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***Review Paper***

# Nosocomial *Klebsiella* infection and its virulence factor associated with drug resistance

**Jitendra Chandra Devrari[1*] and Vidya Pai[2]**
Department of Microbiology, Yenepoya Medical College, Mangalore, Karnataka, India
jitendradevrari87@gmail.com

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## Abstract

*Klebsiella species are gram negative bacilli; non-motile bacteria belong to the Enterobacteriaceae family. They are opportunistic pathogens responsible for diseases like urinary tract infection, pneumonia, septicemia, meningitis and soft tissue infections. Chromosomal mutation in the DNA gyrase and DNA topoisomerase IV leads to the plasmid mediated quinolone resistance (PMQR) in Klebsiella spp. The Klebsiella spp. primarily isolated from different clinical samples by the conventional methods. Isolates are further identified by standard biochemical methods and susceptibility testing of PMQR along with MIC is determined by the CLSI guidelines. Phenotypically type 1 and 3 fimbriae can be determined by the hemagglutination assays. Genotypic methods have been proved to be a great tool for detection of virulence and drug resistance genes associated with the Klebsiella infection. This studyalso focus on the virulence factors and most common drug resistant mechanism for quinolone group of antibiotics used in treatment of Klebsiella infection.*

**Keywords**: *Klebsiella pneumoniae*, Plasmid mediated quinolone drug resistance, Virulence factor.

## Introduction

*Klebsiella species* are belongs to the family *Enterobacteriaceae* and tribe *Klebsiella*. *Klebsiella spp.* causes opportunistic infection such as urinary tract infection, pneumonia, septicemia, soft tissue infection, meningitis, and bacteremia[1-3]. Most of the nosocomial infections are caused by the *Klebsiella* spp. which are associated with a high mortality rate in infants and older patients[4,5]. *Klebsiella species* increase in number during endemic and epidemic outbreaks in pediatric wards and Hospital outbreaks of multidrug-resistant are often caused by an ESBL producer strains. There are several virulence factors which contribute to the pathogenesis of *K. pneumoniae*, such as capsule, liopopolysaccharide, and fimbriae which are prominent and have ability to colonize and to form biofilms[6-9].

## Pathogenicity factor

Capsule is complex of lipid polysaccharide which helps to make a biofilm formation and has antiphagocytic activity to resist the host immune systems. Biofilm is a common virulence factor which is the mode of growth for bacteria in natural and clinical environments. Bacterial extracellular polysaccharides have been shown to mediate closely packed with cell-to-cell and cell-to-surface interactions that are required for the formation, cohesion and stabilization of bacterial biofilms[10].

Fimbria is another common virulence factor which is non flageller, filamentous projection on the bacterial surface. It has adherence property which attaches to the host cell surface. In Klebsiella species there are mainly two different types of fimbriae (pili) - type 1 and type 3[10]. Type 1 fimbriae have mannose-sensitive hemagglutinins, while type 3 fimbriae have mannose-resistant hemagglutinins[11]. Type 1 fimbriae is the most common adhesive organelle in *Enterobacteriaceae* and its adhesive subunit FimH, plays an important role in UTI, caused by *K. pneumonia*[12,13]. In Type 1 fimbriae adhesion protein (pilus type) is located on the fimbrial shaft and is capable of binding to mannose-containing trisaccharides of the host glycoproteins to cause UTI[14]. FimH mutations are pathoadaptive in nature, and found in a highly virulent uropathogenic strain of *Enterobacteriaceae*. The abundance of type1 fimbriae in *Enterobacteriaceae* finds that fimH gene is importance for adhesion in *K. pneumoniae*[15]. FimH and R plasmid gene from the spinal cord injury (SCI) and non-SCI patients which have a adhesion property of *K. pneumoniae* which is the cause of multidrug resistance antibiotics, nosocomial infection and UTIs[16]. The gene (mrkD) encoding the adhesion of *K. pneumoniae* type 3 fimbriae was identified by transcomplementation analysis with the fimbrial gene cluster of *Escherichia coli*. However type 3 fimbriae are less important for disease production in *Klebsiella species.*

The new virulent hypermucoviscous *Klebsiella spp.* strains associated with magA and rmpA genes have mainly emerged in Asia. The *mag*A (mucoviscosity associated gene A) is a chromosomal gene which plays an important role in serious infection of *Klebsiella* such as septicemia, bacteremia, and pneumonia as well as lung and liver abscesses. The

chromosomal *mag*A gene has hyperviscous phenotype, and characterized by forming a muscoviscous string of >5 mm diameter during passing loop through a colony. It also causes increased levels of resistance to phagocytosis. The r*mp*A, (regulator of the mucoid phenotype A, A1 and A2 gene), *rmp*A1 and *rmp*A2 are responsible for regulating the synthesis of the extracellular polysaccharide capsule[17].

