



Frequency and Antimicrobial Susceptibility of Multidrug-resistant *Klebsiella pneumoniae* Isolated From Wound Samples in Isfahan, Iran

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Abstract

Background: *Klebsiella pneumoniae* is one of the most important opportunistic enteric bacteria and is a major cause of pneumonia and urinary tract infection. In addition, the serotype capsules of K1 and K2 can cause intense diseases. Further, the acquisition of plasmid that codes the production of extended-spectrum β -lactamases (ESBLs) confers *K. pneumoniae* resistance on a number of broad-spectrum antibiotics posing a global public health problem. Accordingly, this study aimed to identify 120 *K. pneumoniae* isolates that were detected from infected wound samples in Isfahan hospitals in Iran.

Methods: Capsular serotypes and antibiotic resistance genes were studied in 120 isolates of *K. pneumoniae* from different clinical cases in Isfahan, Iran. To this end, the frequency of resistance genes at the presence of specific primers was examined and all resistant isolates were tested for the detection of capsular serotypes genes using special primers.

Results: The results demonstrated that 120 isolates had serotype K2 with the redundancy of 78% and most cases had serotype K5 with the redundancy of 63%. Based on the results, *aac (3)-IV* gene was observed in most isolates with the redundancy of 54.1% and *tetA* with the redundancy of 75.86%. In this study, the highest resistance belonged to ceftazidime (74.3%), ciprofloxacin (78.5%), and tetracycline (72). Furthermore, the results revealed that serotype K2 is one of the most important serotypes of *K. pneumoniae*. Finally, there seems to be a strong relationship between the presence of integron and increased resistance to different antibiotics.

Conclusions: In general, this was the first extensive study regarding the distribution and antimicrobial resistant profile of *K. pneumoniae* and related genes. Therefore, the continued monitoring of the antimicrobial resistance establishment of a surveillance system is urgently needed to prevent further dissemination in Iran.

Keywords: *Klebsiella pneumoniae*, Wound samples, Multiplex polymerase chain reaction, Iran

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Background

Klebsiella pneumoniae is a gram-negative, aerobic, nonmotile bacilli and is a common cause of a wide range of infections in humans and animals. In addition, it is one of the most prevalent enteric bacteria responsible for up to 10% of all nosocomial infections and is also involved in pneumonia and urinary tract infections causing severe morbidity and mortality (1). A recent report is also available regarding the highly invasive *K. pneumoniae* that causes primary liver abscesses in humans (2). These invasive, abscess forming strains of *K. pneumoniae* are associated with the so-called hypermucoviscosity (HMV) phenotype, which is a bacterial colony trait identified by a positive string test. The HMV phenotype is found in *K. pneumoniae* expressing either the capsular serotypes K1 or K2. The K1 serotypes of *K. pneumoniae* have 2 potentially important genes of *rmpA* and *magA*. The first one is a

transcriptional activator of colanic acid biosynthesis and the second one encodes a 43-kD outer membrane protein (3). Further, the serotype capsules of K1 and K2 can cause intense diseases and based on the studies on these serotypes, *magA* and *rmpA* genes, related to HMV “in charge of the positive synthesis of outside-capsule polysaccharide” are both useful tools in knowing such serotypes (4,5). Most *K. pneumoniae* isolates have a chromosomally encoded SHV-1 β -lactamase (6). Since 1983, plasmid-encoded extended-spectrum β -lactamases (ESBLs) derived from the TEM and SHV families have been extensively reported in some Gram-positive bacilli such as *Staphylococcus aureus* and Enterobacteriaceae, especially in *Klebsiella* spp. (7). Furthermore, the emergence and spread of multidrug-resistant *K. pneumoniae*, specifically the ESBL-producing strains, are often responsible for the failure of antibiotic treatment in hospital settings.

