Review Article :

Carbapenem-resistant biofilm-producing multidrug resistant Klebsiella pneumoniae induced hospital-acquired infections and analysis of resistant genes

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Received on 22-9-2022 , accepted for publication on 18-10-2022, dx.doi.org/10.5455/mjhs.2023.01.013

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Abstract

In recent years, drug resistance has caused a significant number of fatalities. This review focuses on the emergence of medication resistance in Klebsiella pneumoniae strains associated with biofilm development, neonatal sepsis, aging, and secondary K. pneumoniae infection in COVID-19 cases. This review is an exclusive survey of >200 research articles and >100 review articles indexed in the Web of Science list with the keywords “K. pneumoniae,” “drug resistance,” “sepsis,” “neonatal sepsis,” “sepsis in older age,” “secondary infection,” “biofilm production,” “secondary infection in COVID-19,” “drug resistance genes,” and “whole genome sequencing”. In order to protect antibiotic susceptibility and to provide an overview of the biofilm forming mechanisms among gram-negative bacteria, the review discusses the function of multidrug-resistant K. pneumoniae in the production of biofilms. Additionally, K. pneumoniae’s role in contemporary cases of sepsis, spread of medication resistance in hospitals, and sepsis caused by K. pneumoniae in the geriatric age group are discussed. A summary of current approaches and the identification of drug resistance genes utilizing a whole genome sequencing technique are also reviewed in this article.

Keywords:

Klebsiella pneumoniae; Biofilm; Drug resistance; carbapenemresistance; plasmid; gene .

Introduction

Antibiotic resistance is considered as a major evolutionary response of micro-organisms, including, bacteria, fungi, and viruses to survive antibiotic contaminated environment. Antibiotics are broadly classified based on the mode of action and chemical structure. Most of the currently using antibiotics were discovered between 1950s and 1960s[1]. These antibiotics were derived from actinomycetes, fungi or bacteria. The antibiotics classes such as glycopeptides, aminoglycosides, carbapenems and lipopeptides were derived from actinomycetes[2]. Cyclic lipopeptides were

considered as the novel antibiotic discovered in 1990s and introduced in 2003. Bacteria showed resistant toward various commercially available antibiotics due to their activity of efflux pumps, cell wall structure and porins. Bacteria have several mechanisms including, mutations in existing genes, and acquisition of drug resistance gene from other bacteria, mutation in metabolic genes, new metabolic pathways, and also through horizontal gene transfer. Bacterial transmission of antibiotic gene transfer is involved in antimicrobial resistance pandemic. Antibiotic resistance gene was reported before the discovery of medicine, and unrestricted application of antibiotics in aquaculture, agriculture, animal husbandry and in health care sectors lead to global spread of drug resistant genes[3]. Most of the antibiotic resistant genes were coded in plasmids and the plasmid mediated spread of carbapenemase genes increased threat throughout the globe. Most of the multidrug resistant infections are nosocomial origin and these variants can tolerate in hospital environment for several years. Health-care-associated infection (HAI) is a major threat to the patients admitted in the hospitals. It is associated with healthcare centres or from hospitals. An average of 7% hospitalized patients in developing countries and 10% from developed countries encountered HAI acquired infections[4]. Therefore, sanitation in hospitals, the surrounding area, wastewater treatment, equipment hygiene, the use of efficient disinfectants, and the sanitation of medical devices are all related to the spread of these drug-resistant bacteria. Controlling drug-resistant bacteria requires a sterile environment in hospital wards and thorough hospital cleaning[5]. These drug resistant bacteria colonized on medical devices and produced biofilms[6]. Novel therapeutic interventions, improved hygiene protocol in hospitals and health centres, rapid and precise diagnostic tools to detect resistant bacteria, analysis of drug resistant genes by whole genome sequencing may combat drug resistant bacterial infection.