## Antibiotics Use against in Klebsiella infection

Most commonly beta lactam and quinolone group of drugs are used against the infection caused by *Klebsiella spp.* infection.

**Beta lactam group of drugs are classified as[18]:** i. Penicillin group and ii. Cephalosporin group.

The cephalosporin group is further subdivided under: i. First generation: Cephalothin, Cefazoline, ii. Second generation: Cefuroxime, Cefoxitin, iii. Third generation: Ceftazidime, Ceftriaxone, Cefotaxime, Cefoperazone. iv. Fourth generation: Cefepime, v. Fifth generation: Ceftoroline.

**Quinolone group of drugs:** i. First generation: Nalidixic acid, Oxonilic acid ii. Second generation: Ciprofloxacin, Ofloxacin, Lomefloxacin, Norfloxacin, iii. Third generation: Levofloxacin, Moxifloxacin, Clinofloxacin.

## Drug resistance in Klebsiella infection

**Extended spectrum beta lactamase (ESBLs):** ESBLs are able to hydrolyze oxyimino cephalosporins and usually inhibited by beta lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam or etc. Existence of ESBLs and quinolone resistance in the *K. pneumoniae* infection suggests that care should be taken for the choice of antibiotic therapy[19].

**Characteristics of beta lactam antibiotics:** Resistance to beta lactam has become a selected amongst bacteria since its clinical use. Resistance mechanisms of beta lactams are: due to the altered receptors (alteration of penicillin binding proteins), production of beta-lactamases enzyme which will binds to the drugs and inactivate these drugs, and altered antibiotic transport[20]. Extended-spectrum beta lactamase producing *K. pneumoniae* has a significant source for mortality and morbidity in infants. Use of oxyimino antibiotics has a significant risk factor for infection[21]. The phenotypic resistance of cefotaxime, ceftazidime, and ceftriaxone of ESBL-producing *K. pneumoniae* isolates have correlate well with the presence of blaSHV, blaCTX-M, blaVEB-1-like genes, but not the blaTEM gene. Bla SHV genotype are positive among 35.3% of MDR *K. pneumoniae* isolates because the SHV-type enzyme shows stronger hydrolytic activity for ceftazidime than cefotaxime, so it has contributes to increased ceftazidime resistance in *K. pneumoniae*[22,23]. ESBL producing Klebsiella species from various clinical specimens was found 26.66% of isolates to be ESBL producers.[24] Higher rate of ESBLs producing *Klebsiella spp*. were found to be associated with the urinary tract infection compared to the other clinical sites[25,26].

**Plasmid mediated quinolone drug resistance (PMQR):** Plasmid mediated quinolone resistance genes (qnr, aac(6')ib-cr, qepA) involved in producing drug resistance to quinolone groups of antibiotics in *Klebsiella* infection. PMQR are commonly present in ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* infections[27,28]. The transferable, plasmid-mediated quinolone resistance are associated with *qnr* and *aac(6')-Ib-cr, qepA* genes, all these genes are implicated in low-level fluoroquinolone resistance which play a significant role in the generation of resistant and therapeutic failure effect. The emergence of plasmid-mediated quinolone resistance contributes to the rapidly increase of quinolones resistance. Co-transmission of *qnr* with *aac(6')-Ib-cr*, ESBLs, and plasmid-mediated AmpC genes rapidly formation of multidrug resistance in *Enterobacteriaceae*[29]. High frequency of PMQR genes indicates the early detection and routine screening of ESBL producing *K. pneumoniae* to prevent infection[30]. Increase frequency of quinolone resistance in *Enterobacteriaceae* associated with an increased prevalence and diversity of PMQR genes in clinical isolates. These factors together with increase the emergence of highly quinolone-resistant associated with MDR[31].

