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Analyzed the genotypic and phenotypic antibiotic resistance patterns of *Klebsiella pneumoniae* isolated from clinical samples in Iran.

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Abstract

Klebsiella pneumoniae **is one of the most important opportunistic enteric bacteria and is a major cause of pneumonia and urinary tract infection. Serotype capsules of K1 and K2 can cause intense diseases. Acquisition of plasmid that codes the production of ESBLs confers on *K. pneumoniae* resistance to number of broad spectrum antibiotics posing a global public health problem. Integron is one of the important factors of multi resistance in gram negative microorganism's especially intestinal bacteria. The *magA* gene *rmpA* gene was studied in 90 isolates of *K. pneumoniae* from different clinical cases in Shahrekord city, Iran. The frequency of resistance genes *qnr, sul 1, tetB, tetA* and *aac* (3) IIa at the presence of specific primers were examined and all resistant isolates were tested for detection of *sul1, sul2, sul 3* and *int1* genes using special primers. Of the 90 isolates, 13 had serotype K1A with redundancy of 14.44% and 15 cases had serotype K2A with the redundancy of 16.66%. *rmpA* gene was observed in 10 isolates the redundancy of 11.11%. In this study 33 isolates resistance to cotrimoxazole, aren't finding *sul1* gene in 15 isolated cases, *sul2* gene in 20 isolated cases, *sul3* gene in 2 isolated cases, respectively. Also there were 27 demonstrating *int1* genes for Cotrimoxazol. The study has revealed that serotype K1 is one of the most important serotypes of *K. pneumonia.* Also there seems to be a strong relationship between presence of Integron and increased resistance to different antibiotics.**

Keywords: Antibiotic resistance genes, Capsular antigen, Integron, *Klebsiella pneumoniae*, *rmpA* gene.

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Introduction

Klebsiella pneumoniae is a gram-negative, aerobic, non motile bacillus and is a common cause of a wide range of infections in humans and animals [1,2] and one of the most common enteric bacteria responsible for up to 10% of all nosocomial infections and also involved in pneumonia and urinary tract infections causing severe morbidity and mortality [1,3]. Recently, a highly invasive *K. pneumoniae* causing primary liver abscesses in humans has also been reported [4-6]. These invasive, abscess-forming strains of *K. pneumoniae* are associated with the so-called hypermucoviscosity (HMV) phenotype, a bacterial colony trait identified by a positive string test [7-9]. The HMV phenotype is seen in *K. pneumoniae* expressing either the capsular serotypes K1 or K2. K1 serotypes of *K. pneumoniae* have 2 potentially important genes, *rmpA*, a transcriptional activator of colanic acid biosynthesis, [10] and *magA*, which encodes a 43-kD outer membrane protein [7]. K2 serotypes of *K. pneumoniae* also have *rmpA* but do not have *magA*. Serotype capsules of K1 and K2 can cause intense diseases and based on studies of these serotypes, it has been revealed that *magA* gene, related to Hypermocoviscosities and *rmpA* gene, in charge of positive synthesis of outside-capsule polysaccharide, are both useful tools in knowing such serotypes. Most *K. pneumoniae* isolates have a chromosomally encoded SHV-1 β-lactamase [11]. Since 1983, plasmid-encoded extended-spectrum β-lactamases (ESBLs) derived from the TEM and SHV families have been extensively reported in Enterobacteriaceae, especially in *Klebsiella* spp. [12,13].

Emergence and spread of multidrug resistant *K. pneumoniae*, specifically the ESBL producing strains, is often responsible for the failure of antibiotic treatment in hospital settings [14]. In many countries, however, the presence of resistance to Trimethoprim-sulfamethoxazole can lead to treatment failure in cases of UTIs [15]. 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 [16]. Currently, three different types of DHPS genes (*sul1, sul2*, and *sul3*) are known [15]. The *sul1* gene is found linked to other resistance genes in class 1 integrons and on large conjugative plasmids [17], while *sul2* is usually located on small nonconjugative plasmids [18], large transmissible multi-resistance plasmids [15], or through insertion element common region (ISCR2) element [19]. Although rare, *sul3*, a

plasmid-borne sulfonamide resistance gene, is also present [17].

