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An Overview of Virulence, Antimicrobial Resistance, Biofilm Formation and Antibiofilm Strategies of *Klebsiella* Species

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Abstract | *Klebsiella* spp. are Gram negative opportunistic pathogens, which cause many diverse community acquired and nosocomial infections. The infections caused by multidrug-resistant (MDR) bacteria are difficult to treat. In addition, *Klebsiella* spp. have several virulence factors, which play their role in pathogenesis and infectivity involving capsule, lipopolysaccharide, fimbrial and non-fimbrial adhesins, siderophores and its ability to form biofilms. *Klebsiella* biofilm is formed on biotic and abiotic surfaces like medical devices. It can protect the bacteria from antibiotics and host immune system leading to chronic infections and mortalities. Therefore, it is necessary to investigate new antibiofilm methods better than the conventional methods to eradicate the biofilm and thereby control *Klebsiella* infections. In this review, we will discuss *Klebsiella* virulence and its antibiotic resistance to give more insights into its capability for biofilm production and the application of the antibiofilm approaches for controlling its infection.

Keywords | *Klebsiella*, Virulence factors, Antibiotic resistance, Biofilm, Antibiofilm methods

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INTRODUCTION

Klebsiella species are Gram-negative, non-motile, encapsulated, lactose-fermenting and facultative anaerobic bacteria and they belong to *Enterobacteriaceae* family. *Klebsiella* spp. are important opportunistic pathogens those exist as a normal flora in the gastrointestinal tract of humans and animals. However, under favorable conditions, this bacterium is responsible for a variety of diseases in humans and animals (Brisse et al., 2006) such as pneumonia, urinary tract infections and mastitis.

Klebsiella species possess copious virulence factors involving capsule, lipopolysaccharides (LPS), fimbrial and non-fimbrial adhesins and iron-scavenging systems (siderophores) (Paczosa and Mecsas, 2016). In addition to this, pathogenicity of *Klebsiella* is increased by its biofilm forming ability (Azzopardi et al., 2013).

The ability of *Klebsiella* spp. to develop the multi-drug resistance (MDR) and form biofilm makes the treatment of infections extremely difficult and highlights the demanding necessity for using new anti-biofilm approaches (Sadekuzzaman et al., 2015). Hence, this review offers an overview virulence attributes of MDR *Klebsiella* strains, their capacity for biofilm formation and alternatives approaches to combat biofilm development.

CLINICAL FEATURES OF *KLEBSIELLA* SPP.

Klebsiella species are classified into *K. pneumoniae* with its subspecies *(pneumoniae, ozaenae and rhinoscleromatis)*, *K. oxytoca* with its two subgroups and *K. varricola* (Martinez et al., 2004). In humans, *Klebsiella pneumoniae* is the most common causative agent of nosocomial and community acquired infections (Podschun and Ullmann, 1998) such as pneumonia, urinary tract, blood stream and surgical wound

infections, peritonitis, septicemia, meningitis (Namratha et al., 2015) and pyogenic liver abscess (Lederman and Crum, 2005).

Klebsiella can also cause different types of diseases in different animal hosts like bovine mastitis (Zadoks et al., 2011) and hematogenous osteomyelitis in cattle (Saunders, 2011), metritis (Frontoso et al., 2008), pneumonia (Ainsworth and Cheetham, 2010) and pyothorax (Saunders, 2011) in horses, urinary tract (Jarvinen, 2002) and respiratory infections (Ayodhya et al., 2013) and meningoencephalomyelitis (Radaelli and Platt, 2002) in dogs.

VIRULENCE FACTORS OF *KLEBSIELLA* SPP. CAPSULE

Capsule is the main *Klebsiella* spp virulence factor that encases the bacterial cell. *Klebsiella's* virulence is greatly influenced by the capsule, because it protects the bacterium from the host immune response by different ways involving (i) it prevents phagocytosis by immune cells (Domenico et al., 1994) (ii) it suppresses the bactericidal effect of antimicrobial peptides such as human beta defensins 1 to 3 and lactoferrin by attaching these molecules distal from the bacterial outer membrane (Fang et al., 2004) (iii) it inhibits the lysis of bacteria that transpires by the complement via obstructing the interaction between the complement components like C3 and the bacterial membrane (Paczosa and Mecsas, 2016) (iv) it reduces the reactive oxygen species (ROS), IL-8, IL-6 and TNF-α production, increases the production of the anti-inflammatory cytokine IL-10, aids in the activation of a NOD-dependent pathway and defends LPS from detection by immune cell receptors and thus, it frustrates the activation of the immune response (Yoshida et al., 2000, 2001).

