Klebsiella pneumoniae Infections and Antimicrobial Drug Resistance

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Ashwath Priyanka, Kotian Akshatha, Vijaya Kumar Deekshit, J. Prarthana, and Dharnappa Sannejal Akhila

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A. Priyanka · K. Akshatha · V. K. Deekshit · D. S. Akhila (✉)
Nitte University Centre for Science Education and Research, NITTE (Deemed to be University), Mangaluru, Karnataka, India
e-mail: akhila@nitte.edu.in

J. Prarthana
SDM PG College, Ujire, Karnataka, India

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Abstract

Klebsiella species is ubiquitous in nature and is an important pathogen of humans and animals. *K. pneumoniae* is the most notorious opportunistic pathogen mainly affecting the hospitalized immunocompromised patients and accounts for urinary tract, respiratory tract, blood, and wound infection. They are the chief source of ventilator-associated pneumonia (VAP) and is accountable for 83% of hospital-acquired pneumonia (HAP). The antimicrobial resistance to human pathogens is being reported globally and has become a critical public health issue. In comparison to the susceptible pathogens, the rate of morbidity, and mortality is high with the infections caused by drug-resistant bacteria. Multidrug-resistant *K. pneumoniae* with diverse resistance determinants causing hospital and community acquired infections are of a major concern.

The mechanism of resistance known to date involves the production of beta-lactamases, such as extended-spectrum ß-lactamases, cephalosporinases, and carbapenemases. Constant horizontal transfer of antibiotic resistance genes via mobile elements essentially plasmids and transposons aid the production of extended spectrum beta-lactamases and other mechanisms of resistance that facilitate the survival of the *Klebsiella* in nosocomial environments. Currently, the emergence of *Klebsiella* strains that acquire plasmid-mediated resistance to ESBLs are of particular concern and are accountable for the failure of antibiotic treatment. The multidrug-resistant phenotype of the nosocomial *K. pneumoniae* caused by the presence of carbapenemases and extended-spectrum beta-lactamases make the therapeutic options limited.

The unending effort to develop new antibiotic has been outrun by the incidence of multidrug-resistant microbes and failed to replace the armamentarium required to combat the problem. Since discovering antibiotics takes a longer time, it is better to boost the activity of the existing antibiotic by inhibiting the mechanism that prevents the drug from acting will be an effective alternative. This chapter focuses on *K. pneumoniae* infections, pathogenicity, antibiotics, mode of action, antimicrobial resistance, therapies or alternative strategies for controlling drug resistance.

Keywords

K. pneumoniae · Multidrug resistance · Pathogenicity · Antimicrobial resistance · Nosocomial infections

12.1 Introduction

Klebsiella species belonging to Enterobacteriaceae are Gram-negative bacilli and are ubiquitous in nature. *Klebsiella* have two common habitats, one is the environment, where they are found in surface water, drinking water, sewage, industrial effluents, vegetation, soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses, or swine in which they colonize. The four-recognized species of the genus *Klebsiella* include *K. pneumoniae*, *K. oxytoca*, *K. terrigena,* and *K. planticola* (Donnenberg et al. 2005). Among these, *K. pneumoniae* and *K. oxytoca* are the most common human pathogen capable of causing neonatal infections of the bloodstream, urinary tract, central nervous system, lung, and soft tissue infections (Podschun and Ullmann 1998). *K. pneumoniae* was first isolated in the nineteenth century and were familiar as Friedlander's bacteria (Felson et al. 1949). *K. pneumoniae,* a nonmotile bacterium is a member of human gastrointestinal tract causes opportunistic infections such as urinary tract infections, wounds and respiratory infections, and causes ventilator-associated *pneumoniae* in hospitalized patients (Booker et al. 2008).

12.2 Classification of the Genus *Klebsiella*

As the taxonomy became increasingly refined due to the development of new methods such as numerical taxonomy, the species classification in this genus was continually revised. In the early 1980s, *Klebsiella* isolates from the environment, which was previously classified as "*Klebsiella*- like organisms" (groups J, K, L, and M), was later classified into provisional taxa. These groups led to the identification of four new species such as *K. terrigena* (Izard et al. 1981), *K. ornithinolytica* (Sakazaki et al. 1989), *K. planticola* (Bagley et al. 1981), and *K. trevisanii* (Ferragut et al. 1983). Since the DNA homology of the last two species was similar, they were combined as one and named *K. planticola* in the year 1986 (Gavini et al. 1986). Although *K. terrigena* and *K. planticola* are restricted to aquatic and soil environment, now are being reported in human clinical specimens. Since these isolates were obtained from polymicrobial specimens, it is not possible to distinguish them from other *Klebsiella* spp. In addition to *K. pneumoniae* and *K. oxytoca*, *K. granulata* is also now included in the list of human pathogens.

12.2.1 Differentiation of *Klebsiella* Species

Based on the biochemical reactions, *Klebsiella* species are identified and distinguished from other pathogens. It is a Gram-negative, nonmotile, encapsulated rod-shaped bacteria, produces lysine decarboxylase and shows a positive reaction for Voges-Proskauer test (Podschun and Ullmann 1992).

12.2.2 Typing of *Klebsiella* Isolates

It is essential in determining the clonality of the strains as it is mandatory in the management of endemic and epidemic nosocomial outbreaks of *Klebsiella* infections. Various methods adopted in the typing of *Klebsiella* are discussed below.

12.2.2.1 Biotyping

Biotyping is mainly based on the morphology of *Klebsiella* on a culture plate and the biochemical assay is performed in the laboratories as a gold standard method for the identification of *Klebsiella*. Biotyping can be performed by macro tube tests or by a combination of commercially available systems viz. API 20E with the additional macro tube methods. The biotyping of the *Klebsiella* is not much appreciated as an epidemiological tool due to the drawback of longer cultivation time of about 90 days for the confirmation of gelatinase production (Mitrophanov et al. 2008).

12.2.2.2 Serotyping

Serotyping is the most accepted technique for the typing of *Klebsiella* spp. and is based on the number of capsule antigens on the surface of the pathogen (Orskov and Orskov1984). The bacteria have a well-developed polysaccharide capsule and show a characteristic mucoid colony on the culture plate. Of the 82 capsule antigens described, 77 antigens are recognized and form the basis for the capsule antigen scheme. Regardless of the recognition of 12 different O-antigen types of *Klebsiella*, their classification is being held up by the heat-stable capsules (Ørskov and Fife-Asbury 1997). The method is very convenient as it aids in the differentiation of clinical isolates. The main drawback of the serotyping is the cross-reactions among the 77 capsule types. In addition, the procedure of typing is cumbersome due to the time-consuming longer procedures and difficulty in the interpretation of results due to the exhibition of weak reactions. The procedure is practiced in specialized laboratories since the anti-capsule antisera are not commercially available. However, neither the capsule typing, biochemical typing, phage typing, nor the biocin typing is singly sufficient to obtain results for epidemiological purpose. The combination of biotyping, as well as capsule typing, facilitates the differentiation of a large number of bio serotypes (Slopek et al. 1967).

12.2.2.3 Phage Typing

Discovered in the 1960s the phage typing is easy to read and is most accepted. The relative typing rate is as poor as 19–67% (Ślopek and Chapter 1978). Since it is just a method of typing and not an alternative to capsule typing, the procedure is not popular and may be used as an accessory method in addition to the serologic testing (Pieroni et al. 1994).