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 [20-22]. 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 [23]. Different classes of integrons are characterized by sequence differences in the *intI* gene encoding an integrase. Class 1 integrons possess two conserved segments (CSs), the 5'-CS and the 3'-CS, separated by a variable region, which includes integrated antibiotic resistance gene cassettes of different lengths, arrangements, and sequences [23]. In the clinical environment, three main groups or classes of integrons associated with antibiotic resistance have been described. Class 1 integrons are most frequently found in clinical isolates of Gram-negative bacteria [22]. Although several literatures studied sul and/or dfr genes in relation to class 1 integron in *E. coli* [15,24-26], there are limited reports investigating the antigenic capsules of *K. pneumoniae*, the phenotypic genotypic antibiotic resistance patterns in *K. pneumoniae* and sul genes in relation to class 1 integrons and *sul* genes in *Klebsiella* in Iran. Therefore, in this present study, we investigated the genotypic and phenotypic antibiotic resistance patterns of strains of *K. pneumoniae* isolated from clinical samples in Iran

Materials and Methods

Bacterial strains and identification

We examined 90 *K. pneumoniae* clinical isolates from hospitals of Shahrekord, Iran. Clinical isolates were mostly from urine, blood culture, eye secretion, trachea and wound. Prior to molecular-serotyping, all clinical isolates were biochemically identified by conventional bacteriology tests as detailed previously [27].

The PCR method was used to detect the 16S–23S internal transcribed spacer unit of *K. pneumoniae* subsp. *pneumoniae*, facilitating identification of this subspecies, as described previously [28]: F: ATTTGAAGAGGTTGCAAACGAT and R: TTCACT CTGAAGTTTTCTTGTTTC (amplicon size: 130 bp). Cycling conditions were as follows: Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min followed by a final extension at 72°C for 7 min. *K. pneumoniae* ATCC13883 was used as positive control.

Antimicrobial susceptibility testing

The antibiotic susceptibility was determined by disk diffusion method on Mueller-Hinton agar plates (Merck, Darmstadt, Germany) as recommended by the Clinical Laboratory Standards Institute (CLSI) [29]. The disks containing the following antibiotics were used (Padtan-Teb, Iran): amoxiciline (10 µg), amikacin (30 µg), kanamycin (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), co-trimoxazole (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). E. coli ATCC 25922 was used as quality control for antimicrobial susceptibility test.

Polymerase chain reaction assay

The DNA template was extracted using phenol and chloroform method. The total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell [30]. The reverse and forward primers, size of product and PCR programs (temperature and volume) as previously published used for the detection of capsular K1 and K2 serotypes in *K. pneumoniae* in this study are presented in Table 1 [28]. In addition, The primers, size of product and PCR conditions as previously published used for the detection of resistant genes and *sul* genes of *K. pneumoniae* are presented in Table 2 and Table 3, respectively [15,31-34]. Reference strains of *K. pneumoniae* AY762939 and *K. pneumoniae* D21242 were used as positive controls for PCR reactions of K1 and K2 serotypes respectively.

Table 1. *Primers used for genes in K. pneumoniae.*

Gene	Primer name	Primer Sequence (5'-3')	Size of product (bp)	PCR program	PCR volume (50 µl)	Reference
K1A	aac(3)-IV	(F) GGTGCTCTTTACATCATTGC (R) GCAATGGCCATTTGCGTTAG	1283	1 cycle: 95°c ---------- 10 min. 34 cycle: 95°c ---------- 30 s 58°c ---------- 60 s 72°c ---------- 90 s 1 cycle: 72°c ---------- 5 min	5 µl PCR buffer 10X 2.5 mm Mgcl2 200 µM dNTP (Fermentas) 0.5 µm of each primers F & R 2 U Taq DNA polymerase (Fermentas) 3 µl DNA template	[28]
K2A	sul1	(F) GACCCGATATTCATACTTGACAGAG (R)CCTGAAGTAAAATCGTAAATAGATGGC	641			
rmpA	blaSHV	(F) ACTGGGCTACCTCTGCTTCA (R) CTTGCATGAGCCATCTTTCA	536			