Antibiotic resistance and global challenge

Antibiotic resistance is a global challenge, involving the transfer of resistance genes and bacteria between animals, humans, and the surrounding environment. There are various barriers affect the flow of both genes and bacteria, bacterial and or fungal pathogens acquire new resistance genes from other pathogenic strains, thereby minimizing the effect of antibiotics. Analysis of drug resistance factors in bacteria from evolutionary events is very rare and very challenging to predict the event. However, in transmission events,

widespread drug resistant genes were predicted and quantified. Analysis of drug resistance pathways is bottlenecks for the determination of transmission of antibiotic resistance and environmental evolution to manage and understand drug resistance. Antibiotics are broadly classified based on their mode of action and chemical structure, including DNA replication inhibition, cell wall synthesis inhibition, metabolic pathway inhibition and protein synthesis inhibition[1].Bacteria acquired resistance because of either mutations in existing genes, or core metabolic genes, through horizontal gene transfer or in intracellular targets[7].

The spread of antibiotic resistance genes, whether between species or within species, is to contribute to the current situation. The unrestricted use of commercialized antibiotics in health care and animal husbandry, poor sanitation in low income countries, unsuitable treatment of sewage water has led to multidrug resistant bacteria across the world[8]. Among antibacterial resistance genes, the global spread of antibacterial resistance genes are mainly plasmid-mediated genes and the genes are resistance to colistin and detected in Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterobacteriaceae, vancomycin resistance gene (vanA) in Staphylococcus aureus and Klebsiella pneumoniae[9].

Carbapenem-resistant Klebsiella pneumoniae

Klebsiella pneumoniae is a non-motile, Gram-negative bacilli, found in soil, sewage, plants and water. K. pneumoniae was common in gastrointestinal tract and nasopharynx of healthy individuals. It causes both community-acquired and hospital-acquired infections. K. pneumoniae is one of the drug resistant bacteria characterized from hospital environment, including reception, equipments, and intensive care units and involves various bacterial infections such as, urinary tract infection, respiratory tract infections, endocarditis, endophthalmitis, and invasive liver abscesses[10]. K. pneumoniae colonized in respiratory tract, gastrointestinal tract or urinary track leads to sepsis in humans[11]. A multi-locus sequence typing (MLST) method is widely used to distinguish bacteria based on DNA sequences of house-keeping genes in K. pneumoniae. In this organism, MLST is based on housekeeping genes such as, tonB, rpoB, phoE, pgi, mdh, infB, and gapA)[12]. Virulence of K. pneumoniae was also classified based on its 78K antigens, also referred as capsular antigens. The pathogenic K. pneumoniae is associated with capsule and it protects from phagocytosis and se-

rum resistance. The fimbriae or pili are an important part of the pathogenic bacteria that mediated the adhesion and colonization of the organism to epithelial cells of respiratory tract, lower urinary tract[13]. K. pneumoniae consists of aerobactin, rmpA, rmpA2 genes and these virulence genes involved in the development of hypervirulent strains[14].

Antibiotic resistant genes and mobile elements

Klebsiella pneumoniae is one of the ESKAPE pathogens responsible for the widespread of various antibiotic resistances in most of the hospital-acquired infections in humans. In K. pneumoniae, antibiotic resistance are plasmid mediated in most of the cases. Most pathogenic strains carry 3 or more antimicrobial resistance plasmids and increased stability was reported compared to drug resistant E. coli[15]. In Klebsiella pneumoniae, replicons such as IncL, IncHI2, IncHIB-FIB, IncHI1-FIA, IncX3, IncN1 and IncFIIK were reported. Other than transfer of disease resistant genes via plasmids, mobile genetic materials such as, transposons, and integrons and were characterized in the plasmids, mediated mobilization of the antibiotic resistant genes between chromosome and plasmids or between different plasmids[16].

Biofilm– a biological shield

K. pneumoniae forms biofilms on gastrointestinal tract mucosa, urinary tract and respiratory tract, as well as, on various abiotic surfaces including, catheters and medical devices.There are several factors involved in the development of biofilm in K. pneumoniae.These include pili, fimbriae, cell wall, the presence of other bacterial species and iron metabolism. The polysaccharide content of the bacteria significantly influenced on biofilm formation, including, adhesion on cell surface and maturation[17]. However, mutant bacterial strains with impaired capsule production showed poor biofilm forming ability[18]. The role of bacterial capsular polysaccharide to biofilm development was reported previously. The pathogenic K. pneumoniae strains involved in bacteremia and showed the presence of virulence gene (wcaG) and in capsule production[17]. K. pneumoniae strains involved in the formation of biofilm and detected the presence of treC gene in bacterial colonization and infection in humans[19].