12.2.2.4 Bacteriocin Typing

Though capsule typing is suggested as a method for identifying *Klebsiella*, it is preferable to combine with a supplementary method independent of the capsule type for the epidemiological considerations. Several studies recommend typing of

Klebsiella via bacteriocins (Tomás et al. 1986; Bauernfeind 1984). Bacteriocins are bactericidal substances, made up of proteins with antimicrobial activity. Characterization of the isolates can be done by its competency in inhibiting specific indicator strains or by its susceptibility to bacteriocins. Instability of bacteriocin preparations, low type ability of strains and poor reproducibility are the major drawback of these methods (Bauernfeind et al. 1981). The limitations of these methods are summarized by the modification of the "scrape-and-point" procedure, thus avoiding the use of potentially unstable pre-produced and stored bacteriocins. The bacteriocins are synthesized on an agar medium prior before the strains to be typed and are inoculated by a multipoint inoculator. This procedure is proven effective as a bacteriocin typing for both clinical, environmental *Klebsiella* strain and also for nosocomial outbreaks of *Klebsiella* (Podschun and Ullmann 1993).

12.2.2.5 Molecular Typing Methods

Some of the most commonly used molecular typing methods for *Klebsiella* species are plasmid profiles (Combe et al. 1994; Nouvellon et al. 1994), ribotypes (Bingen et al. 1994), multilocus enzyme analysis, and pulsed-field gel electrophoresis (Poh et al. 1993; Gouby et al. 1992; Kitchel et al. 2009). The procedure followed varies from laboratory to laboratory and due to the lack of standardization it becomes laborious in the comparison.

12.2.2.6 Serotypes of *Klebsiella*

Klebsiella pneumoniae is recognized as an urgent threat to human health because of the multidrug-resistant strains and hypervirulent strains associated with hospital outbreaks. Virulence factors thought to be associated with invasive community-acquired infections include siderophores, specific polysaccharide capsule serotypes, and *rmpA* genes that are associated with hypermucoidy. Classic non-virulent *K. pneumoniae* (c-KP) strains are associated with pneumonia, urinary tract infection, and neonatal sepsis in immune compromised individuals. Being first recognized in Taiwan, the classic nonvirulent *K. pneumoniae* (c-KP) causes liver abscesses, meningitis, and endophthalmitis. K1 and K2 are the major capsular serotypes that cause liver abscesses. Genes like *mag*A and *K2*A are serotype specific genes for the K1 and K2 serotype, respectively.

The most important determinants of virulence in *K. pneumoniae* for liver abscesses are hyper mucoviscosity, rmpA (regulator of mucoid phenotype), aerobactin (an iron siderophore), kfu (an iron uptake system), alls (associated with allantoin metabolism), and K1/K2 capsules, respectively. The *rmpA* genes enhance the extracapsular polysaccharide (CPS) synthesis and thus confers highly mucoviscous phenotype of *K. pneumoniae* (Holt et al. 2015). It has been speculated that the structural genes for *Klebsiella* CPS synthesis are located near *his* on the chromosome (Liao et al. 2011).

K1 serotype is the major cause of primary liver abscesses and has greater potential for causing metastasis, whereas, K2 is a major cause of secondary liver abscesses (Yu et al. 2008). According to these capsular polysaccharides, *K. pneumoniae* can be classified into 77 serological K antigen types. High virulence of hv-KP is

correlated with the enhanced capsule production often triggered by the regulator of the mucoid phenotype (*rmpA*) gene and mucoviscosity-associated gene A (*magA*) (Chung et al. 2007).

12.3 Transmission

The nosocomial pathogens are transmitted in the hospital either by direct or indirect contact. The direct contact requires physical contact between the infected individuals or the contaminated source and the susceptible host. Indirect contact is by the mechanical transfer of the pathogens between the hospitalized patients via hospital personnel or by contaminated medical equipment (Nazir and Kadri 2014).

12.3.1 Airborne Route

This route of transmission of the infectious agent is via dissemination of airborne droplet nuclei or the dust particles containing the infectious agents. The nosocomial pathogens carried in this fashion may be dispersed widely by the surrounding air and inhaled by the susceptible host in the same area or distant places depending on the environmental factors.

12.3.2 Droplet Route

The droplets produced as a result of sneezing, coughing, or even by talking can settle on the surrounding surfaces or the mucosal surfaces and can be transmitted to others (Beggs 2003).

12.3.3 Common Vehicle Transmission

This implies to the pathogens transmitted to the healthy host via contaminated sources such as food, water, medical equipments that include catheters, ventilators, and also hands of the hospitalized personnel (Reybrouck 1983).

12.3.4 The Four Most Common Nosocomial Infections Caused by *K. pneumoniae*

12.3.4.1 Urinary Tract Infections

UTI are the most common and frequent of all the nosocomial infections. The main reason being the indwelling urethral catheters. The effective, proven intervention in preventing nosocomial UTI is limiting the duration of catheter usage and maintaining aseptic insertion and maintenance of closed drainage. Usage of the catheters

may be avoided unless and until there is a serious medical complication and are proven effective against nosocomial infections. Systemic antibiotic prophylaxis, bladder irrigation, the addition of antiseptics to the drainage bags, and antimicrobial coated catheters are proved to be ineffective (Warren 1997).

12.3.4.2 Surgical Wound Infections

There are several factors that influence frequency of surgical wound infection, to name a few techniques employed in the surgical course, the degree of endogenous contamination of the wound at the time of surgical procedure, duration of the surgical procedure, status of the underlying patient and the environment of the operative room (Owens and Stoessel 2008). Preventive measures in preventing surgical wound infections include optimal surgical technique, maintenance of a clean operative room environment with the restricted entry of staff members, sterile surgical equipment, preoperative preparation of the patients, and surgical wound surveillance program (Anderson et al. 2014).

12.3.4.3 Operative Room Environment

Airborne bacteria might be a cause of nosocomial respiratory tract diseases and hospital-acquired pneumonia (Johanson 1984). The infection can be minimized by maintaining clean operative and postoperative surroundings. Before any surgical interventions, all the surfaces must be disinfected thoroughly by using the recommended disinfectant. Unnecessary movement and conversations must be avoided within the operative and postoperative wards (Mangram et al. 1999).

12.3.4.4 Operative Room Attire

Usage of sterile gloves is recommended for all the operative staff. Hospital personnel entering the operative theater must and should wear the surgical attire restricted within the surgical ward. Coverage of the mouth and nose areas with the surgical mask within the operative ward must be mandatory. Therefore, maintenance of hygiene in the hospital and nursing settings are a prerequisite in the control and spread of any nosocomial infection.

12.4 Diagnosis

12.4.1 Conventional Methods

Usually, the identification of *K. pneumoniae* infection is confirmed by the culture of blood, sputum, urine, and aspirated body fluid, which includes pleural effusion, pericardial effusion, abscess material, and cerebrospinal fluid (Garner et al. 1988). In the identification of bacterial pneumonia, Gram staining may serve as presumptive identification, but the sensitivity is as less as 50% (Anitha 2012). Bacteria belonging to the genus *Klebsiella* appear as straight, short and Gram-negative encapsulated bacilli (Yu et al. 2008). Hence, capsule staining can be performed as

one of the diagnostic methods in the confirmation of the species from the clinical specimens.