Capsule is composed of strain-specific capsular polysaccharides called K antigens those are classified into 78 serological types (Pan et al., 2008). The production of capsule in *K. pneumoniae* is occurred by the genes, which are situated on a chromosomal operon, *cps* (Shu et al., 2009). The *cps* gene cluster accommodates a number of genes participated in capsule production comprising *wzi, wza, wzb, wzc, gnd, wca, cpsB, cpsG,* and *galF* (Pan et al., 2013).

Lipopolysaccharide

It is an essential constituent of the outer membrane of *Klebsiella* and it is renowned also as an endotoxin. It consists of three parts: An O antigen, a core oligosaccharide and lipid A. The genes required for formation of these parts are present in the *wb*, *waa* and *lpx* gene clusters, respectively (Raetz et al., 2009). LPS has an important role in the virulence of this bacterium, where *K. pneumoniae* can transform its lipid A leading to inactivation of the inflammatory response (Llobet et al., 2015) and also lipid A hinders the bactericidal impact of the antimicrobial peptides (Clements et al., 2007). LPS is the principal way of defense against complement, where strains that have a full-length O antigen or smooth LPS are impervious to complement interceded killing, while those with truncated or absent O chains or rough LPS are sensitive to complement-mediated killing even in the existence of capsule (Merino et al., 1992). This is attributed to the role of O antigen in thwarting the attachment of C1q to bacteria, which hinders successive stimulation of the complement pathway along with fixing C3b apart from the outer membrane of bacteria and thus, frustration of bacterial lysis by the complement membrane attack complex (Paczosa and Mecsas, 2016).

Adhesins

Fimbrial adhesins (Fimbriae or pili)

They are protein structures that aid the bacteria to target particular tissue surfaces in the host (Klemm and Schembri, 2000). *K. pneumoniae* has three types of fimbriae; 1, 3 and Kpc (Clegg et al., 2011).

Type 1 fimbriae

Type I fimbriae are thin, firm and filament-like projections on the bacterial cell surface. They are collected by chaperone/usher pathway and they are encoded by the *fim* gene cluster (Clegg and Murphy, 2016). The *fim* A gene codes the main structural subunit *fim* A, while the *fim* H gene codes the tiny tip adhesin subunit *fim* H (Struve et al., 2008). *K. pneumoniae* contains *fim* K gene that does not exist in *E. coli*. This gene participates in the regulation of type 1 fimbriae and it is important to note that the removal of *fim K* gene results in the failure of type 1 fimbriae expression (Rosen et al., 2015).

Genes of type 1 fimbria are expressed in the urinary tract, but not in the gastrointestinal tract or lungs, so these fimbriae can invade the urinary bladder cells and form the biofilm in the bladder (Struve et al., 2009).

Type 3 fimbriae

Type 3 fimbriae are spiral like threads encrypted by the *mrk*ABCD gene cluster, which may be chromosome-or plasmid-borne (Ong et al., 2010). They are also collected by chaperone/usher pathway (Allen et al., 2012). These genes consist of the chief structural part (*mrk* A) and the small adhesin tip (*mrk* D) (Paczosa and Mecsas, 2016), while *mrk* B, C, and E are implicated in gathering and regulation of fimbriae expression and *mrk*F is included in the fimbrial surface constancy (Allen et al., 1991).

These fimbriae are expressed by all *K. pneumoniae* isolates (Paczosa and Mecsas, 2016) and their expression is regulated by intracellular levels of cyclic di-GMP through using a combination of a phosphodiesterase (mrk J) and a

c-di GMP-binding protein (mrk H) in conjunction with a DNA-binding protein (mrk I) (Wu et al., 2012).