**qnr gene:** Quinolone resistance is usually caused by mutations in the chromosomal genes for DNA gyrase and DNA topoisomerase IV. The co-transmission of qnr with aac(6')-Ib-cr and ESBL CTX-M genes spread up the formation of multidrug resistance in Enterobacteriaceae. The spread of PMQR qnr genes is high among *K. pneumoniae* isolates, whereas aac(6')-Ib-cr and qepA1 are restricted to *E. coli* isolates from adult patients[33].

qnrA determinant is found in ESBL producing *Enterobacteriaceae* isolates. Possible chromosomal location of the *qnrA1* gene in *E. cloacae*, suggested that the *qnrA*-structure could have integrated into the chromosome of that isolate, possibly by a transposition process[34]. qnrB can co-transfer with either blaCTX-M-15 or blaSHV-12, whereas qnrS is a nontransferable plasmid, and aac(6')-Ib-cr can co-transfer with either blaCTX-M-3 or blaCTX-M-4. High prevalence rate of PMQR genes among ciprofloxacin-resistant ESBL producing *K. pneumoniae* and reduced fluoroquinolone susceptibility such as ciprofloxacin, norfloxacin, and enrofloxacin[35,36]. *qepA* encodes an efflux pump belonging to the major facilitator subfamily (MSF)[37]. The *qepA* gene, together with the *qnr* family and *aac(6')Ib-cr*, is the third recently detected plasmid-borne determinant of resistance to the fluoroquinolones.

These genes serve only low-level resistance, but their presence could potentially facilitate higher levels of resistance by mutational alterations of type II topoisomerase[38]. Various enzymes, efflux pump, and alteration of outer membrane proteins displayed their respective roles and contribute the drug resistant strains. Horizontal gene transfer and clonal spread were responsible for the transmission of drug resistance *K. pneumoniae*[39].