Apart from the conventional detection techniques, accurate, and rapid identification of the bacterium in the hospital settings is very crucial. Although real-time PCR is rapid, sensitive, and specific, the technique is not cost effective (Chen et al. 2011). Despite recent advances in the field of molecular biology and advancement in the availability of phenotypic identification kits, identifying the bacterial strains remains a difficult task in many routine microbiological laboratories (Vaneechoutte et al. 2009).

12.4.2 Molecular Methods of Diagnostics

12.4.2.1 Polymerase Chain Reaction

PCR detection of the genus-specific gene (*gyrA*) (Aly et al. 2014) and species detection by targeting the 16s rRNA for the identification of *K. pneumoniae* can be a basic rapid diagnostic tool in the molecular level. Other virulence genes of *Klebsiella* can also be used as an identification remark viz. *magA*, the mucoviscosity-associated gene; *kfu*, iron uptake system gene; *rmpA*, the extra polysaccharide synthesis regulator gene (Nassif et al. 1989); and *fimH*, fimbrial gene encoding type 1 fimbrial adhesion (Schembri et al. 2005).

PCR-ELISA can also be employed in the detection of *K. pneumoniae* clinical strains by using the 16S rDNA gene-based specific primers. PCR-ELISA is known to be accurate and a rapid method for the detection of infectious agents and has the advantage of being specific and a sensitive approach for the detection of *K. pneumoniae* strains (Mousavi et al. 2008).

12.4.3 Imaging Studies

12.4.3.1 Chest Radiography

Klebsiella usually affects the upper lobes, the involvement of the lower lobe is usually not common (Knight et al. 1975). The affected lobe is usually seen swollen producing a bulged fissure. But the clinical presentation is not exclusive for *Klebsiella* infections, *Haemophilus influenzae* also produces a similar radiographic appearance (Qureshi et al. 2014).

12.4.3.2 Chest Tomography

The patients under treatment for pneumonia, responding slowly are usually recommended for chest tomography. The findings help in excluding entities that are treatable with drainages such as empyema and respiratory tract obstruction caused by *K. rhinoscleromatis* (Qureshi et al. 2014).

12.4.3.3 DNA Microarray Technology

This method may be employed for the rapid detection of TEM, SHV, and CTX-M ESBLs, since the identification of the etiological agent is critical in the diagnosis of the disease (Fevre et al. 2011). Specific PCR assay was developed to discriminate the *K. pneumoniae* subspecies. This technique provides a platform for rapid and simple detection of rhinoscleroma.

12.4.3.4 Susceptibility Testing for ESBL-Producing Organisms

The increase in the incidence of ESBL-producing organisms has given rise for the effective screening methods for detection and has a sensitivity of as much as 98% for the detection of ESBL (Falagas and Karageorgopoulos 2009). The Vitek ESBL test is an automated broth microdilution method and has a sensitivity of 99.5% and a specificity of 100% (Spanu et al. 2006). It is the most reliable substitute.

It is the responsibility of all the individuals and the services providing health care in preventing the spread of nosocomial infections. Cooperation between the hospital personnel providing hospital care and hospital management is very necessary for reducing the risk of infections for the patients and the staff (Hawley 1985). Methods used for the diagnosis of *K. pneumoniae* are summarized in Table 12.1.

12.5 Virulence Factors Present in *Klebsiella pneumoniae*

K. pneumoniae harbors different virulence factors in order to grow and overcome the immune response by the host. *K. pneumoniae* uses pathogenic factors like capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins, adhesins, and siderophores, for the survival and immune evasion during infection.

12.5.1 Capsule

The capsule is dense, approximately 160 nm in thickness consisting of a highly structured layer of surface-associated acidic polysaccharides, mainly composed of repeating three to six units of sugars. The composition is mainly dependent on the strain and is considered a dominant virulence factor (Shankar-Sinha et al.

Table 12.1 Methods used for diagnosis of *K. pneumoniae*

Diagnostic tools		
Conventional methods	Molecular methods	Imaging studies
Gram's staining	Polymerase chain reaction	Chest tomography
Capsule staining	PCR-ELISA	Chest radiography
Indole test	DNA microarray technology	Cystography
Citrate utilization test	LAMP assay	Computed tomography
Urease test	Real-time PCR assay	Magnetic resonance and imaging

2004; Li et al. 2014; Doorduijn et al. 2016). Capsule in the bacteria is known to show two pathogenic mechanisms: (1) protecting the bacteria from phagocytosis and (2) directly modifying the immune response. Production of the capsule in *K. pneumoniae* is important to cause infections in the host. The production of a capsule comprising of acidic polysaccharides by the Wzy-dependent polymerization pathway is the important characteristic of the genus *Klebsiella*. The complete ORF of *K. pneumoniae* capsule (*cps*) harbors around 16–25 genes with clusters ranging from 21 to 30 kb. Around 77 different types of capsule (K) antigen have been identified in *Klebsiella,* but only a few types have been systematically studied. Strains expressing K1 and K2 are highly virulent and the degree of virulence depends on the mannose content of capsular polysaccharides. K2 serotype is the prime serotype associated with UTI, pneumonia, or bacteremia and is hardly encountered in the environment sources. Presence of capsule protects the bacteria from opsonization and phagocytosis by macrophages during internalization into the host cell (Cortes et al. 2002). In addition, excess production of capsule helps them escape the neutrophil-mediated intracellular killing and leads to the development of abscess at different sites, such as the liver (Shankar-Sinha et al. 2004).

12.5.2 Lipopolysaccharides

Lipopolysaccharides (LPS) are considered a major and essential component of the cell membrane of every Gram-negative bacterium. Lipopolysaccharide is also an important pathogenic determinant in *K. pneumoniae* causing pneumonia, UTI, and bacteremia (Clements et al. 2007; Lugo et al. 2007; Llobet et al. 2011). Lipid A, core oligosaccharide (OS), and the O antigenic polysaccharide (O-PS) are three structural domains of LPS. O-antigen is the outermost component of LPS whereas core oligosaccharides anchor lipid A and O antigen. Capsular polysaccharides and the O-antigen portion of the lipopolysaccharides are the first molecules to come across the host immune system (Tomás et al. 1986).

Nine antigenic groups such as O1, O2, O2ac, O3, O4, O5, O7, O8, and O12 have been identified in *K. pneumoniae*. O1 is the common familiar among the invasive strains than noninvasive clinical strains (Dorman et al. 2018). *K. pneumoniae* O-antigen blocks the availability of complement components to activators and protects the bacteria against the complement-mediated killing process. Core polysaccharide of types 1 and 2 is identified in *Klebsiella* species. Lipid A and core polysaccharide protects mouse alveolar macrophages and contributes to resistance. Modification in the gene of lipid A and core polysaccharides leads to the attenuation of virulence of *Klebsiella* species and is proved in an animal model (Struve et al. 2009). LPS is known to have a dual effect on *K. pneumoniae,* infecting the host is beneficial and harmful because LPS also act as a strong immune activator.