F- Forward; R- Reverse

Table 2. *Primers and PCR conditions of resistant genes of K. pneumoniae.*

Antibiotic	Resistant gene	Sequence	Size (bp)	Anealing	PCR program	References
Tetracycline	*tetA*	GTGAAACCCAACATACCCC GAAGGCAAGCAGGATGTAG	888	55	1 cycle: 94°c ---------- 5 min cycle: 30 49°c ---------- 15 s	[31]

					55°c ---------- 60 s 72°c ---------- 60 s 1 cycle: 72°c ---------- 5 min	
Tetracycline	*tetB*	CCTTATCATGCCAGTCTTGC ACTGCCGTTTTTTCGCC	774	55	1 cycle: 95°c ---------- 5 min. 30 cycle: 95°c ---------- 30 s 55°c ---------- 60 s 72°c ---------- 60 s 1 cycle: 72°c ---------- 5 min	
Fluoroquinolone	*qnr*	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	55	1 cycle: 95°c ---------- 5 min. 30 cycle: 95°c ---------- 30 s 55°c ---------- 60 s 72°c ---------- 60 s 1 cycle: 72°c ---------- 5 min	[32]
Gentamicin	*aac(3)IIa*	CGGAAGGCAATAACGGAG TCGAACAGGTAGCACTGAG	740	55	1 cycle: 94°c ---------- 5 min cycle: 30 49°c ---------- 15 s 55°c ---------- 60 s 72°c ---------- 60 s 1 cycle: 72°c ---------- 5 min	[31]
Sulfonamide	*Sul1*	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	65	1 cycle: 94°c ---------- 5 min. 34 cycle: 94°c ---------- 60 s 65°c ---------- 60 s 72°c ---------- 90 s 1 cycle: 72°c ---------- 8 min	[33]

Table 3. *Primers used for sul genes.*

Gene	Sequence (5'–3')	Annealing temp (°C)	Size of product (bp)	Reference
Sul 1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	65	433	[15]
Sul 2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	65	293	[15]
Sul 3	F: GCCTATGCATCTACACAATC R: TGAGAAATGGACAATGTCCG	65	750	[34]
Int1	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	53	160	[34]

The 2% agarose gel in TBE buffer was used for PCR products separation. 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-Document. The expected PCR products for 16S–23S, Capsular K1, K2 and *rmpA* were 130, 1283, 641 and 537 base pair (bp) in length, respectively.

Results

Serotyping and antimicrobial susceptibility patterns of K. pneumonia

During the study period, a total of 90 *K. pneumonia* clinical isolates, were collected. The molecular serotyping was performed and showed in Table 4. Among 90 *K. pneumonia* clinical isolates, 13 had serotype K1A with redundancy of 14.44% and 15 cases had serotype K2A with the redundancy of 16.66%. *rmpA* gene was observed in 10 isolates the redundancy of 11.11% (Figure 1 and Figure 2). Of the total 90 K. pneumonia clinical isolates, 55 were collected from females and 35 isolates were from males. There was widespread resistance of the isolates to Amoxicillin 87.8%, Cephalothin 53.3%, Kanamycin 45.5%, Tetracycline 43.3%, Ceftriaxon 41.1%, Nitrofurantoin 41.1%, Cotrimoxazole 36.7%, Amikacin 32.2%, Cefepime 34.4%, Gentamicin 26.7% (Table 5).

Table 4. *Serotype K1, K2 and rmp isolates from samples.*

Isolate source	165rRNA	K1A	k2A	*RmpA*
urine (N= 76)	76	11	12	10
Blood culture (N= 5)	5	2	1	0
Eye secretion (N= 5)	5	0	2	0
Wound (N= 2)	2	0	0	0
Trachea (N= 2)	2	0	0	0
Total (N= 90)	90 (100%)	13 (14.44%)	15 (16.66%)	10 (11.11%)

Table 5. *Antimicrobial resistance profiles of K. pneumoniae isolates against 90 antimicrobial agents.*