However, the presence of resistance to trimethoprim-sulfamethoxazole can lead to treatment failure in cases of urinary tract infections in many countries (8). Sulfonamide resistance in Gram-negative bacilli generally arises from the acquisition of dihydropteroate synthase (DHPS) genes in integrons that are not inhibited by the drug. Three different types of DHPS genes have been currently identified, including *sul1*, *sul2*, and *sul3* (9). The *sul1* gene is found linked to other resistance genes in class 1 integrons and on large conjugative plasmids while *sul2* is usually located on small nonconjugative plasmids, large transmissible multi-resistance plasmids, or through insertion element common region (ISCR2) element. *sul3*, as a plasmid-borne sulfonamide resistance gene, is also present although it is rare (10,11). Recent studies have shown that mobile and mobilizable DNA elements such as integrons play an important role in the development and dissemination of antibiotic resistance. Integrons are defined as site-specific recombination systems that are capable of integrating and expressing open reading frames contained in modular structures called mobile gene cassettes. Moreover, different classes of integrons are characterized by sequence differences in the *intI* gene encoding an integrase (12). Additionally, class 1 integrons possess two conserved segments (CSs), namely, the 5'-CS and the 3'-CS, which are separated by a variable region including the integrated antibiotic resistance gene cassettes of different lengths, arrangements, and sequences (13,14). Three main groups or classes of integrons associated with antibiotic resistance have been described in the clinical environment. Therefore, the present study investigated the genotypic and phenotypic antibiotic resistance patterns *K. pneumoniae* strains isolated from clinical samples in Iran.

Materials and Methods

Bacterial Strains and Identification

This study set out to determine 120 *K. pneumoniae* isolates which were detected from infected wound samples in Isfahan hospitals. All clinical isolates in addition to molecular serotyping were biochemically analyzed by conventional bacteriology tests. Furthermore, the polymerase chain reaction (PCR) method was used to detect the 16S-23S internal transcribed spacer unit of *K. pneumoniae* subsp. *pneumoniae*, facilitating the identification of the following subspecies:

F: ATTTGAAGAGGTTGCAAACGAT and **R:** TTCACT CTGAAGTTTTCTTGTTTC (amplicon size: 130 bp).

Further, cycling conditions were as follows:

Initial denaturation at 94°C for 5 minutes, 35 cycles of 94, 58, and 72°C each for 1 minute, respectively, followed by a final extension at 72°C for 7 minutes. *K. pneumoniae* ATCC13883 was used as the positive control as well (15).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar plates (Merck, Darmstadt, Germany) as recommended by the Clinical Laboratory Standards Institute (CLSI). The disks containing the following antibiotics were used (Padtan-Teb, Iran):

Amoxicillin (10 µg), amikacin (30 µg), kanamycin (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), cephalothin (30 µg), norfloxacin (10 µg), ceftriaxone (30 µg), nitrofurantoin (10 µg), imipenem (10 µg), cefepime (30 µg), and gentamicin (10 µg). Finally, *Escherichia coli* ATCC 25922 was used as quality control for the antimicrobial susceptibility test (15,16).

Polymerase Chain Reaction Assay

DNA template was extracted using the phenol and chloroform method and total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell. The reverse and forward primers, the size of the product for PCR programs, and volume, as previously published, were used for the detection of *K. pneumoniae* (17-19). The 1.5% agarose gel in the Tris-Borate-EDTA buffer was used for PCR product separation. Then, the gels were run at a constant voltage of 100 V for 1 hour, stained in 2 µg/mL ethidium bromide for 10 minutes, and photographed under UV by gel documentation system (20). Eventually, the reverse and forward primers, the size of the product for *K. pneumoniae* capsular serotypes were used (20).

Results

Serotyping and Antimicrobial Susceptibility Patterns of *Klebsiella pneumoniae*

During the study, 120 *K. pneumoniae* were isolated from wound samples in Isfahan hospitals in Iran, followed by performing molecular serotyping. Then, the disk diffusion method was used according to the CLSI (2017) in order to determine antibiotic resistance by the phenotypic pattern.