12.5.3 Pili

Pili also familiar as fimbriae is filamentous projections on the bacterial surface. *K. pneumoniae* generally contains four types of fimbriae, namely type 1, type 3 fimbriae, Kpc fimbriae, and KPF-28 adhesin (Ong et al. 2008; Rosen et al. 2008). Types 1 and 3 pili are the predominant fimbrial adhesins present in *Klebsiella* species. Type 1 fimbriae are common in all the members of *Enterobacterial* species, but type 3 is specific to *Klebsiella* species though few studies have reported the expression of type 3 adhesins in *E. coli.* The genes required for structure and assembly of fimbriae are encoded on a gene cluster (*fim*). Types 1 and 3 fimbriae aids in the adherence to epithelial cells of the urogenital tract as well as intracellular biofilm formation within bladder umbrella cells in urinary tract infection and on abiotic surfaces. It has been assessed that most of the nosocomial infections (80%) are associated with an indwelling medical device. These devices offer a site for biofilm formation and the mechanical insertion of these devices further causes host cellular damage which provides attachment sites for the bacteria. Several studies have shown that types 1 and 3 fimbriae of *Klebsiella* are significant colonization factors required for biofilm formation (Miethke and Marahiel 2007).

12.5.4 Siderophores

Iron is an essential component in the growth of *K. pneumoniae*, required during the infection and is acquired from the environment (Raymond et al. 2003). As this metal is not available readily during the infection, the majority of the bacteria induce high-affinity iron-transport systems so that they can prevail over the low availability of the element. To acquire iron from the host during infection, these bacteria employ tactics by the secretion of siderophores, which have a higher affinity toward iron than the host transport proteins, which can steal iron from host iron-chelating proteins or obtain it from the environment (Bachman et al. 2009).

Most of the Gram-negative bacteria secrete small iron-chelating molecules called siderophores for their growth, virulence, and replication. The ability of the bacteria to produce siderophore depends on the iron and carbon contents in the culture medium. Different siderophores are expressed in *K. pneumoniae* viz. enterobactin, salmochelin, yersiniabactin, and aerobactin and have different roles in the infection process. Enterobactin produced by the members of Enterobacteriaceae has a greater affinity toward iron than host molecules (transferrin and lactoferrin). To overcome the effects, the host produces antimicrobial protein like lipocalin-2, which helps them in preventing the uptake of iron by the bacteria. To combat the effect of host antimicrobial protein, bacteria have acquired additional mechanism like stealth siderophores (salmochelin and yersiniabactin) which helps them to evade lipocalin-2-mediated iron starvation and proliferate within the host cell during overexpression of lipocalin-2 (Raffatellu et al. 2009). Existence of diversity in the siderophores impacts the replicative niche and pathogenesis of *K. pneumoniae* in the host (Bachman et al. 2011). Siderophores also modulate the host responses by activating

the transcriptional factor HIF-1α in the Peyer's patches, epithelial and endothelial cells of human (Holden et al. 2016). It also promotes the dissemination of *K. pneumoniae* to the spleen by inducing stabilization of HIF-1α in lung epithelial cells. Hence, inactivation of siderophores may be helpful to the host not only by inhibiting pathogens from acquiring protein-bound iron but also by preventing pathogen dissemination through modulating the immune system.

12.5.5 Outer Membrane Proteins

Outer Membrane Proteins (OMPs) are considered as an important factor for the virulence in *K. pneumoniae,* which includes outer membrane protein A (OmpA), peptidoglycan-associated lipoprotein (Pal), and murein lipoprotein (LppA). OmpA protects the innate immune response of the host. These proteins contribute to the selective impermeability of the cell membrane in an LPS- and capsule-independent manner, integrity and also protect *K. pneumoniae* against certain antibiotics and anionic detergents (Sugawara et al. 2016). Outer membrane porins that exist as trimers act as water-filled protein channels allowing transportation of small hydrophilic molecules like iron, antibiotics and nutrients, and are also important in both virulence as well as antibiotic resistance in the bacteria. OmpK35 and OmpK36 are the two classical trimeric porins, which are produced by *K. pneumoniae.* Porin channels act as the route of penetration for the antimicrobial drugs, which should first penetrate the outer membrane to reach the periplasm (Tsai et al. 2011). Porins even serve as receptors for phages and bacteriocins also in combination with lipopolysaccharide and peptidoglycan, their structural role is to maintain the integrity of the cells.

12.5.6 Efflux System

Efflux system is proved to be vital mechanism of antibiotic resistance and found exclusively in Gram-negative bacteria. These systems allow the microorganisms to balance their internal environment by removing toxic substances, such as antimicrobial agents, metabolites and quorum-sensing-regulated expression of virulence determinant. Efflux pumps are grouped into resistance-nodulation-division (RND) family, the small multidrug resistance (SMR) family, the multi-antimicrobial extrusion (MATE) family, the major facilitator superfamily (MFS), and the ATP-binding cassette (ABC) superfamilies (Eswaran et al. 2004). To date, AcrAB, KexD, and OqxAB efflux systems have been identified to be involved in antibiotic resistance in *K. pneumoniae*. AcrAB efflux system encoded by *acrRAB* operon while *acrR* encodes the AcrAB repressor, where, *acrA* and *acrB* encode a periplasmic lipoprotein is attached to the inner membrane which bridges the outer, inner membranes, and an integral membrane protein situated in the cytoplasmic membrane. AcrB connects with outer membrane protein TolC, found in all Gram-negative bacteria is vital for the expulsion of dyes, detergents, and antimicrobial agents (Buckley et al.

2006). Several studies have proved that bacteria with deleterious efflux systems lost their pathogenicity in animal model experiments. The KpnEF efflux pump of *K. pneumoniae* mediates resistance to several dyes, detergents, and antimicrobial compounds such as SDS, deoxycholate, EtBr, cefepime, benzalkonium chloride ceftriaxone, colistin, erythromycin, rifampin, tetracycline, streptomycin, acriflavine, chlorhexidine, and triclosan. Thus, studies have shown that KpnEF efflux pump plays a key role in providing resistance toward broad-spectrum antimicrobial compounds (Bunikis et al. 2008).

12.6 Antimicrobial Resistance in *K. pneumoniae*

The discovery of antibiotics is an important milestone in the history of therapeutics, which led to effective control of infectious agents. Antibiotics are increasingly being thought the wonder drugs because of their selective inhibitory nature at minimum concentration. However, indiscriminate use of antibiotics in medical, veterinary, and agriculture sectors, has led to the emergence of antimicrobial-resistant strains. *Klebsiella* being an important human pathogen is found to be resistant to most of the present generation drugs and has been classified as one among the list of ESKAPE organisms (Boucher et al. 2009). The worldwide contribution of drug-resistant strains of *K. pnuemoniae* to the burden of antimicrobial resistance is increasing throughout the year. Multifactorial dissemination processes through mobile genetic elements play a very prominent role in the spread of multidrug-resistant *K. pnuemoniae* (Navon-Venezia et al. 2017).