Antimicrobial agent	Resistant		Intermediate resistant		Susceptible	
	Number	%	Number	%	Number	%
Amoxicillin	88	97.8	1	1.1	1	1.1
Nalidixic acid	22	24.4	8	9	60	66.6
Nitrofurantoin	37	41.1	22	24.5	31	34.4
Imipenem	4	4.4	2	2.2	84	93.4
Cefepime	31	34.4	3	3.3	56	62.2
Tetracycline	39	43.3	17	18.8	34	37.9
Co-trimoxazole	33	36.7	2	2.2	55	61.1

Ciprofloxacin	6	6.7	4	4.4	80	88.9
Kanamycin	41	45.5	26	28.9	23	25.6
Ceftriaxone	37	41.1	5	5.5	48	53.4
Cephalothin	48	53.3	6	6.7	36	40
Norfloxacin	15	16.7	3	3.3	72	80
Amikacin	29	32.2	4	4.5	57	63.3
Gentamicin	24	26.7	1	1.1	65	72.2

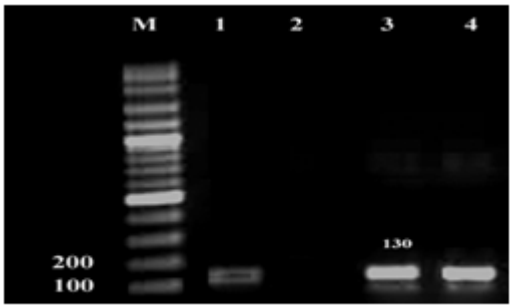

Figure 1. *Result of the PCR Assay for Identification of 16S rRNA K. pneumoniae. M: DNA size ladder 100 bp (Fermentas), number 1: reference strain for 16S rRNA K. pneumoniae; number 2: negative control; number 3 and 4: positive samples.*

Distribution of Klebsiella pneumonia antimicrobial resistance pattern and antibiotic resistance genes

The PCR Assay Result for resistance genes of *K. pneumoniae* is presented in Figures 3-5 and the frequency of genes reported to *tet A* 79.48%, *tet B* 64.10%, *sul1* 21.21%, aac (3) IIa 83.33%, qnr in the antibiotic nalidixic acid 18.18%, Norfloxacin in three isolates (20%) and ciprofloxacin 16.66% (Table 6). The Antimicrobial resistance pattern of *K. pneumoniae* isolates are presented in Table 7.

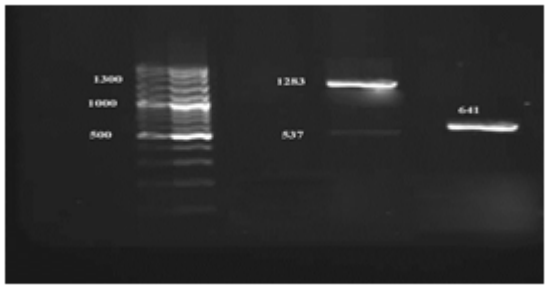

Figure 2. Result of the PCR Assay for Identification of *K. pneumoniae* Capsular K1, K2 and *rmpA*. M: DNA size ladder 100 bp (Fermentas), number 1: negative control; number 2: number 2 and 3: positive samples.

Table 6. *Distribution of antibiotic resistance genes in K. pneumoniae strains isolated.*

Gene	Antimicrobial agent	Resistance by disc	Resistance by PCR
tet A	Tetracycline	39 (43.30%)	31 (79.48%)
tet B	Tetracycline	39 (43.30%)	25 (64.10%)
Qnr	Nalidixic Acid	22 (22.40%)	4 (18.18%)
Qnr	Norfeloxacine	15 (16.70%)	3 (20%)
Qnr	Ciprofloxacin	6 (6.70%)	1 (16.16%)
sul 1	Sulfonamide	33 (36.70%)	15 (45.45%)
aac (3)lia	Gentamycine	24 (26.70%)	20 (83.33%)