Lipopolysaccharide (LPS) involved in the formation of biofilm. It has been reported that LPS promoted the attachment to available abiotic surfaces, and is one of a major factor in the early stages of biofilm development. Moreover, bacteria lacking wbbM gene (associated with LPS produc-

tion) showed delayed biofilm formation[18]. Two types of fimbriae (type I and III) were reported from K. pneumoniaeand showed various functional properties, including, adhesion; promote binding on biotic and abiotic surfaces[20]. The genetic material of K. pneumoniae harbours about 10 gene clusters encoding, chaperones, and adhesin proteins required for the assembly of fimbriae, including kpa, ecp, kpg, and mrkgenes[21].The genes, kpf, ecp, mrk and fim were encoded with type I and type III fimbriae, common pilus and the type I-like fimbriae[22].The type I and II fimbriae influenced on biofilm formation, and helped early adhesion and initiate biofilm formation. It has been reported that the regulation of fimbriae expression is influenced by various factors including DNA binding regulators, oxidative stress and iron availability[23,24].Whereas, regulation of type III fimbrial genes and development of biofilm is controlled by MrkI and MrkH regulators, moreover mutant bacteria lacking these regulator genes have no biofilm forming ability[25].

Whole-genomic sequencing (WGS) and surveillance of virulence factors

Whole genome analysis was used to determine the suspected epidemiological link between patients. It is used for the determination of virulence factors, plasmids, capsular types, and phage region by bioinformatics approach. Enany et al[26] isolated 65 virulence genes and 15 antimicrobial resistance genes from Klebsiella pneumoniae from the clinical samples from Egyptian patients. The resistance genes such as aph(6)-Id, aph(3'')-Ib, tet(D), fosA6, and fosA were determined and were involved in resistance mechanism against fosfomycin, aminoglycoside and tetracycline. Moghnia and Al-Sweih (2022) analyzed the whole genome sequence of multidrug resistant Klebsiella pneumoniae. Whole genome sequencing was performed using an Illumina MiSeq platform. β-lactamase genes, blaCMY-4/-6, blaKPC-2/-29, blaOXA-1/-48, and blaEC were identified. Resistance genes on quinolone antibiotics, aminoglycoside modifying enzymes genes, trimethoprim-sulfamethoxazole resistance genes, fosfomycin and tetracycline resistance genes, chloramphenicol resistance and colistin resistance genes were detected.

The multidrug resistance K. pneumoniae strains were isolated from the non-clinical (n=29) and clinical (n=48) samples and WGS analysis revealed intermix between non-clinical and clinical isolates. The WGS of 77 Thai genome of K. pneumoniae was closely related with a total of 286 global K. pneumoniae sequences. These isolated drug resistance strains were

emerged before 150 years ago. The number of β-lactamase resistance genes was high in clinical isolates than in environmental samples. Moreover, the clinical isolates were closely related with environmental strains[27]. In 2011, an outbreak of carbapenem-resistant K. pneumoniae was reported in United States. A total of [18] patients were affected and 11 were died in that outbreak. Whole-genome sequencing has been performed to determine virulence and drug resistant genes. The pathogenic bacteria were originated from the different sites of single patients and not from different sources. Contamination of medical devices affected the entire patient's health[28].Tamma et al.[29] used Nanopore whole-genome sequencing method to predict drug resistance genes from multi drug resistant K. pneumoniae. A total of nine carbapenem-susceptible K. pneumoniae isolates, 10 non-carbapenemase-producing carbapenem-resistant K. pneumoniae and 21 carbapenemase-producing carbapenem-resistant Klebsiella pneumoniae were used and WGS was analyzed. Nanopore sequencing method was very rapid and within 14 h single-nucleotide polymorphism and annotation were carried out.

