacetazone (Miotto et al. 2018). Rifampicin (RIF) resistant MTB strains have mutations in the codons 450, 445, and 435 of β-subunit of RNA polymerase (rpoB) (Jamieson et al. 2014). PZA resistance results from mutations in pncA. EMB resistance results due to missense mutations at codons 306, 406, and 497 of embCAB operon. Enhancement of EMB resistance results due to missense mutation in Rv3806c (ubiA) V188A, A237V, R240C, and A249G. (Miotto et al. 2018). FQ resistance in MTB is caused by mutations at

Table 6.6 Key MDR genes involved in MDR TB

Resistance-related genes	Function of gene	Name of the drugs
rpoB	RNA polymerase subunit B	Rifampicin
rpsL	Ribosomal protein S12	Streptomycin
gyrA and *gyrB*	DNA gyrase subunit A DNA gyrase subunit B	Quinolones
embB	Arabinosyl transferase	Ethambutol
Rrs	16S rRNA	Kanamycin/amikacin
ahpC	Alkyl hyperperoxide reductase	Isoniazid

codons 90, 91, and 94 of gyrA (Lu et al. 2014) (Table 6.6). KAN and AMK resistance are caused by mutations at nucleotide positions 1401 and 1402 of rrs gene (Georghiou et al. 2012). MDR-TB patients developed resistance against isoniazid and rifampicin whereas extensively drug-resistant (XDR) TB patients developed resistance against kanamycin, amikacin, or capreomycin. *M. tuberculosis* developed resistance due to chromosomal mutations. The rate of resistance mutations was estimated at 10^{-8} and 10^{-9} mutations/bacterium/cell division for isoniazid and rifampicin, respectively (Müller et al. 2013).

6.11 Antimicrobial Resistance Genes in Human Microbiome

Antimicrobial resistance genes (ARGs) are distributed through horizontal gene transfer (HGT), conjugation, phage transduction, or transformation. Human microbiome is considered as a reservoir of ARGs. Human microbiome is the source of about 3.3 million non-redundant genes. Human gut has 10^{14} microbial cells which represent 400 different bacterial phylotypes (Brinkac et al. 2017). Genes resistant to tetracycline (e.g., *tet*(*M*), *tet*(*O*), *tet*(*Q*), and *tet*(*W*)), amoxicillin, and erythromycin are predominant in oral microbiome. Streptococci are the principal carriers of AMR (tet genes) in the oral cavity of children. Veillonella showed resistance towards ampicillin and penicillin (Seville et al. 2009). Aminoglycoside and β-lactam antibiotics (BLr), tetracycline (Tcr), and methicillin (mecA) resistance genes are present in fecal samples of newborns (Gosalbes et al. 2016). Infants acquired AMR bacteria from mother. Infants and mothers showed presence of sulphonamides, spectinomycin, streptomycin, and trimethoprim resistance integrase genes (*int1*) and Tcr genes (Ravi et al. 2015). Plasmid-mediated quinolone resistance qnrA and extended-spectrum-β-lactamase resistance blaCTX-M are derived from non-pathogenic bacteria (Poirel et al. 2005).

6.12 Conclusion

Bacterial pathogens develop resistance to all antibiotics through mutation, transcriptomic alteration, and acquisition of resistance genes. Therefore, understanding the molecular mechanisms and evolution of multidrug-resistant bacteria need to be

studied. Multidrug-resistant (MDR) bacteria are involved in increased mortality and produce economic burden in world. It has now become greatest threats of the twenty-first century in public health. Multidrug resistance of the infected bacterial pathogens is common in clinical settings. Development of antibiotic resistance should be considered as an adaptive response in Darwinian's principles of evolution. Research has to be focused on the development of antibiotics. It is better to understand the antibiotic resistance mechanisms in bacteria to design novel antibiotic to encounter this global threat. To solve this AMR issue, research and development are to be enhanced. It is essential to develop new antibiotics and understand the response of microbes to new antibiotics.

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