They are not required for urinary tract infections or in lung or gastrointestinal tract infections (Struve et al., 2009). They are the main fimbriae those are essential for formation of biofilm and attaching to tissues and medical devices in *K. pneumoniae*, where *mrk* A can bind to abiotic surfaces like medical devices before and after insertion into the body of patients, while *mrk* D can bind to the extracellular matrix such as that present in damaged tissues (Paczosa and Mecsas, 2016).

Kpc fimbriae

Kpc fimbriae are most often related to K1-positive strains of *K. pneumoniae* producing disseminated pyogenic infections and the formation of these fimbriae is occurred by the *kpc* ABCD gene cluster. The Kpc fimbriae are greatly related with hypermucoviscous *K. pneumoniae*. They also have an important role in the incidence of infection and production of *Klebsiella* biofilms (Wu et al., 2010).

Non-fimbrial adhesins, CF29K

It is present on R virulence plasmid encoding the CAZ-1/TEM-5ESBL and the aerobactin siderophore correlated with hypervirulent *Klebsiella* strains. It can adhere to the gut epithelial cells (Darfeuille-Michaud et al., 1992).

Siderophores

Siderophores have a higher iron attraction than host transport proteins (Transferrin). They are produced by *K. pneumoniae* to obtain iron from host iron chelating proteins or from the environment to live and proliferate during mammalian infection (Miethke and Marahiel, 2007). Enterobactin, yersiniabactin, salmochelin and aerobactin are siderophores those are produced by *K. pneumonia* (Brock et al., 1991).

Biofilm formation

The biofilm is an accumulation of adhered microbial cells those are irrevocably related with abiotic and/or biotic surfaces and are inserted in a self-formed matrix of extracellular materials including polysaccharides, proteins and DNA (Piperaki et al., 2017). Biofilm formation includes main four stages. The first stage is the initial alterable connection of bacterial cells to a surface. Throughout this step, the free-natant planktonic cells recognize a surface for attachment and then they begin the procedure of attachment. The second stage is the permanent binding of the bacterial cells to the substrate surface, which is interceded by the expression of quorum sensing (QS) signaling molecules and by the figuration of extracellular polymeric substances. The third stage is the construction of microcolonies and a mature biofilm with a 3-dimensional assembly. The fourth stage is the separation and scattering of cells from the biofilm and development of new biofilm.

Biofilm has a great role in the virulence of the bacterium, where it increases the tolerance of bacteria to harsh environmental circumstances and preserves microbes from opsonization by antibodies, phagocytosis and elimination through the ciliary of epithelial cells (Costerton et al., 2005). Within the biofilm, the horizontal gene transmission and plasmid transfer are happened at elevated levels (Diaz et al., 2009).

The production of biofilm is controlled by a process through which bacteria interconnect by a means of the release of type-2 QS regulatory molecules in the extracellular partition called autoinducers, AI-2 (Balestrino et al., 2005). The production of AI-2 is adjusted at the transcriptional level of *lux* S gene in the AI-2 synthesis pathway (Zhu et al., 2011).

EMERGENCE OF ANTIBIOTIC RESISTANCE IN *KLEBSIELLA* SPECIES

Several mechanisms have been developed by Gram-negative bacteria to resist the antimicrobial drugs. Horizontal transfer is one of the most effective mechanisms for transmitting MDR among bacterial pathogens (Munoz-Price and Quinn, 2009). Moreover, the ability of resistant strains for acting as mobile genetic elements (MGEs) donor is an important way for transmitting resistance among different bacterial pathogens. The mobile elements like transposons and plasmids may be transferred vertically to other strains, species or genera (Woodford et al., 2011). Consequently, MDR isolates spread has been due to commonly shared plasmids across bacteria such as *K. pneumonia* and *K. oxytoca* (Miro, 2010).