The complete genome of multidrug resistant K. pneumoniae HS11286 was analyzed. Whole genome sequence analyses revealed six plasmids, one chromosome and a total of seven circular replicons. Bioinformatics analysis revealed coded genes for 5,316 putative proteins, 8 copies of 16S-23S-5S rRNAs, 1 tmRNA and 87 tRNAs. In silico analysis was performed and the strain specific genes were determined using mGenomeSubtractor and the results were analyzed using H value. The genesp-KPHS3, pKPHS2 involved in the spread of drug resistance among various genera[30]. Jiang et al[31] screened sample from the hospital environment for the isolation of K. pneumoniae and revealed the presence virulence genes.The selected bacteria showed at least one drug resistance plasmid. Recently, the genetic relationship of carbapenem-resistant K. pneumoniae was studied. Whole genome sequence analysis revealed colonization and transmission routes of carbapenem-resistant K. pneumoniae and between hospital facilities[32]. The isolated strain showed hypervirulence factor and genome showed a very close homology with published genomic sequences and the sequence was almost similar with the bacteria isolated from United States[5]. In Colombia, a total of 425 carbapenem-resistant Klebsiella pneumoniaewere isolated. Among these strains, 91.5% bacteria produce carbapenemase and this finding revealed increased drug resistance among bacteria. Carbapenemase producing genes such as, blaNDM-1, blaKPC-3, blaNDM-9,

blaNDM-1, blaVIM-4, blaVIM-2, and blaVIM-24 were determined. In addition to these genes, mobile genetic elements were detected[33]. Whole genome analysis revealed the presence of virulence factors, antibiotic resistant genes and was hypervirulent[34].

Neonatal sepsis

K. pneumoniae is an important hospital pathogen and reported periodically in the intensive care units and involved in respiratory tract infection, bacteremia, endophthalmitis, endocarditis and invasive liver abscesses[15]. It has been previously reported that, K. pneumoniae is a major bacterial pathogens involved in neonatal sepsis[35].Neonatal sepsis is caused by K. pneumoniae at the neonatal stage. It was further classified intoearly-onset sepsis (infection within seven years of birth) and late-onset sepsis (infection after 7 years of birth). In neonatal intensive care units, the rate of sepsis was extremely high and it ranges from 28 to 59% and bloodstream infections were also high[36]. This organism initially colonized urinary tract, or respiratory tract and further disseminates into the blood of patients and formation of sepsis. In recent years, the increase in meningitis and septicaemia in newborns caused by drug resistant Klebsiella is considered as a great concern. In neonatal sepsis, complications such as, respiratory failure, disseminated intravascular coagulation, brain lesions, septic shock, cardiovascular dysfunction and renal failure were reported[37].

Disseminated intravascular coagulation is a major cause of death, followed by sudden respiratory failure. Increased sepsis results in the damage of central nervous system, hypoxemia, and septic shock in infants. However, neonatal sepsis is mainly associated with hospital stay. Despite effective treatment, neonatal sepsis caused mortality among infants. About 28% of deaths associated with neonatal sepsis and this number declined slowly. The incidence of sepsis varied widely among developing countries. The increased antibiotic consumption, increased prescriptions, are the major causes of multidrug resistance among neonates. The characterization of drug resistance bacteria and treatment of sepsis were less satisfactory. Early determination of risk factors is associated with increase cure rates while decreasing neonatal mortality and morbidity[38]. More than 50% sepsis cases have been reported from the middle- and low-income countries. These septic cases are preventable in these countries[39].

Sepsis in aged patients

Sepsis is one of the leading causes of death and it ranked 10th place in patients