12.6.1 Emergence of Antimicrobial Resistance in *K. pneumoniae* to Different Classes of Antibiotics

12.6.1.1 Cell Wall Synthesis Inhibitors

One of the most selective and potent classes of all the antibiotics is the cell wall synthesis inhibitors. Bacterial cell wall is synthesized in three different stages viz. synthesis of precursor molecules in the cytoplasm, transfer of precursor molecules through a lipid carrier and transpeptidation and carboxy peptidation reactions. All three stages can be inhibited by different groups of antimicrobial agents namely fosfomycin, bacitracin, and vancomycin or β–lactams, respectively. *K. pneumoniae* is known to display resistance toward most of the *β*-lactam drugs by producing *β*-lactamase enzymes such as cephalosporinases, extended-spectrum *β*-lactamases and carbapenemases. The existence of β-lactamase was first reported in 1940, which was before the commercial use of antibiotic penicillin (Abraham and Chain 1988). This could be a reason for the intrinsic resistance to penicillin in many of the environmental isolates. The chromosomally mediated *β*-lactamase such as penicillinase was first discovered in *K. pneumoniae* as a part of intrinsic resistance (Labia et al. 1979). The enzymes were later identified as class A group 2b *β*-lactamases (Petit et al. 1992). In addition to natural resistance, the massive use

of β-lactam antibiotics against different groups of human bacterial pathogens, including *K. pneumoniae* initiated the emergence and spread of β-lactamase enzyme (Bush 2010). The first β-lactamase enzyme described was Temoneria (TEM)-1 followed by a sulfhydryl variable (SHV)-1 conferring resistance to penicillin but not to cephalosporins (Hæggman 2010). The derivatives of these enzymes were later classified as ESBL (extended-spectrum β-lactamases) shown to produce activity against oxyimino-β-lactam antibiotics through the modifications in the active site (Rawat and Nair 2010). Soon after the introduction of third-generation cephalosporins in 1982, resistance to oxyimino β-lactam was evident in *K. pneumoniae* and *Serratia marcescens* (Knothe et al. 1983). Since the first recorded outbreak of ESBL-producing strains in French hospitals, there was a rise in the outbreaks of *K. pneumoniae* and *E. coli* (Lewis et al. 2007). Later on, a shift has occurred from the initial predominance of TEM and SHV class of β-lactamases to the emergence of Cefotaximase-Munich (CTX-M)-type, which is the most commonly detected ESBL (van der Bij and Pitout 2012). To treat the infections caused by these ESBL producers, β-lactams of carbapenem group was more commonly used (Rawat and Nair 2010). This further caused the emergence of carbapenem resistance in Enterobacteriaceae worldwide (Tofteland et al. 2013; Storberg 2014). The widely distributed strain *K. pneumoniae* sequence type 258 harboring KPC (*K. pneumoniae* carbapenemase) belonging to the class A type β-lactamase caused national and international epidemics (Coetzee and Brink 2012). First reported in the late 1990s, to date more than ten different KPC variants have been identified (Walther-Rasmussen and Høiby 2007). These enzymes mainly provide resistance to cephalosporins, cephamycins, monobactams, carbapenems, and are weakly inhibited by clavulanic acid and tazobactam (Pitout 2012). KPC-producing *K. pneumoniae* isolates are the most important cause of nosocomial infections and are also endemic in certain parts of world such as Greece, northeastern USA, Colombia, Puerto Rico, Israel and China (Nordmann et al. 2009). Most of the resistance determinants of KPC were carried on transferable plasmids of variable size such as $bla_{\text{KPC-2}}$ on 100 Kb plasmid and $bla_{\text{KPC-3}}$ on 120 Kb plasmid (Samuelsen et al. 2009) associated with transposable element *Tn4401* which further supports the mobilization and spread of KPC among different human pathogens. The carbapenem-resistant strains also initiated the spread of NDM (New Delhi Metalloβ-lactamase) belonging to class B β-lactamases and OXA-48 type belonging to class D β-lactamases showing resistance to virtually all class of β-lactams including present generation carbapenems (Hirsch and Tam 2010; NICD, 2013). In addition, *K. pneumoniae* occasionally harbors plasmid-mediated AmpC- β-lactamases. These enzymes are first reported in 1980s and are derived from the chromosomally encoded *AmpC* cephalosporinases, which are difficult to get inhibited by the first-generation β-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam (Philippon et al. 2002). These resistance determinants along with other β-lactamases $bla_{NDM\text{-}1}$, $bla_{CMY\text{-}16}$, $bla_{OXA\text{-}48,}$ and $bla_{CTX\text{-}M\text{-}15}$ present on different plasmids in a single strain of *K. pneumoniae* pose a great challenge to the clinicians to treat the infection with the available antibiotics (Nordmann and Poirel 2014).

12.6.1.2 Protein Synthesis Inhibitors

Antimicrobial agents that inhibit several stages of protein synthesis are known to have a profound effect on cellular processes of bacteria. Tetracyclines, phenicols, aminoglycosides, MLSKs (macrolides, lincosamides, streptogramins, and ketolides), ansamycins, and oxazolidinones are the widely used classes of antibiotics under this category. Most of the antibiotics in this category work by inhibiting either transcription or translation initiation process during protein synthesis. Many affect either 50s or 30s ribosomal subunits during assembly and translation process. Aminoglycosides such as amikacin, tobramycin, and gentamicin in combination with *β*-lactams are the most frequently used antimicrobial agents against Gram-negative pathogens including *K. pneumoniae* until they were replaced by third-generation cephalosporins, fluoroquinolones, and carbapenems (Krause et al. 2016). Aminoglycosides irreversibly bind to the 30s-ribosomal subunit causing complete inhibition of protein synthesis leading to bacterial cell death (Shakil et al. 2008). Over clinical use of these antimicrobial agents has resulted in the emergence of resistance among *K. pneumoniae.* Bacteria can become resistant to aminoglycoside through a variety of ways including alterations of the ribosomal binding sites, drug inactivation by aminoglycoside modifying enzymes, reduced uptake of aminoglycoside due to the downregulation of porin proteins and overexpression of efflux pumps (Nasiri et al. 2018). Among these enzymatic modifications is the most commonly encountered mechanism. The aminoglycoside-modifying enzymes are classified into three major classes such as aminoglycoside acetyl transferase (*aac*), aminoglycoside nucleotidyl transferase (*ant*) and aminoglycoside phosphoryl transferase (*aph*) encoded either by chromosomal or plasmid-mediated resistance determinants (Kim et al. 2008). Of these, aminoglycoside acetyltransferase enzyme catalyzes the transfer of an acetyl group of acetyl coenzyme A to an amine group of aminoglycoside thereby making it inactive. While other two modifying enzymes (*aph* and *ant*) catalyze the transfer of γ-phosphate and nucleotide monophosphate to hydroxyl portions of aminoglycosides (Ramirez and Tolmasky 2010). Among these, aac(3)-II and aac(6′)-Ib group of modifying enzymes are most commonly reported in *K. pneumoniae* (Liang et al. 2015).