Table 7. *Antimicrobial resistance pattern in K. pneumoniae isolates.*

Isolate	Number of Multidrug-Resistant	Resistance pattern
2	7	IPM / FEP / CP/ CRO / AN / GM
3	11	FM , IPM , FEP, TE , SXT, CP , CRO , CF, NOR , AN , GM
4	7	AM , FEP, K , CRO , CF, AN , GM
5	4	AM , TE , SXT, CF,
6	9	FM , AM , FEP, K , CRO , CF, NOR , AN , GM
7	6	FM / AM/ CRO / CF / AN / GM
8	9	K / FEP / FM / AM / CRO / CF / AN / GM / NOR
9	1	NA , IPM , FEP, TE , SXT, CP , K , CRO , CF, NOR , AN , GM
10	7	FM / AM / K/ CRO/ CF / GM / SXT
11	5	FM / AM / CF / TE / NOR
12	7	K / AM / CF / CRO / GM / FEP / AN
13	3	TE / AM / CF
14	8	FM , AM , FEP, TE , K , CRO , CF, NOR , AN , GM
15	2	FM , NA , IPM , FEP, SXT, CP , CRO , NOR , AN , GM
16	7	CF/K/AM/GM/CRO/FEP/AN
17	13	FM, AM , NA , IPM , FEP, TE , CP , K , CRO , CF, NOR , AN , GM
18	10	FM//CF/K/AM/GM/CRO/FEP/AN/TE/NA
19	4	AM/TE/NA/SXT
20	3	AM/FM/SXT
21	5	AM/K/SXT/TE/NA
22	8	FM/AM/SXT/TE/NA/NOR/CF/CRO
23	8	FEP/FM/AM/SXT/TE/NA/CF/CRO
24	3	K/FM/AM
25	4	K/AM/SXT/NA
26	7	K/AM/FEP/CF/CRO/GM/AN

27	7	AM/FEP/CF/CRO/FM/SXT/NA
28	7	AM/FEP/CF/CRO/FM/SXT/NA
29	4	AM/CF/SXT/TE
30	8	AM/CF/K/AN/FEP/CRO/GM/NOR
31	9	K/AM/FM/CF/AN/FEP/CRO/GM/SXT
32	8	K/AM/CF/AN/FEP/CRO/GM/SXT
33	7	K/AM/CF/AN/FEP/CRO/GM
34	9	K/AM/CF/AN/FEP/CRO/GM/TE/NOR
35	8	K/AM/CF/AN/FEP/CRO/GM/SXT
36	10	NA/K/TE/AM/CF/FEP/CRO/NOR/IPM/CP
37	9	NA/K/TE/AM/CF/CRO/NOR/CP/SXT
38	4	AM/NA/K/FM
39	4	AM/K/TE/SXT
40	4	AM/K/TE/FM
41	4	AM/FM/SXT/AN
42	3	TE/AM/FM
43	9	FM/K/AM/AN/CF/CRO/NOR/FEP/GM
44	4	AM/AN/TE/SXT
45	6	TE/CF/AM/AN/SXT/NA
46	3	FM/TE/AM
47	4	TE/AM/NA/SXT
48	8	TE/AM/SXT/K/FM/CRO/CF/FEP
49	3	AM/SXT/CF
50	4	TE/AM/FM/NA
51	8	K/CF/TE/AM/CRO/FEP/AN/GM
52	4	K/AM/AN/FM
53	11	K/AM/NA/CF/TE/CRO/FEP/SXT/IPM/CP/NOR
54	8	AN/K/AM/CF/TE/CRO/FEP/GM
55	5	FM/AM/TE/FEP/NA
56	6	CF/AM/NA/AN/CP/NOR
57	4	TE/AM/FM/SXT
58	10	K/CP/NOR/TE/AM/SXT/CF/NA/CRO/IPM
59	6	K/TE/FM/AM/SXT/NA
60	9	CF/K/TE/FM/AM/AN/FEP/CRO/GM
61	5	CF/TE/FM/AM/SXT
62	8	TE/AM/AN/K/CF/FEP/CRO/GM
63	5	AM/K/CF/FEP/CRO
64	5	AM/K/CF/CRO/SXT
65	7	K/AM/CF/CRO/FEP/AN/GM
66	5	AM/CF/CRO/TE/SXT
67	3	AM/TE/SXT
68	3	AM/FM/SXT
69	7	K/AM/CF/CRO/FEP/AN/GM
70	6	AM/CF/CRO/FEP/FM/SXT
71	4	AM/CF/SXT/TE