Of the total 120 *K. pneumoniae* wound samples, 63 and 57 isolates were collected from females and males, respectively. In this research, the highest resistance was related to ceftazidime (74.3%), ciprofloxacin (78.5%), and tetracycline (72%). Furthermore, there was widespread resistance of the isolates to the antibiotic which is shown in Figure 1 ($P < 0.05$). Tables 1 and 2 present the PCR results for capsular genes and the frequency of antimicrobial-related gens in *K. pneumoniae*.

The levels of antibiotic resistance are shown in the samples. Based on the results, the highest resistance belonged to ceftazidime (74.3%), ciprofloxacin (78.5%), and tetracycline (72). The investigated antibiotics

Antimicrobial susceptibility patterns of *K. pneumoniae*



Figure 1. Serotyping and Antimicrobial Susceptibility Patterns of *Klebsiella pneumoniae*.

The PCR Results of Genotypic Antibiotic Resistance Patterns

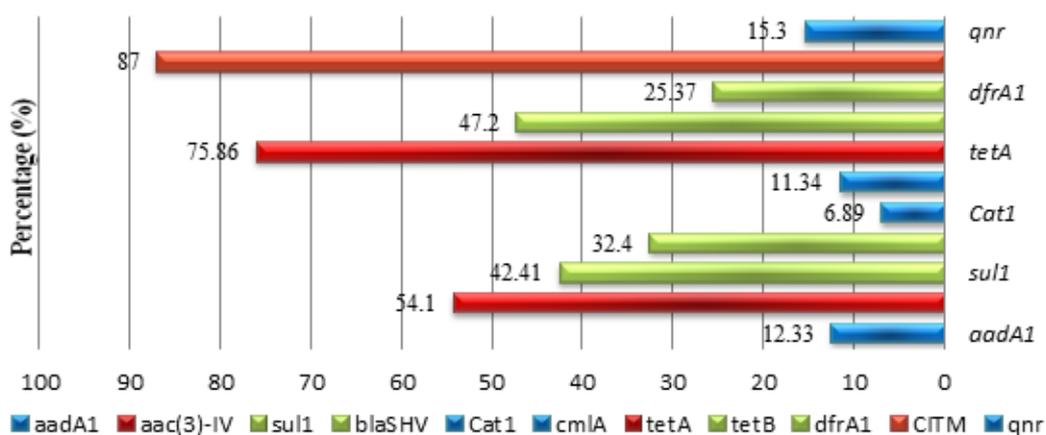


Figure 2 . Antimicrobial Susceptibility Patterns of *Klebsiella pneumoniae*.

included ciprofloxacin, ceftazidime, tetracycline, trimethoprim, norfloxacin, gentamicin, azithromycin, meropenem, erythromycin, cotrimoxazole, tobramycin, cefalotin, levofloxacin, amikacin, rifampin, imipenem, streptomycin, chloramphenicol, and nitrofurantoin.

The PCR results represent the frequency of antimicrobial-related genes and the percentage for genotypic antibiotic resistance patterns in *K. pneumoniae*. According to the results, *aac (3)-IV* (63%) had the highest frequency among the examined genes.

Discussion

The results of the experiment found clear support for the multidrug-resistance patterns of *K. pneumoniae*, along with the frequency distribution of *K. pneumoniae* genes and their capsular genes. Our findings

with regard to the overall high resistance of *K. pneumoniae* strains to antibiotics such as ceftazidime (74.3%), ciprofloxacin (78.5%), and tetracycline (72%) are in agreement with those of other recent studies. Similar to our results, Salimizand et al found the high rate of resistance to imipenem (100%) and meropenem (100%). Although other beta-lactam groups including aztreonam (20%), ceftazidime (20%), and cefotaxime (20%), indicated more antibiotic resistance, which contradicts our results (21). On the other hand, Mansury et al (22) reported that the highest rate of resistance belonged to amoxicillin (100%), cefotaxime (50%), and gentamicin (42.3%) and the lowest rates were observed for meropenem (11.8%), imipenem (15.9%), and amikacin (15.9%), which is in line with the results of previous studies.