The efflux pump systems contribute a main cause of the MDR pattern (Meletis et al., 2012). Efflux pump systems in *K. pneumoniae* include *AcrAB* and *mdt K*. The AcrAB-TolC pump is composed of an outer-membrane channel (TolC), a secondary transporter located in the inner membrane (AcrB), and a periplasmic component (AcrA) (Du et al., 2014). This pump is responsible for resistance to quinolones, tetracyclines and chloramphenicol in various MDR isolates (Okusu et al., 1996). The MATE (Multidrug and Toxic Compound Extrusion) pumps such as the *mdtK* system transport some of those antimicrobial agents (Sun et al., 2014).

In *K. pneumoniae*, intrinsic resistance to some β-lactams is found, because the extended spectrum beta-lactamase (ESBL) enzyme is encoded in the core genome of the species. For example, SHV (cromosomally encoded species specific enzyme) is consistently found in *K. pneumoniae*

chromosome and therefore the corresponding ampicillin resistance is a property of this species (Bialek-Davenet et al., 2014). *K. pneumoniae* are also known to carry plasmid-mediated β-lactamases such as AmpC enzymes, which gives resistance to most penicillin antibiotics (Jacoby, 2009). Carbapenems have been the drug of choice to treat serious infections caused by ESBL-producing bacteria. Due to the selective pressure of treating ESBL infections with carbapenems, carbapenem resistance has emerged and *K. pneumoniae* is the most common carbapenem-resistant *Enterobacteriaceae* (CRE). In 2013, the centers for disease control and prevention (CDC) declared that the CRE is an urgent threat to public health in the United States (CDC, 2014).

Carbapenem resistance is primarily driven by the accessory genome and sometimes in combination with mutations in the core genome. Carbapenem resistance in *K. pneumoniae* can be mediated through up-regulation of efflux pumps (Filgona et al., 2015), alteration of outer membrane porins in the core genome (Kaczmarek et al., 2006) and hyperproduction of ESBL enzymes or AmpC β-lactamases in the accessory genome (Bush and Jacoby, 2010).

Colistin is among the polymyxin class of antibiotics, which is used to treat Gram negative infections in the 1960s and 1970s. Its use was discontinued due to renal- and neurotoxicity (Jerke et al., 2016). However, the recent emergence of CRE has made a necessity to return to colistin as a drug of a last resort. Colistin resistance in *K. pneumoniae* typically occurs through mutations in regulatory genes such as *mgrB* that regulates the modification of bacterial lipid A, the target of polymyxin antibiotics leading to a decrease in the ability of polymyxins to interact (Wright et al., 2015).

BIOFILM-DEPENDENT MECHANISMS LEAD TO ANTIBIOTIC RESISTANCE IN *KLEBSIELLA* SPECIES

Bacterial infections associated with biofilm formation are of a main public health concern, because the microbial cells growing in the biofilm are highly resistant to antibiotics and host immune defenses. These bacterial cells might develop a biofilm-specific biocide-resistant phenotype. Because of biofilm heterogeneous nature, it is expected that there are several resistance mechanisms work within a single community (Mah and O'Toole, 2001).

Biocide resistance development is not understood, but modern studies have used a diversity of model systems to determine how and why biofilms are resistant to different antimicrobial agents as following:

Failure of antimicrobial penetration into the biofilm

Exopolysaccharide matrix or glycocalyx production is one of the characteristic features of biofilm. It has been suggested that the matrix function is to prevent the access of antibiotics to the bacterial cells embedded in the community (Stewart, 1996).

Slow growth and stress response

Starvation of the bacterial cell culture for a particular nutrient slows its growth. Transition from exponential to slow or no growth is generally accompanied by an increase in resistance to antibiotics (Tuomanen et al., 1986). Slow growth of the bacteria has been observed in mature biofilms (Wentland et al., 1996).

Heterogeneity

Any given cell within the biofilm will experience a slightly different environment compared with other cells within the same biofilm and thus will grow at a different rate. Gradients of nutrients, waste products and signaling factors allow for this heterogeneity within the biofilm (Mah and O'Toole, 2001).

General stress response

It has been suggested that the slow growth rate of some cells within the biofilm is not owing to the nutrient limitation, but to a general stress response initiated by growth within a biofilm (Brown and Barker, 1999). This idea is possible because the stress response results in physiological changes that act to protect the cells from various environmental stresses. Thus, the cells are protected from the detrimental effects of heat shock, cold shock, changes in pH and many chemical agents (Hengge-Aronis, 1996).