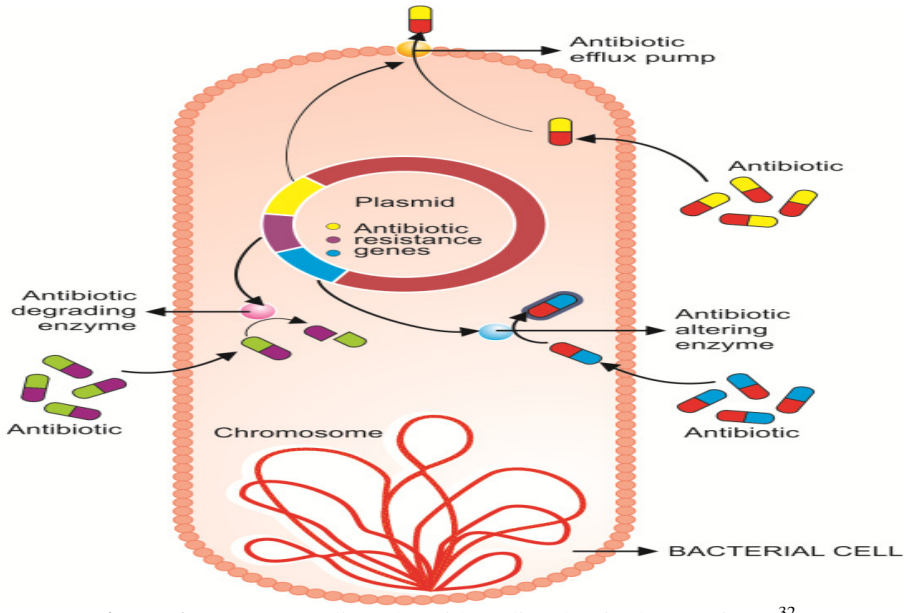


**Figure-1:** Genes encoding plasmid mediated quinolone resistant[32].

**aac(6')Ib–cr gene:** A new Plasmid mediated drug resistance mechanism has been identified the based on enzymatic modification of some fluoroquinolones drugs (norfloxacin and ciprofloxacin). The aminoglycoside acetylteransferase variant, *aac (6')–Ib–cr* capable of acetylating and subsequently reducing the activity of norfloxacin and ciprofloxacin against the bacteria which shows the resistance[40]. The aac(6′)-Ib-cr gene was detected for the first time in the chromosome, although a plasmid location was the most frequently found with differentiation of plasmids types in *E. coli* versus *Klebsiella spp*[41]. Low-level fluoroquinolone resistance conferred by *aac(6')-Ib-cr* gene associated with reduced bactericidal activity of ciprofloxacin in vivo and lead to ciprofloxacin therapeutic failure in pyelonephritis. aac*(6')-Ib-cr* is the most prevalent PMQR gene detected in clinical isolates, especially in ESBL producing strains[42]. The co-existence of PMQR genes with KPC on the same plasmid belong a serious epidemiological, clinical and public-health threat[43]. Three known plasmid-mediated quinolone resistance genes among the 16S rRNA methylase producing *Enterobacteriaceae* isolates of human origin and their prevalence was found to be high with *qnrB*, *aac(6')-Ib-cr*, and *qepA* in the decreasing order of their frequency. It was found that *qnrB4*, *aac(6')-Ib-cr*, and *qepA* genes are principally disseminated among them through spread of Inc FIIAs, IncL-M, and IncF conjugative plasmids, respectively[44]. aac*(6')-Ib-cr* gene was detected in three genera of *Enterobacteriaceae* (*E. cloacae*, *E. coli*, and *K. pneumoniae*), indicating horizontal transfer among the *Enterobacteriaceae*. The *aac(6')-Ib-cr* gene showed a high association with β-lactamase gene, including OXA-1, CTX-M-3 and TEM-1 in isolates from Korea[45]. Epidemiological survey evaluating the low prevalence of the plasmid-mediated quinolone resistance determinant in a European hospital. Identified the qepA with widespread ESBL CTX-M-15 on a broad-host-range plasmid and associated with ISCR-mediated genetic structure may point toward its further spread[46]. High prevalence and possible transmission of drug resistance *K. pneumoniae* isolates among hospitalized patients. Thus demonstrate that high degree of awareness and monitoring the drug resistance are needed to better control the emergence and transmission of drug-resistant *K. pneumoniae* isolates[47]. Prevalence of PMQR mediated by *qnrA* and *qnrB, aac(6')–1b-cr* mutant gene showed multi drug-resistant *K. pneumoniae* isolates. A multidrug-resistant plasmid conferring high resistance to ciprofloxacin was found to another PMQR gene, that is *aac(6')–1b-cr* mutant gene[48]. The potential risk spread of multidrug resistant bacteria among Indian patients thus demanding their pre-screening before treatment and development of new drugs for effective treatment of these resistant pathogenic bacteria. Bla genes were detected in *K. pneumoniae* isolates with prevalence in the following order: blaTEM>blaSHV>blaCTXM[49]. Level of ciprofloxacin resistance is currently low in European populations; it may rapidly increase by clonal spread[50]. Monitoring and surveillance, and molecular typing of strains with multiple resistances are necessary procedures to control the emergence of MDR strains in hospital settings and the occurrence of related outbreak events[51].

## Methods of detection

There are two way of detecting of PMQR: phenotypic methods and genotypic methods.

**Phenotypic methods:** Use of non molecular techniques detects the ESBLs by the combine disc diffusion method and PMQR detect by the Kirby Bauer disc diffusion methods. According to CLSI guidelines the isolates are screened and then confirmed for ESBL and PMQR production[52].

**Minimum inhibition concentration (MIC) detection:** MIC detect by the agar/broth dilution technique[53]. Detection would be maximized by screening with ciprofloxacin or norfloxacin by both MIC determination and disk diffusion assays. Furthermore, a low concentration of ciprofloxacin in the disks seemed to increase the sensitivity of the disk diffusion assay[54].

**Hemagglutination method detects:** The type 1 and type 2 fimbriae and will show the Mannose sensitive hemagglutination (MSHA) and mannose resistant hemagglutination (MSHA). Ability of sequence variation between fimH gene of *K. pneumoniae* and *E. coli* to colonize the urinary tract and produce a disease. The sequence of TOP52 fim h was almost identical to those of other sequenced *K. pneumoniae* fim h proteins. The inability of sequence of TOP52 fim h of *K. pneumoniae* to agglutinate guinea pig RBCs is not an isolated finding. Additionally, expression of *E. coli* type 1 pili at similar levels to TOP52 type 1 pili resulted in a positive MSHA[55].

**Biofilm formation by Microtitre plate assay:** Microtitre plate assay can be used alternatively as an accurate, rapid, reproducible and inexpensive primer screening method for detection of biofilm formation. However, in order to complete and enhance the final results, it would be efficient to carry out other experiment, such as PCR for detection of genes and comparison with the microtitre plate assay results[56].

**Genotypic methods:** Molecular techniques are use to detect the gene responsible for the production of PMQR. Molecular detection methods are Polymerase chain reaction (PCR), and Multiplex PCR. PMQR gens i.e. qnr, aac(6)ib-cr and qepA genes can be detected the use of real time PCR as a tool for their rapid detection. Simple assay could also be used such as pyro-sequencing for the detection PMQR. It is an innovative technique may prove highly useful for the rapid detection of PMQR known to be associated with the decreased bactericidal activity of quinolone[57].

## Conclusion

ESBLs producing PMQR *Klebsiellae* infection are current problem in hospitalized patients and immunocompromised patients. It has important implication as carbapenems, imipenem (10µg), meropenem (10µg) remain the only choice of drugs for infection caused by this organism. PMQR genes (qnr, aac(6)ib-cr and qepA) mediate the drug resistant to quinolone group of antibiotics due to the mutation of DNA gyrase and topoisomerase IV, enzyme modification of Fluoroquinolone drugs and efflux pump protein activation, which is highly responsible for nosocomial infections.

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