Decrease in the usage of aminoglycosides slowed down the emergence of new aminoglycoside resistance mechanisms until the discovery of plasmid-mediated enzyme 16S rRNA methyltransferase (16S RMTase) and is gaining importance due to its high prevalence in recent times (Liang et al. 2015). This enzyme methylates the binding site of the drug, thereby making the drug inactive, causing high-level resistance to aminoglycosides (Doi et al. 2016). Currently, eight 16s RMTase genes namely *armA*, *npmA, rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE,* and *rmtF* have been identified among Gram-negatives with high prevalence of *rmtB* and *armA* (Xia et al. 2016). *armA* was the first 16sRMTase identified in *K. pneumoniae* in France in 2003 and is being increasingly reported worldwide along with *β*-lactam resistance among *Klebsiella* species and in other Gram-negative human pathogens (Nasiri et al. 2018; Costello et al. 2019). Recent studies have shown that the genes *rmtB* and $bla_{\text{CTX-M-55}}$ were carried on transferable plasmids harboring *IS26* and *Tn3* transposons in *K. pneumoniae* ST37 isolated from dogs and cats (Xia et al. 2017). However, further

studies are needed to understand the transfer of these plasmid-mediated aminoglycoside genes from animals to humans. Chromosomal aminoglycoside resistance mechanisms include alterations in the cell membrane permeability due to the modifications in AcrAB-TolC and KpnEF efflux pumps or due to the loss of porin protein KpnO. However, different membrane apparatus exhibits variable affinities to different aminoglycosides. For instance, disruptions in AcrAB-TolC apparatus increased the sensitivity of *K. pnuemoniae* to tobramycin and spectinomycin (Padilla et al. 2010), while, mutant of KpnFF (Δ*kpnEF*) shows strong change in resistances to tobramycin and spectinomycin but exhibit low resistances to gentamycin and streptomycin (Srinivasan and Rajamohan 2013). Besides, it was also observed that the loss of porin protein caused resistances to tobramycin, spectinomycin and streptomycin (Srinivasan et al. 2012).

Although macrolides, lincosamides, streptogramins, ketolides and ansamycins are not considered for treating clinically relevant Gram-negative bacterial infections, a reasonable number of Gram-negative pathogens act as a reservoir of MLS resistance determinants. This can be successfully transferred to other pathogens of clinical importance through mobile genetic elements (Nguyen et al. 2009).

12.6.1.3 Cell Membrane Synthesis Inhibitors

The second most potent group of antimicrobial agents are cell membrane inhibitors. Although, not as selective as β-lactams, cell membrane inhibitors can work at stages where all other antimicrobial agents fail to control the infections caused by MDR strains. Polymyxins are the only drugs that are approved for human therapy under this category. Polymyxins are positively charged cations that are attracted toward the negatively charged LPS of the bacteria, modifies its structure and make the membrane more permeable. This further disrupts the osmotic balance through the displacement of cations (Ca^{2+}/Mg^{2+}) causing leakage of cellular molecules leading to cell death (Falagas and Kasiakou 2005). Colistin (polymyxin E) among polymyxins is considered as the drug of last reserve for treating Gram-negative MDR infections (Olaitan et al. 2014). The extensive use of colistin sulfate in veterinary medicine has led to the emergence of colistin-resistant strains. The first clinical case of colistin-resistant *K. pneumoniae* was reported in the late 1960s during the initial period of its use (Davis et al. 1969). However, during 1980s to 2000 use of polymyxin was restricted in human medicine due to its associated toxicity. Later in the early 2000s, with the increasing prevalence of XDR (extensively drug resistance) carbapenemase-producing *K. pneumoniae* (CPKP), the treatment mainly relied on polymyxins as the drug of last resort (Antoniadou et al. 2007). Soon after its comeback in clinical use, the first nosocomial outbreak of colistin-resistant MDR *K. pneumoniae* was reported from Greece in the year 2004 (Antoniadou et al. 2007) and since then there is an increasing trend of colistin-resistant clinical isolates (Marchaim et al. 2011). The main mechanism of polymyxin resistance in *K. pnuemoniae* is chromosomal-mediated target site modifications. This is named as LPS modification system. Thus, the altered the LPS structure in resistant strain decreases the anionic charge interfering with the binding of polymyxins (Navon-Venezia et al. 2017). The alteration in LPS is due to the mutations in certain core genes of lipid A

such as *lpxM* and its regulator *ramA* (De Majumdar et al. 2015). Additional mechanisms like neutralization of lipid A, binding of amino arabinose (*pbgP* and *pmrE*), phosphoethanolamine (*pmrC*) to lipid A portion of cell membrane has also been reported (Llobet et al. 2011). Similarly, the enhanced activity of LPS-modifying gene regulators like *phoPQ*, *pmrA,* and *pmrD* could be another reason for resistance in *K. pneumoniae* (Navon-Venezia et al. 2017). Certain pathways such as CrrAB regulatory and TupA-like/glycosyltransferase system also involved in LPS modification leading to colistin resistance in *K. pneumoniae* (Wright et al. 2015).

The plasmid-mediated mcr (mobilized colistin resistance) genes can also lead to target site modification. *mcr* codes for phosphatidylethanolamine transferase, which is an enzyme that transfers phosphatidylethanolamine residue to the lipid A portion of Gram-negative bacteria, thereby modify its structure. This further results in reduced affinity of colistin and related polymyxins to lipid A portion of the cell membrane. The first acquired colistin resistance gene *mcr-1* has been detected in *E. coli* and *K. pneumoniae* in China and was present on an insertional element *Incl2* (Liu et al. 2016). Recently, three multidrug-resistant strains of *K. pneumoniae* isolated from chickens harboring *mcr 7.1* and $bla_{CTX\text{-}M\text{-}55}$ gene on an Incl2 conjugative plasmid was characterized (Yang et al. 2018). Among the three isolates, one isolate was found to carry other antibiotic resistance determinants such as oqxAB for quinolone resistance, *fosA* for fosfomycin resistance and *aph(3′)-Ia* for aminoglycoside resistance. Although mcr genes are of recent origin, the rapid spread of different classes of this gene could pose a serious threat if associated with pan-drug-resistant (PDR) strains (Karaiskos et al. 2017).

12.6.1.4 Nucleic Acid Synthesis Inhibitors

Most of the bacteriostatic agents belong to the category of nucleic acid synthesis inhibitors. One of the earliest nucleic acid synthesis inhibitor is a quinolone antibiotic, nalidixic acid, widely used for UTI infections and nosocomial infections. Later its potency was increased by the addition of fluorine group in its chemical structure. Nearly all modern quinolone antibiotics are fluoroquinolones with ciprofloxacin being most widely used. Fluoroquinolones mainly work by inhibiting the normal functioning of topoisomerase II (*gyrA* and *gyrB*) and topoisomerase IV enzymes (*parC* and *parE*) which are involved in DNA replication. Fluoroquinolones bind to the DNA–enzyme complex during replication and prevent the negative supercoiling of replication fork. However, other mechanisms of resistance such as presence of plasmid-mediated quinolone resistance (PMQR) genes and overexpression of efflux pumps have also been reported in certain Gram-negative pathogens. Quinolones have been in use since 1960s, but their clinical use increased rapidly after the introduction of fluoroquinolones in 1980s. The extensive use of fluoroquinolones led to the development of resistance in bacterial pathogens (Naeem et al. 2016). In *K. pneumoniae*, all the known mechanisms of quinolone/fluoroquinolone resistance have been reported including QRDR (quinolone resistance determining regions) mutations, PMQR, or MDR efflux pumps (Redgrave et al. 2014). The first chromosomal resistance mechanism by *K. pneumoniae* was observed against the first quinolone antibiotic, nalidixic acid and the first fluoroquinolone, norfloxacin (Davis

et al. 1969). Like in other members of Enterobacteriaceae, the first QRDR mutation was seen in *gyrA* and *parC* subunits (Deguchi et al. 1997) followed by *gyrB* (Nam et al. 2013) and *parC* (Guillard et al. 2015) in MDR strains of *K. pneumoniae*. Another important resistance mechanism is the presence of PMQR genes. These genes are composed of Qnr pentapeptide proteins that protect topoisomerases II and IV from the action of quinolone/fluoroquinolone antibiotics. The first plasmid-mediated *qnr* was discovered in *K. pneumoniae* isolated from a clinical sample in the USA (Martínez-Martínez et al. 1998). Since then multiple genes (*qnrA1*, *qnrB1, qnrB4, qnrS1, oqxAB,* and *aac(6′)-1b-cr*) associated with PMQR have been discovered in MDR strains of *K. pneumoniae* (Yang et al. 2014). *aac(6′)-1b-cr* confer resistance to both aminoglycosides and fluoroquinolones and *qepA* encodes an efflux protein that recently identified in *K. pneumoniae* (Heidary et al. 2016). PMQR genes alone known to produce low or moderate level of resistance to fluoroquinolones (Fabrega et al. 2009). However, the combination of quinolone/fluoroquinolone-resistant mechanisms such as QRDR mutation and presence of PMQR determinants play a very important role in high-level fluoroquinolone resistance in *K. pneumoniae* (Azargun et al. 2019).