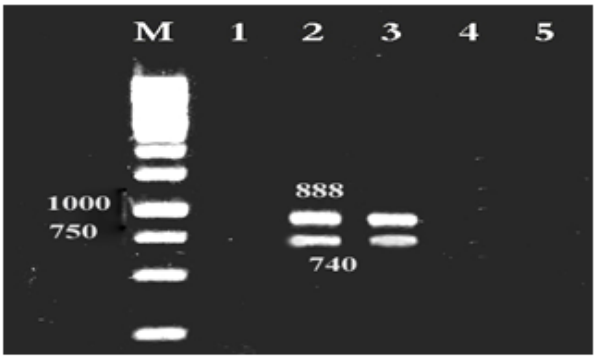

Figure 3. Result of the PCR Assay for resistance genes of *K. pneumoniae*. M: DNA size ladder 100 bp (Fermentas), number 1: negative control; number 2 and 3: positive samples and positive control; number 4 and 5: negative samples.

Prevalence of sulfonamides resistance-encoding sul genes and their relatedness to class 1 integrons

In this study 33 isolates resistance to cotrimoxazole, aren't finding *sul1* gene in 15 isolated cases, *sul2* gene in 20 isolated cases, *sul3* gene in 2 isolated cases, *sul1* and *sul2* gene in 2 isolated cases, *sul1* and *sul3* gene in 1 isolated cases and *sul1* and *sul2* and *sul3* gene in 15 isolated cases, respectively. Overall the most prevalent *sul* gene was *sul2*, found in 20/33 (60.60%) strains, followed by sul1 15/33 (45.45%) and *sul3* 2/33 (6.06%) (Table 8). Also there were 27 demonstrating *int1* genes for cotrimoxazol. The sulfonamides resistance-encoding *sul 1* genes in relation to class 1 integrons were found in 22/33 (66.66%) of the *K. pneumoniae* strains (Table 9).

Table 8. *Prevalence of sul genes in K. pneumoniae isolates resistant to sulphonamides.*

	No. of isolates with genes					
Strain Characteristics	*Sul 1*	*Sul 2*	*Sul 3*	*Sul 1* *Sul 2*	*Sul 1* *Sul 3*	*Sul 1* *Sul 2*

						Sul 3
Sulfonamide Resistance	15	20	2	2	1	-
N=33	45.45%	60. 60%	6. 06%	6. 06%	3. 03%	0%

Table 9. *Prevalence of in1 genes in K. pneumoniae isolates resistant to sulphonamides.*

	No. of isolates with genes		
Strain Characteristics	*Sul 1* + *Int 1*	*Sul 2* + *Int 1*	*Sul 3* + *Int 1*
Sulfonamide Resistance	22	3	2
N=33	66. 66%	15. 15%	6. 06%

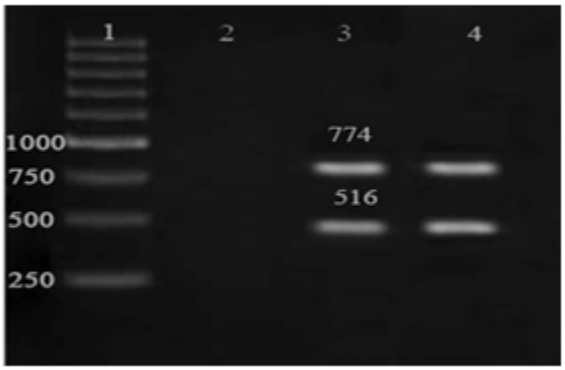

Figure 4. *Result of the PCR Assay for resistance genes of K. pneumoniae (tetB and qnr). number 1: DNA size ladder 100 bp (Fermentas), number 2: negative control; number 3 and 4: positive samples and positive control.*