Induction of a biofilm phenotype

Up till now, the mechanisms discussed have been based on general strategies to slow down the effect of antimicrobial agents on cells growing in the biofilm. An emerging idea is that a biofilm-specific phenotype is induced in a subpopulation of the community that results in the expression of active mechanisms to combat the detrimental effects of antimicrobial agents (Cochran et al., 2000). Moreover, it was noted that intrinsic resistance to antimicrobial agents dramatically increases when *K. pneumoniae* strains grow in the biofilm (Vuotto et al., 2014).

ANTIBIOFILM APPROACHES FOR COMBATING *KLEBSIELLA* BIOFILM

Natural compounds

Many natural molecules and plant extracts have antibiofilm activities against *Klebsiella* biofilm as followings:

A. Casbane diterpene: it is isolated from the extract of *Croton nepetaefolius* (a native plant in Brazil) and

it can reduce *Klebsiella* biofilm by 45% when used in sub-minimum inhibitory concentrations (sub-MIC) (Carneiro et al., 2011).

B. Reserpine: It is an efflux pump inhibitor found in the roots of *Rauwolfia serpentina* and *R. vomitoria* and it is efficient in biofilm inhibition at a concentration of 0.0156 mg/mL, the 64-fold inferior concentration than its MIC.
C. Linoleic acid: It is an essential fatty acid that is potent in biofilm prevention at 0.0312 mg/mL, 32-fold lesser than its MIC.
D. All of Berberine, which is an antimicrobial compound extracted from a variety of plants like barberry and goldenseal, chitosan, which is a natural polycationic linear polysaccharide derived from chitin and eugenol, which is a naturally arising phenolic molecule found in numerous plants such as cinnamon, clove and bay leaves and it can preclude biofilm formation at a minimum concentration of 0.0635 mg/mL.
E. Curcumin: It is a natural phenolic chemical compound produced by *Curcuma longa* (turmeric) plants and it is effectual in inhibition of biofilm at 0.25 mg/mL that is 50 fold fewer than its MIC (Magesh et al., 2013).

Essential oils

Cumin seed essential oil obtained from a medicinal aromatic plant of the *Apiaceae* family was proven to decrease *K. pneumoniae* biofilm development and to enhance also the effectiveness of ciprofloxacin antibiotic (Safoura et al., 2010). Another study demonstrated that the eucalyptus oil displayed the highest inhibitory effect among tulsi, garlic, neem and clove oils on biofilm formation on the surface of the catheter at sub-MIC concentrations of 200 µl/mL (Mathur et al., 2013).

Deoxyribonuclease1 (DNase1) enzyme

This enzyme is capable of decreasing the 24 h active biofilm biomass of *K. pneumoniae* almost by 40% at the concentration of 5.0 µg/mL. Moreover, there is a synergistic effect between DNase1 and azithromycin, rifampin, levofloxacin, ampicillin and cefotaxime antibiotics. The additive DNase improves the impact of antibiotics and decreases the formation of biofilm due to the cleavage of extracellular DNA, which results in the growth of distorted biofilm that allows the increased dissemination of antibiotics (Tetz et al., 2009).

Bacteriophage

They are the viruses, which attack the bacteria. They are recently used for obliteration of biofilm, because they have numerous advantages upon antibiotics as antibiofilm agents as they are specific, non-toxic, self-replicating and they are of a self-limiting nature (Parisien et al., 2008). Phages that produced polymerases are able to destruct biofilm more than non-depolymerases producing phages, because depolymerases can damage exopolysaccharide matrix of biofilm that hinders the penetration of antimicrobial compounds and thereby they lead to massive distraction of biofilm (Hughes et al., 1998). Meanwhile, the phages do not have the capability to pierce the old biofilm that include many metabolically dormant cells due to their removal speedily from the circulation once ingested or injected (Azeredo and Sutherland, 2008).