12.6.1.5 Antimetabolites

Antimetabolites are the chemicals which inhibits metabolites. They are also referred to as antifolates that inhibit folic acid synthesis in bacteria which otherwise required for the synthesis of adenine. The two important antimicrobial agents trimethoprim and sulfonamide belong to this category. Trimethoprim binds to dihydrofolate reductase enzyme and inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid in the folate pathway. Similarly, sulfonamide inhibits dihydropteroate synthase thereby preventing the conversion of para aminobenzoic acid to dihydrofolic acid. Sulfonamide was first put into clinical use in the year 1935 (Sköld 2000). Since then, it has been extensively used although it resulted in serious side effects. Later, a new compound referred to as trimethoprim first used in the year 1962 in England for the treatment of urinary tract infection in combination with sulfonamide due to its synergistic effect in vitro (Bushby 1980). However, overuse of these drugs has resulted in the development of resistance among Enterobacteriaceae. The usual trimethoprim-resistance genes encountered in *K. pneumoniae* are *dfrA14*, *dfrA1dfrA5*, *dfrA8*, *dfrA12*, *dfrA13/21/22/23* family, *dfrA15*, *dfrA16*, and *dfrA17*. Similarly, *sul2* was found to be more common in *K. pneumoniae* followed by *sul2* and *sul3* conferring resistance to sulfonamide (Taitt et al. 2017). Resistance to trimethoprim and sulfonamide (co-trimoxazole) in *K. pneumoniae* is mostly associated with the plasmid IncA/C2 group that carries multiple resistance determinants conferring resistance to *β*-lactams (bla_{NDM-1}), chloramphenicols (*catA1*), aminoglycosides (*armA* or *rmtB* 16S RNA methylases) along with *AmpC* β-lactamase CMY-2 (Carattoli 2013). In general, most of the ESBL-producing *K. pneumoniae* usually show high-level resistance to co-trimoxazole (Somily et al. 2014; Stanley et al. 2018).

As a known cause of nosocomial infection, *K. pneumoniae* plays a prominent role in spreading the burden of antimicrobial resistance worldwide. The most recent

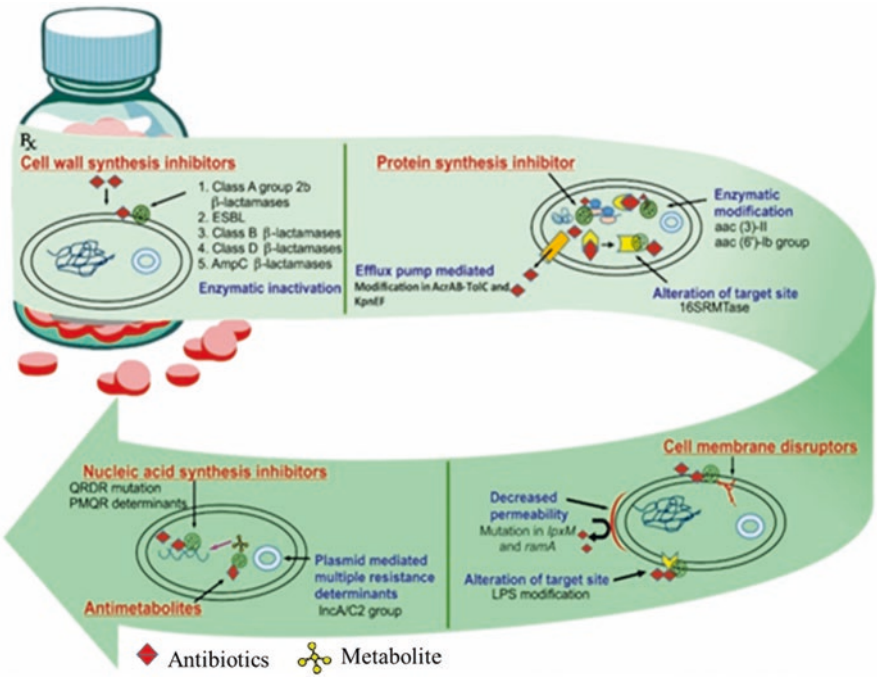

Fig. 12.1 Representative image of antimicrobial resistance mechanisms predominantly observed in *Klebsiella* toward different classes of antibiotics

emergence of XDR (resistant all drugs except cefepime, tigecycline, and ceftazidime-avibactam) and PDR (resistant to all drugs) strains of *K. pneumoniae* (Bi et al. 2017; Li et al. 2018; Krapp et al. 2018) would be a wakeup call for the world to contemplate on more strategic measures to control the spread of drug-resistant human pathogens like *K. pneumoniae.* Figure 12.1 represents an image of antimicrobial-resistant mechanisms predominantly observed in *Klebsiella* toward different classes of antibiotics.

Ideal therapeutic options for multidrug-resistant *K. pneumoniae* infections are not well established (Qureshi et al. 2014). Routine monitoring and prevention are a prerequisite in controlling any infection and their outbreaks.

12.7 Methods to Combat Antibiotic Resistance

12.7.1 Phage Therapy

Infections due to multidrug-resistant bacteria are increasing globally and is a critical issue. Bacteriophages are considered as an effective alternative for the treatment of bacterial infections. Phages are species and strain specific, some are polyvalent (Chibani-Chennoufi et al. 2004). The decline in the effectiveness of antibiotics has

generated a need for a substitute therapy and hence, phage therapy can be one of the alternatives to combat bacterial infections. Frederick d' Herelle coined the term 'bacteriophages', convinced the use of phages as a therapeutic option and demonstrated the first clinical in the year 1919 in Paris at a hospital used to treat cases of pediatric dysentery (Chanishvili 2012). In the food industry, several phage preparations are marked safe and approved by the FDA (Monk et al. 2010).

Investigations of phage treatment on an animal model have proven effective against a range of clinically significant pathogens viz to treat antibiotic-resistant *Pseudomonas aeruginosa* infections of the skin, gastrointestinal tract, and lungs in mice model (Watanabe et al. 2007). Additional results show promising effects on *Vibrio parahaemolyticus, Staphylococcus aureus, Escherichia coli*, and *Acinitobacter baumani*. Human trails also have been proved effective against common pathogens such as *S. aureus*, *P. aeruginosa*, *E. coli, Proteus, Enterococcus*, and *Salmonella* species (Kutateladze and Adamia 2008). They also have concluded the therapeutics did not have any effect on the normal flora of the mice, thus proving it as a better alternative (Bogovazova et al. 1991). It has proven effective against pneumonia caused by multidrug-resistant *K. pneumoniae* experimented on animal model and thus considered a potent alternative to combat drug resistance (Fang et al. 2004).