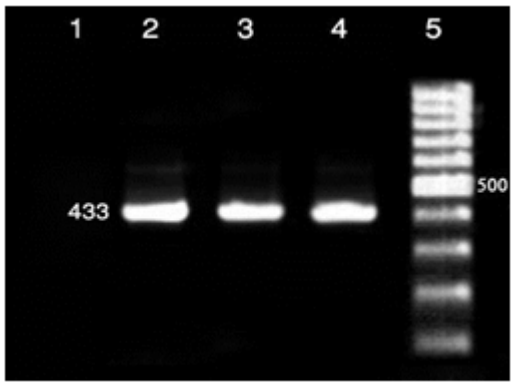

Figure 5. *Result of the PCR Assay for resistance genes of K. pneumoniae (sul1). number 1: negative control; number 2, 3 and 3: positive samples and positive control.), number 5: DNA size ladder 100 bp (Fermentas).*

Discussion

In this study, we evaluated the antibiotic resistance patterns of *K. pneumoniae* and the frequency distribution of *K. pneumoniae* genes and their relatedness with the class 1 integron in *K. pneumoniae* and *sul* genes. Integrons have become an important means of horizontal transfer of resistance genes in clinical isolates [22,35]. The present study showed that the most common *K. pneumoniae* serotype was K2 (15/90; 16.66%), followed by K1 (13/90; 14.44%). *magA* has been confirmed to be located in the cps (capsular polysaccharide synthesis) gene cluster of serotype K1 of *K. pneumoniae* and is restricted to serotype K1 isolates, regardless of their sources [36-38]. Our present data show that A total of 10 (11.11%) *K. pneumonia* isolates carried *rmpA* which is in contrary with human isolates of *K. pneumoniae*, in which the *rmpA* gene is present in both K1 and K2 capsular serotypes, as well as nearly 67% of non–K1/K2 serotypes,17 but the *magA* gene appears restricted to isolates of the K1 serotype [15]. Therefore, *magA* is a good tool for molecular typing rather than a major virulence determinant. In contrary, a study conducted in Singapore and Taiwan showed that the most common serotype was K1 (34/73; 46.6%), followed by K2 (15/73; 20.5%). *magA* was restricted to serotype K1. All K1 or K2 isolates and 66.7% (16/24) of isolates that were neither serotype K1 nor serotype K2 (non-K1/K2) carried *rmpA* [39]. In addition, another study also showed that Serotype K2 *K. pneumoniae* is the second most prevalent serotype next to serotype K1 as a cause of pyogenic liver abscess and is also frequently reported in community acquired *pneumonia* [40].

The treatment of infectious diseases is an important issue for human wellbeing and the daily increase in bacterial resistance has elevated patients' costs in recent years. In our study, markedly high resistance to Amoxicillin and Cephalothin was noticed in clinical isolates of *K. pneumoniae*. *K. pneumoniae* isolates were considerably resistant to cephalosporin has been reported from other parts of the world [41]. Our study, along with other studies, have also demonstrated that the rates of ESBLs production in our country are different from other countries such as; India (57.1%), Turkey (57%) and South Korea (30%), which showed a higher prevalence of ESBL-producing isolates [42-45]. Feizabadi et al. found that the rates of resistance for amikacin, ciprofloxacin, cefepime, ceftazidime, and cefotaxime were; 21.4%, 28%, 76% and 84.0%, respectively [46]. The comparison of our study results