It is better to use phages in amalgamation with other chemically discrete antimicrobial compounds such as iron chelating molecules like cobalt that decreases the accessibility of iron. The iron is considered an important agent for growth of bacteria as it contributes in oxygen and electron transport processes (Banin et al., 2006), regulates the motility of bacterial surface, promotes the formation of biofilm by stabilizing the polysaccharide matrix (Berlutti et al., 2005) and helps in conversion from planktonic to sessile cells (Chhibber et al., 2013).

There was a study showing that the addition of copper sulphate in combination with depolymerase producing phage leads to a complete eradication of younger biofilm (1st and 2nd day biofilms), but they had a very little inhibitory effect on the older biofilms of *K. pneumoniae* (4th day onwards). On the other hand, the combination of copper sulfate and non-depolymerase producing phage had no additive effect on biofilm extermination (Chhibber et al., 2013).

Another study demonstrated that the combination of high concentrations of amoxicillin (512 µg/ml) and multiplicity of infection (MoI = 0.01) of specific phage leads to a larger damage of mature biofilm (8-day old biofilm) (Bedi et al., 2009). Another study showed that the use of ciprofloxacin in combination with depolymerase producing lytic bacteriophage (kpo1k2) leads to an enhanced destruction of old biofilm and this indicates that the effect of antibiotics is boosted by the bacteriophage (Verma et al., 2010).

Nanotechnology

It is the manipulation of matter on an atomic, molecular and supramolecular scale with at least one dimension sized from 1 to 100 nanometers. Lately, the nanotechnology has become a good implement for biofilm hindrance and hegemony (Sadekuzzaman et al., 2015).

Silver is known as an antimicrobial metal characterized by its non-toxicity (Sadekuzzaman et al., 2015). Silver nanoparticles (AgNPs) exhibited an effective antimicrobial feature due to their tremendously large surface area, which supplies a well connection with the microbes.

The nanoparticles adhere to the cell membrane and

invade the bacteria. The particles then react with the sulfur including proteins in the cell membrane and the phosphorus-comprising compounds as DNA (Rai et al., 2009). As well, silver interrelates with thiol group molecules located in the respiratory enzymes of bacterial cells. So, silver usage obstructs DNA replication, expression of ribosomal and other cellular proteins and it also restricts the respiration process, ultimately resulting in the death of bacterial cell (Yamanaka et al., 2005).

Silver nanoparticles have also a robust anti-biofilm action against most biofilm producing bacteria that causes many infectious diseases at a low concentration (100 µg/mL). Interestingly, *K. pneumoniae* is more susceptible to silver nanoparticles (Ramachandran and Sangeetha, 2017). There is a recent study conducted in Italy indicated that AgNPs can prevent biofilm formation by changing the membrane of *K. pneumoniae*, generating irreparable deterioration on bacterial cells and variation of the membrane permeability and respiration of *K. pneumoniae* (Franci et al., 2015).

Likewise, another research in India elucidated that AgNPs have the ability to extirpate the present biofilm through the water channels (pores), which exist in the biofilm for conveyance of nutrients, where AgNPs may directly spread within the exopolysaccharides layer by these pores and make their antibacterial and anti-biofilm effects (Ansari et al., 2013).

Quorum sensing inhibitors

Quorum sensing (QS) is a process through which the bacteria are connected by a means of the release of type-2 quorum sensing regulatory molecules (autoinducers, AI-2) in the exracellular partition (Balestrino et al., 2005). Several bacterial biological activities involving virulence, motility, luminescence, biofilm formation, sporulation, evolution of genetic proficiency, production of peptide antibiotics, formation of secreted proteolytic enzymes and fluorescence are controlled by this QS system (Rocha-Estrada et al., 2010).

The suppression or degeneration of QS signal molecules is recognized as QS inhibition or quorum quenching (QQ). This can be achieved by numerous methods such as the use of antibodies to QS signal molecules, the enzymatic devastation of QS signal molecules or through factors that obstruct QS (Chan et al., 2011).