over the age of 60. Older age people are highly susceptible to sepsis and mortality rate was also higher. Angus et al.[40]revealed that the incidence of sepsis was higher than young age and the mortality was higher in aged patients than children. Martin et al.[41]reported that the mortality rate of sepsis was only 17.7% if the patients age <65, whereas, the death rate was 27.7% in older age (>65). Dombrovskiyet al[42] found that the incidence rate of sepsis was 0.00705% for children, 0.161% for people between 50 and 64, 0.442% for people between 65 and 79, and 0.992% for people above 85 years. Klebsiella pneumoniae caused Lemierre's syndrome in patients with poorly-controlled diabetes mellitus. In older age, septic emboli were found in various organs and it is complicated to treat using anticoagulant therapy, antibiotics and surgical intervention[43]. Klebsiella pneumoniae-caused liver abscesses are one of the critical diseases, which may cause severe complications and sepsis. Klebsiella pneumoniae-caused liver abscesses in aged people. The limited healthcare access, delayed diagnosis may be severely associated with the increase incidence of sepsis. Patients with age >65 were highly susceptible for the development of pyogenic liver abscess[44]. Moreover, the mean age of pyogenic liver abscess patients was about 60 and maximum cases were observed between 51 and 60 years[45].Generally, men were highly susceptible for sepsis than woman. These previous findings revealed the role of sex hormones in sepsis and female hormone protect from sepsis. Pyogenic liver abscess mediated sepsis was associated with chronic renal insufficiency, fatty liver and hepatic dysfunction[46].

K. pneumoniae: secondary infections in COVID-19 cases

Viral infection in the respiratory system mediates the secondary bacterial infections leading to severe mortality. In 1928, maximum pandemic death was reported in Spanish flu due to Pneumococcalpneumoniae infection[47]. Despite a continuous effort to cure and control COVID-19 across the world after alarming signal by WHO, the incidence of COVID-19 associated secondary infection continued in 2020 and 2021. Klebsiella pneumoniae is considered as the most prevalent bacterial species involved in secondary infection in COVID-19 cases. About 7.1% COVID-19 patients admitted in hospitals were infected with bacteria and 15.5% patients were associated with nosocomial secondary bacterial infections[48,49]. The secondary infection of K. pneumoniae in COVID-19 cases is a serious problem because these strains have developed specific mechanism to resist various antibacterial agents

such as polymyxins, quinolones, aminoglycosides, and β-lactams[16]. The scheme of Carbapenem-resistant biofilm-producing multidrug resistant K. pneumoniae in COVID-19 cases was illustrated in Fig. 1.