The findings of the present study showed that K2 (78%)

Table 1. The PCR Results (Frequency of Antimicrobial-Related Gens) for the Genotypic Antibiotic Resistance Patterns of *Klebsiella pneumoniae*

Gene	Size of Product (bp)	Antimicrobial Agent	Resistance Percentage by PCR (%)
<i>aadA1</i>	447	Streptomycin	12.33
<i>aac(3)-IV</i>	286	Gentamicin	54.1
<i>sul1</i>	822	Sulfonamides ¹	42.41
<i>blaSHV</i>	768	Cephalothin	62.4
<i>Cat1</i>	547	Chloramphenicol	6.89
<i>cmlA</i>	698	Chloramphenicol	11.34
<i>tetA</i>	577	Tetracycline	75.86
<i>tetB</i>	634	Tetracycline	47.2
<i>dfrA1</i>	367	Trimethoprim	25.37
<i>CITM</i>	462	Ampicillin	87
<i>qnr</i>	516	Fluoroquinolone	15.3

Note. PCR: Polymerase chain reaction.

Table 2. The PCR Results for Capsular Genes in *Klebsiella pneumoniae*

Gene for Capsule Type	Size of Product (bp)	Number of Samples	Percentage (%)
<i>K1</i>	1283	7	5.8
<i>K2</i>	641	78	78
<i>K5</i>	280	63	63
<i>K54</i>	881	23	19.16
<i>K57</i>	1037	13	10.83

Note. PCR: Polymerase chain reaction.

was the most common *K. pneumoniae* serotype, followed by K5 (63%). The obtained data further revealed that a total of 87% of *K. pneumoniae* isolates carried *CITM* and 75.86% of them carried *tetA*. This is contrary to the human isolates of *K. pneumoniae* in which the *tetA* gene is present in both K1 and K2 capsular serotypes, as well as nearly 67% of non-K1/K2 serotypes, but the *cat1* gene appears restricted to the isolates of the K1 serotype. This result ties well with that of Jung-Chung, representing that poor glycemic control in diabetic patients plays a significant role in impairing the neutrophil phagocytosis of serotype K1/K2 *K. pneumoniae* (23). Khamesipour and Tajbakhsh (15) found a similar result and reported that serotype K1 is one of the most important serotypes of *K. pneumoniae* and of 13 out of 90 isolates had serotype K1A (14.44%) and 15 cases had serotype K2A (16.66%).

Based on the findings of the current study, more than half of the *K. pneumoniae* strains possessed one or more of these *sul* genes and sulfonamides and gentamicin resistance occurred in 42.41% and 54.1% of these strains, respectively. This result is in line with the findings of another study done among *Escherichia coli* strains where the *sul2* gene was found to be predominant in *E.*

coli strains isolated in urinary tract infection episodes. The results of this study confirm the need for increasing concern regarding therapy for clinical infections caused by *K. pneumoniae* isolates that have resistance-encoding *CITM* and *sul* and *tetA* genes which are related to class 1 integrons. Therefore, the continued monitoring of antimicrobial resistance, the adoption of the prudent use of antimicrobial agents and the establishment of a surveillance system is highly needed for preventing further dissemination in Iran (24).

Conclusions

In general, it is necessary to continuously monitor antimicrobial resistance, adopt the prudent use of antimicrobial agents, and establish a surveillance system to prevent further dissemination in Iran.

Conflict of Interests

The authors declared no conflict of interests.

Authors Contribution

FN: Ideas; evolution of overarching research goals and aims, preparation of the published work.

SB and DD: investigation process experiments, provision of study materials.

ET: Management and coordination responsibility for the research activity planning and execution, acquisition of the financial support for the project.

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