Adverse reactions to antibiotics include anaphylaxis, cardiotoxicity, hepatoxicity, neurotoxicity, and gastrointestinal complications (Granowitz and Brown 2008). In contrast to this, phage therapy is considered safe since the translocation is across the epithelium and subsequently circulate within the blood (Górski et al. 2006). Phages are armed with enzymes on the exterior of the capsid that aid degradation of the extracellular polymeric substances (EPS) and disperse bacterial biofilms and thus allowing the phage to access the bacteria embedded within the EPS matrix (Abedon 2015). In contrast to the antibiotics, phages are more specific toward species and strain. Other complications of antibiotics include increased risk of asthma, diabetes, and obesity. The damage is less in case of phages and still is believed to reduce gut carriage of pathogens such as uropathogenic *E. coli* and *Shigella* (Mai et al. 2015). The available literature on the use of phage as an alternative therapy to combat bacterial infections, especially with respect to multidrug-resistant pathogens is a promising note. The combination of both phage therapy and antibacterial agents is significant in addressing the issue of antibiotic-resistant infections.

12.7.2 Gene Silencing/Knockout

Earlier days antibiotics were referred to as a magic bullet to combat-associated bacterial infection. Unfortunately, bacteria have devised a plethora of mechanisms that cause resistance to several antibiotics. Due to the pacing advent of new resistance mechanisms, there is a decline in the effectiveness of conventional antibiotic therapy, higher expenditures for health care, and immense risk of death. Modification in various drug enablers of bacteria viz increasing bacterial efflux pump, inactivation/

modification of drug, alteration of drug target site collectively contributes to the reduction in antibiotic potency (Tenover 2006). The unending effort to develop new antibiotic has been outrun by the incidence of multidrug-resistant microbes and also failed to replace the armamentarium required to combat this problem. Targeting drug resistance mechanism is the best option to tackle multidrug-resistant strain rather than a synthesis of a new antibiotic. Gene manipulation and gene editing is the best tool to modulate the antibiotic resistance but it failed to be used as therapeutics due to the ethical problem. Treatments of infectious disease by traditional antibiotic therapy have lagged behind the plethora of multidrug-resistant bacteria. Microbes acquire resistance to conventional antibiotic therapy by various drug resistance mechanisms. Efflux pump is considered to be the preferred route to expel diverse class of structurally unrelated drug and also prevent the emergence of drug-resistant mutant (Pérez et al. 2012; Ayhan et al. 2016). Reports have also shown that *Salmonella* Typhimurium strain lacking genes coding for efflux pump were totally avirulent in a mice infection model (Blair et al. 2015). Hence, it is better to tackle the drug-resistant mechanism in bacteria than scavenging for new antibiotics. RNA-mediated interference (RNAi) is an evolutionarily conserved natural phenomenon formerly discovered as an antiviral mechanism in plants and other organisms for the specific silencing of gene expression. Limited studies have used RNAi-based inhibition molecule to induce antibiotic sensitivity in drug-resistant bacteria (Yanagihara et al. 2005; Gong et al. 2013). Further study is required to extend the in vitro study to animal models of infection.

12.8 Prevention of Nosocomial Infections Caused by *Klebsiella*

A manual consolidating all the instructions and practices in the prevention of nosocomial infection prevention is mandatory (Chinn and Sehulster 2008). It is the duty of the infection control squad in developing, revision, and updating the manual.

12.8.1 Responsibility of the Infection Control Team

12.8.1.1 Role of the Hospital Administration

In the prevention of nosocomial infections, hospital administration and management play a crucial role in the establishment of the infection control board. It must also aid in the identification, monitoring, and implicating suitable methods in the control (Zingg et al. 2015). All the staff should be trained about the aspects of control of infection by techniques such as sterilization and disinfection. The hospital personnel including the nurse, housekeeping, laboratory technicians should be conveyed about hospital hygiene and their maintenance (Lówbúrý et al. 2013). Periodically the degree of the hospital-acquired infections and effective medical interventions should be reviewed (World Health Organization 2002).

12.8.1.2 Infection Control Team

The hospital management must have access to specialists in the field of epidemiology (Scheckler et al. 1998). However, the infection control team must ensure the appropriate management of infection control schedule. The infection control squad personnel also have the responsibility in invigilating the day-to-day functions, surveillance, evaluation, and supervision of the necessities viz. disinfectants and other sterilization agents required for the control of infections in the hospital settings (Scott et al. 2005).

12.8.1.3 Duty of the Nursing Staff

Implementing guidelines laid down by the infection control committee to the patient care service is the duty of the nursing staff (Grol et al. 2013). Knowledge of the nursing staff in preventing the spread of nosocomial infection, the practice of appropriate interventions for all the patients during the stay period is mandatory (Hooton et al. 2010).

Senior nursing heads are responsible for actively participating in the training program which includes supervising and implementing techniques in the prevention of the infections in the wards, operation theaters, intensive care units, and maternity units (Drachman 1981).

Maintaining hygiene and adopting good nursing practices in the wards. Aseptic conditions in the wards which include washing hands and reporting of any infections to the physicians in charge (Conly et al. 1989). Isolation of patients with any communicable diseases. Limiting patient visitors, hospital staffs, and equipment used for medical interventions (World Health Organization 2002).

12.8.1.4 Central Sterilization Service

A central sterilization facility should be provided in all the hospital settings. The responsibilities of the central sterilization facilities include cleaning, testing, and decontamination, storage of all the hospital equipment aseptically. This committee works hand in hand with the infection control team. They also monitor the cleaning procedures and decontamination of the contaminated equipment which include wrapping procedure and packing of the equipment according to the sterilization techniques. Sterilization conditions (temperature, humidity, and pressure). Higher authorities supervise the use of different physical, chemical, and biological methods in monitoring the sterilization process. Training the new staff members and periodic training to other staff members about the new technique employed.

There must be frequent training to the hospital personnel regarding hygiene and frequent cleaning and washing hands. Causes of contamination in the hospital premises and ways of minimizing it must be scrutinized by the hospital staff (Emori and Gaynes 1993).

12.8.1.5 Hospital Hygiene Services

They are responsible to overview and coordinate infection control activity and check the effectiveness of the methods employed in disinfection and sterilization, develop methods to improve hygiene in the hospital settings. Inspection and

replacement of filters of all the equipment for ventilation. The hospital hygiene service may also assist and undertake research, which includes hospital hygiene and control of infections (Boyce and Pittet 2002).

12.9 Conclusion

Klebsiella is opportunistic nosocomial pathogen responsible for many hospital-acquired infections. The nosocomial infections are common among the hospitalized and immune compromised patients. The incidence of *Klebsiella* nosocomial infections ranges from 5 to 7% of the hospital-acquired infections and ranks them as the most significant nosocomial pathogen. The morbidity and mortality rates recorded as a result of *Klebsiella*-related infections are very high (as high as 50%). The prevention and control of nosocomial infections have resulted in considerable advancements in managing and controlling infections.

Alternative approaches for the prevention and control of nosocomial *Klebsiella*-related nosocomial infections is a necessity.

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