with the above-mentioned study shows that antibiotic resistance to four of the previously mentioned antibiotics is higher in our study. In addition, in another study, both non-hospitalized and hospitalized isolates were more resistant to first line drugs including; ampicillin, and trimethoprim-sulfamethoxazole [45]. This result, which is comparable with other studies in developing countries, is due to the widespread use of these drugs because of their low cost and easy administration. Long hospital stay and antibiotic pressure select resistant strains which were colonized in susceptible patients [47]. In these conditions physicians have limited drug choices. High percentage of resistant to Amoxicillin, Cephalothin, Kanamycin and the other beta lactams show the high rate of beta lactam prescription. Also, aminoglycosides are used in combination therapy with beta- lactam antibiotics. Therefore, it is expected to reveal high rate of resistance to aminoglycosides as well as beta-lactams. Although, sequencing analyzes show integrated gene cassettes related to aminoglycoside resistant in most isolates, however, in total there are medium rates of resistance for aminoglycosides (gentamicin, 26.7% and amikacin 32.2%). Our findings with regard to the overall high resistance of *K. pneumoniae* strains to antibiotics such as Amoxicillin (87.8%), Cephalothin (53.3%), Kanamycin (45.5%), and others studied are in agreement with those of other recent studies [48-51]. This shows the limited possibility of using these antibiotics in the empirical treatment of patients infected with *K. pneumonia*. Usually sulfonamides resistance is encoded by the *sul1, sul2,* and *sul3* genes. We found that more than half of the *K. pneumoniae* strains possessed one or more of these *sul* genes, and in 60.60% of these strains, sulfonamides resistance occurred. This result is in line with others done among *E. coli* strains, the *sul2* gene has been found to be predominant in *E. coli* strains isolated in UTI episodes [32,52]. In our study, sulfonamides treatment was associated with the occurrence of *sul* genes and with increased phenotypic resistance to sulfonamides. Horizontal gene transfer has been associated with escalated SXT resistance among Enterobacteriaceae [52,53]. The remarkable stability of resistance markers, such as phenotypic resistance patterns and sul genes, among *K. pneumoniae* strains may be a helpful tool for the preliminary differentiation between relapse and reinfection.

The present study characterized class 1 integrons and their gene cassettes in *K. pneumoniae* isolates collected from clinical patients. In this study, we observed lower class 1 integron prevalence in *K. pneumoniae* (66.66%) compared to the previously reported frequencies of 92% in India [54], 93.2% in Shan Dong, China [55], 73% in Australia [56], and 70% in the United States [25]. The class 1 integron was highly prevalent in *K. pneumoniae* (66.66%) and was strongly associated with the *sul1* genes, which was similar in other literature [57]. Thus, class 1 integrons with various gene cassette arrays in association with *sul1* genes were highly prevalent in Enterobacteriaceae, and the variation of the gene cassettes in class 1 integrons may reflect the horizontal transfer of integrons among members of the Enterobacteriaceae family [57]. On the other hand, previous study conducted in Iran showed that Class 1 integrons were more frequent among *K. pneumoniae* isolates in comparison with class 2. Five different resistance gene arrays were also identified among class 1 integrons. Dihydrofolate reductase (dfrA) and aminoglycoside adenyltransferase (aad) gene cassettes were found to be predominant in the class 1 integrons [58].

In conclusion, we report the first extensive study regarding the distribution and antimicrobial resistant profile of *K. pneumoniae* and *sul* genes and the prevalence of sulfonamides resistance-encoding *sul* genes and their Relatedness to Class 1 Integrons among *K. pneumoniae* isolates in Iran. The study has revealed that serotype K1 is one of the most important serotypes of *K. pneumonia*. Also there seems to be a strong relationship between presence of Integron and increased resistance to different antibiotics. In this study serotype K1 or K2 is the major virulence determinant for *K. pneumoniae*. Majority of the isolates are resistance to Amoxicillin and Cephalothin. In addition, resistance to sulfonamides in *K. pneumoniae* was explained by the acquisition of sul1, sul2, and sul3 genes. There is also high rate of antibacterial resistance in *K. pneumoniae* and diverse integrated gene cassettes related to class 1 integrons. In most of the cases, class 1 integrons with various multi-gene cassette arrays in association with sul1 genes were widely disseminated in *K. pneumoniae* so that, there is a strong relationship between presence of class 1 Integron and increased resistance to sulfonamides antibiotics. The wide distribution of integrons in the *K. pneumoniae* isolates and sul genes may be because of the horizontal transfer of antibiotic resistance gene and might become a serious threat to the search for effective antimicrobial therapy in the future. The results of this study reinforce the need for increasing concern for therapy for clinical infections caused by *K. pneumoniae* isolates having resistance-encoding sul genes in relation to class 1 integrons. Therefore, continued monitoring of antimicrobial resistance, the adoption of prudent use of antimicrobial agents and the establishment of a surveillance system is urgently needed to prevent further dissemination in Iran.

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