Quercetin is a flavonoid obtained from a water-soluble plant pigment. It has anti-oxidant and QS inhibitory effects, where there is a survey in India mentioned that quercetin at a concentration of 40 µg/mL hindered the fabrication of exopolysaccharides in *K. pneumoniae* by 80.39% and diminished the biofilm of this bacterium by 13-72% (Gopu et al., 2015).

Antimicrobial peptides

Antimicrobial peptides (AMPs) are counted as new approaches against biofilms (Di Luca et al., 2015). As fundamental constituents of the natural immunity, AMPs are extensively spread through the microbial, animal and plant kingdoms (Grassi et al., 2017). Their amino acid sequences, net-positive charge, amphipathicity and extremely small size permit them to attach to and disturb the membranes of the bacteria (Yeaman and Yount, 2003).

Owing to the low specificity of their molecular intention, AMPs expose a large scale of activity and have a great capacity to affect metabolically latent cells (Batoni et al., 2016). Furthermore, they can prevent the manufacture of cell wall, nucleic acid and protein (Brogden, 2005). As well, AMPs are cell specific and are capable to differentiate host from non-host cells based on their charges (Beckloff et al., 2007). In addition to their antimicrobial effects, they posses immune-modulatory actions, where they can affect processes that boost the antimicrobial impact such as cytokine release, chemotaxis, antigen presentation, angiogenesis and wound healing (Lai and Gallo, 2009) and they also have an anti-tumor activity (Niyonsaba et al., 2010).

Diverse studies is indicated the potency of AMPs to intervene with different phases of biofilm development by inhibiting the early adherence of bacterial cells to surfaces, affecting the planktonic cells before they insert into the biofilm assembly or damaging the old biofilms via the separation and/or extermination of bacteria that formed in the biofilm (Segev-Zarko et al., 2015).

An example of such peptide is the type 1018, which is a powerful anti-biofilm peptide that acts by thwarting the production of guanosine 5´-triphosphate 3´-diphosphate (pppGpp), which is a vital signal in the formation of biofilm. There is a study in Canada showed that this peptide can entirely block the development of biofilm and eliminate the old biofilm when it is used at a concentration of 2 µg/mL. This concentration does not influence the growing of planktonic cells (De la Fuente-Nu´n˜ez et al., 2014).

Another example is DJK-6, which has an anti-biofilm effect and pursues the mature biofilm produced by carbapenemase-producing *K. pneumoniae*. It also promotes the activity of β-lactam antibiotics comprising meropenem to destroy aged biofilms produced by this bacterium (Ribeiro et al., 2015).

An important AMPs is nisin, which has anti-biofilm characteristics. It is a 34 amino acid polycyclic lantibiotic derived from *Lactococcus lactis*. It is broadly used as a natural biopreservative, where it has a bactericidal activity against both Gram-positive and Gram-negative bacteria and it can act synergistically in conjunction with traditional

therapeutic treatments (Shin et al., 2015).

There is a survey in Ireland demonstrated the ability of the antimicrobial peptide nisin to enhance the effectiveness of polymyxin antibiotics (colistin, polymyxin E and polymyxin B), where their combination decreases the concentrations of these polymyxins to hinder the development of biofilm produced by Gram negative bacteria. This will lead to a decline in the toxicity of polymyxins and thereby returning the sensitivity of these bacteria to such antibiotics (Field et al., 2016).

CONCLUSION

Klebsiella spp. are extremely dangerous opportunistic microbes that cause severe chronic infections and death. The danger of this bacterium is due to the emergence of MDR strains and the capability of *Klebsiella* to form and grow in biofilm societies those increase their resistance to antibiotics, host immune system and hard environments. New strategies like using the natural compounds, essential oils, enzymes, bacteriophage, nanotechnology, quorum sensing inhibitors and antimicrobial peptides are innovated for the removal and prevention of biofilm formation and therefore treatment of *Klebsiella* infections.

AUTHORS CONTRIBUTION

NKAE and MIAE made equal contributions to the conception and the design of the review. AMA participated in the study design and data interpretation. NAG and SSM performed the literature search and prepared the manuscript. All the authors contributed in writing of the article, revised the manuscript critically for important intellectual contents and gave the final approval of the version to be published.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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