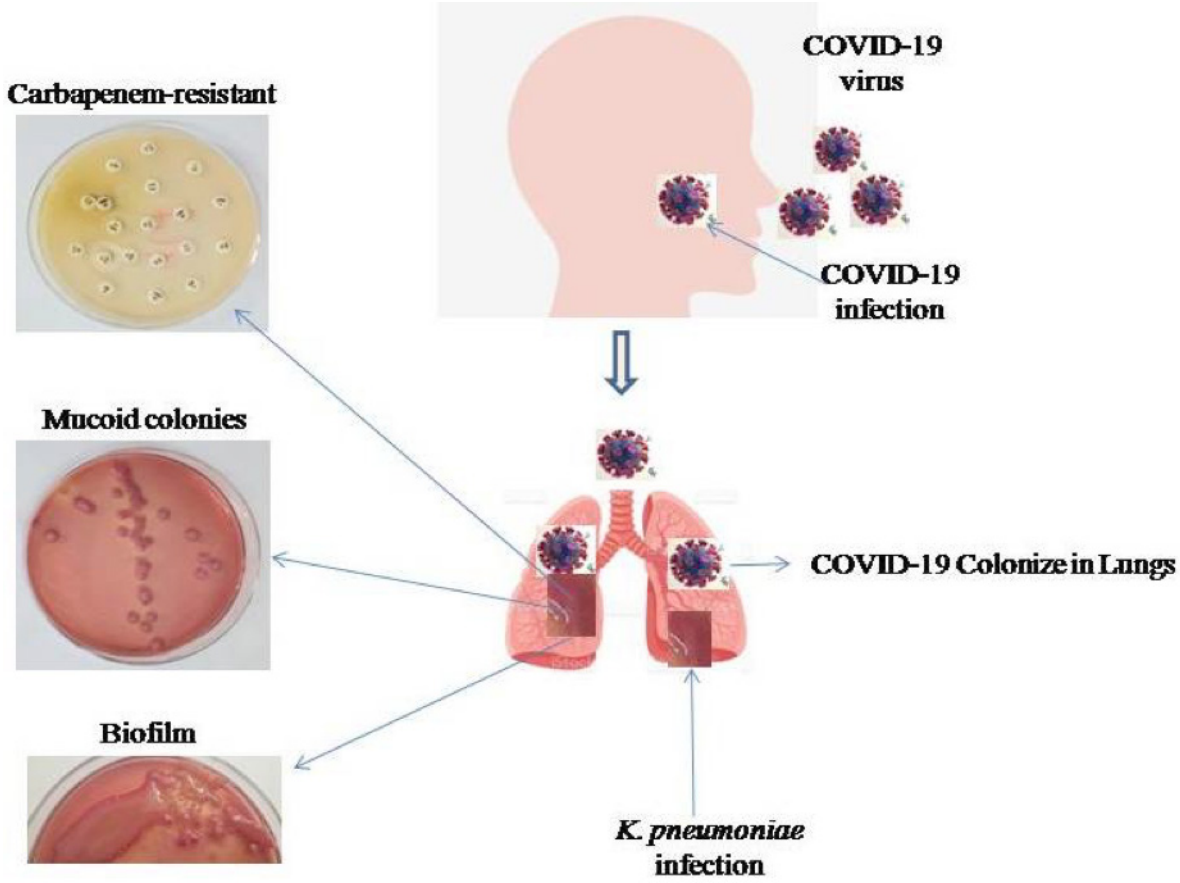


Fig. 1. Carbapenem-resistant biofilm-producing multidrug resistant K. pneumoniae in COVID-19 cases

K. pneumoniae involved in the production of various virulence factors, such as, endotoxins, capsular polysaccharides, biofilm formation, and all these virulence factors increase pathogenicity of the organism[50]. Non mucoid K. pneumoniae are less virulent, whereas the mucoid strains were virulent[51]. Recently, Ali et al.[52]studied secondary infection on 217 patients infected with COVID-19 and a total of 142 patients were associated with secondary bacterial infections. From the 142 cases, 58 patients were associated with K. pneumoniae infection. These bacterial species showed increased drug resistance and reported more than 38% mortality in admitted cases. In the hospital environment, patients admitted in COVID-19 wards and ICUs showed increased percentage of K. pneumoniae infection[53]. Lai et al.[54]reported COVID-19 associated drug resistant Klebsiella pneumoniae along with other bacterial species, fungal strains as well as human immunodeficiency virus, influenza B virus, metapneumovirus, and parainfluenza virus. In

United Kingdom, only 3.2% COVID-19 patients were associated with co-infection, whereas, in France, 28% of co-infections were reported and most of the common bacterial strains were S. pneumoniae, H. influenzae and S. aureus[55,56].

Conclusions

Antimicrobial resistance is a complicated problem in hospitals and multifaceted approach is required to reduce resistance. Moreover, effective approaches are required to treat multidrug resistance pathogens. Standard wastewater treatment protocol, especially treatment of hospital wastewater, proper disinfection in the hospital environment, state-of-art hygiene for healthcare workers and patients will reduce the outbreak of multidrug resistant pathogens from the hospital environment. Carbapenemases-producing K. pneumoniae hydrolyse b-lactam antibiotics and many variants were determined from various sources. Plasmids play a significant role in interspecies and intraspecies transmission of drug resistance genes mainly under antibiotic stress. Within few years, these new variants reach other countries and clone spread mediate transmission of variants. Plasmids associated with carbapenem-resistant genes also carry various resistant genes. The traditional diagnostic methods are not effective in most of the cases and incomplete. K. pneumoniae caused sepsis in neonates and old age groups. The secondary infection of K. pneumoniae in COVID-19 cases is a serious problem because these strains have developed specific mechanism to resist various antibacterial agents. Whole genome sequencing is precise and rapid method for the determination of antibiotic resistance genes from the clinical pathogens.

Key points

- Antimicrobial resistance is a challenging problem in hospitals. A diverse approach is needed to eliminate resistance.
- Most standard diagnostic procedures are ineffective and insufficient.
- Efficientand appropriate strategies are needed to handle multidrug-resistant infections.
- K. pneumoniae secondary infection in COVID-19 cases is an issue since these strains have evolved antibacterial resistance mechanisms.

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