

Molecular characterization, serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the *Klebsiella pneumoniae* strains isolated from different types of hospital-acquired infections

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Reza Ranjbar¹
Ali Fatahian Kelishadroki²
Mohammad Chehelgerdi²

¹Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran; ²Biotechnology Research Center, Shahr-e Kord Branch, Islamic Azad University, Shahr-e Kord, Iran

Purpose: Virulent and resistant *Klebsiella pneumoniae* strains are considered as one of the most significant causes of hospital-acquired infections. The present investigation was done to study the distribution of virulence factors, capsule serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the *K. pneumoniae* strains isolated from hospital-acquired infections.

Patients Materials and methods: Two hundred and sixty different types of hospital-acquired infections were collected and cultured. Antibiotic resistance pattern of *K. pneumoniae* isolates and their molecular characterization were studied using disk diffusion and PCR, respectively.

Results: One hundred and fifty out of 260 (44.22%) hospital-acquired infections harbored *K. pneumoniae*. Urine samples (63.75%) had the highest prevalence of *K. pneumoniae*, while wound (33.33%) had the lowest. *K. pneumoniae* strains harbored the highest prevalence of resistance against ampicillin (100%), cefuroxime (100%), amoxicillin/clavulanic acid (95.65%) and ceftazidime (95.52%). *FimH-1* (93.04%), *traT* (92.17%), *mrkD* (84.34%), and *entB* (80.86%) were the most commonly detected virulence genes. *AcrAB* (96.52%) and *tolC* (85.21%) were the most commonly detected antibiotic resistance genes. Prevalence of *ompK35* and *ompK36* virulence genes were 75.65% and 79.13%, respectively. Prevalence of K1 and K2-positive serotypes were 27.82% and 6.96%, respectively.

Conclusions: High prevalence of resistance against several types of antibiotics and simultaneous presence of some virulence factors and multi-drug resistance genes pose an important public health issue.

Keywords: *Klebsiella pneumoniae*, antibiotic resistance pattern, antibiotic resistance genes, capsule serotypes, virulence genes, hospital infections

Introduction

Hospital-acquired infections including urinary tract infections (UTIs), respiratory, skin and soft tissue, blood, gastrointestinal and finally reproductive infections are considered as the most important causes of hospitalization. Hospital-acquired infections are estimated for around 10–60% of hospitalizations and more than

Correspondence: Reza Ranjbar
Molecular Biology Research Center,
Systems Biology and Poisonings Institute,
Baqiyatallah University of Medical
Sciences, Tehran, Iran
Email Ranjbar@bmsu.ac.ir;
Ranjbarre@gmail.com

800,000 deaths annually in the USA.^{1,2} The presence of multi-drug resistant pathogenic bacteria has increased the impact of hospital-acquired infections.^{1,2}

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, and rod shape bacterium belonging to the Enterobacteriaceae family.^{3,4} *K. pneumoniae* is also an important pathogen in nosocomial infections such as pneumonia, bacteremia, UTIs, pyogenic liver abscesses, wounds and burns infections around the world.³ Furthermore, *K. pneumoniae* infections are difficult to treat because of the presence of certain virulence factors and also the emergence of antibiotic resistance.³⁻⁶

Pathogenicity of *K. pneumoniae* is due to the presence of certain virulence genes which encode specific virulence factors that allow the bacterium to attack the immune system and cause many kinds of diseases.^{3,5,6} A set of virulence factors contributing to virulence and pathogenicity in *K. pneumoniae* strains, including the capsular serotype (particularly capsule serotypes K1 and K2), lipopolysaccharide, iron-scavenging systems, and adhesions, have been introduced in the *K. pneumoniae* strains isolated from hospital-acquired infections. Iron acquisition systems are essential for the growth of pathogenic bacteria. Furthermore, the iron chelator siderophore allows bacteria to take up protein-bound iron from the host cells.^{6,7} Virulence-associated genes include those encoding regulators of mucoid phenotype A (*rmpA*), type 1 and type 3 adhesins (*fimH-1*, *mrkD*), aerobactin (iron siderophore) synthase (*iucC*), bacteriocin biosynthesis [enterobactin (*entB*), and yersiniabactin (*irP-1*)], and serum resistance-associated outer membrane lipoprotein (*traT*) have a predominant role in pathogenicity of *K. pneumoniae* strains isolated from hospital-acquired infections.^{6,8} The above-mentioned virulence factors have an essential role in adhesion and invasion of *K. pneumoniae* strains to host tissues.

K. pneumoniae strains isolated from hospital-acquired infections have emerging antibiotic resistance. Epidemiological investigations revealed that *K. pneumoniae* strains isolated from hospital-acquired infections harbored a high prevalence of resistance against commonly used groups of antibiotics, especially penicillins, aminoglycosides, tetracyclines, macrolides, lincosamides, folate inhibitors, fluoroquinolones, and phenicolis.^{9,10} Gram-negative bacteria like *K. pneumoniae* have developed several mechanisms of resistance against commonly used antimicrobials. The presence of certain

antibiotic resistance genes is one of the most important causes of the occurrence of antibiotic resistance. Genes coding for the multi-drug efflux pump system *AcrAB-TolC* and *MdtK* and porin coding genes (*OmpK35* and *OmpK36*) have high clinical importance in the occurrence of antibiotic resistance in *K. pneumoniae* strains.^{9,10} These genes predominantly cause the occurrence of resistance against several kinds of antibiotic groups. Thus, it is important to detect antibiotic resistance genes in the *K. pneumoniae* strains isolated from hospital-acquired infections.

Data on the prevalence, antibiotic resistance properties and distribution of virulence genes of *K. pneumoniae* strains are scarce in Iran. Therefore, the current research was done to assess the prevalence rate, distribution of virulence genes and phenotypic and genotypic evolution of antibiotic resistance of *K. pneumoniae* strains isolated from different types of hospital-acquired infections.

Materials and methods

Samples

From April to October 2017, a total of 260 hospital-acquired infections including urine (n=80), wound (n=45), blood (n=64) and sputum (n=71) samples were randomly collected from hospitalized patients. All specimens were collected from hospitalized patients of Shohada Lenjan, Imam Khomeini Falavarjan, Sadooghi and Al-Zahra hospitals of Isfahan province, Iran. Midstream urine was collected in a sterile condition to decrease potential bacterial, cellular and artifactual contamination. Samples were immediately transferred to the laboratory in coolers with ice-packs.

Bacterial isolation

All samples were immediately streaked onto MacConkey Agar (MCA, Merck, Germany) and incubated at 37°C for 24 h. Bacterial colonies were streaked again on Brilliant Green Agar (BGA, Merck) and xylose lysine deoxycholate agar (XLD, Merck) and incubated at 37°C for 24 h. Colonies were streaked again on HiCrome UTI agar (HiMedia Laboratories, Mumbai, India). All isolates were subjected to standard confirmatory tests, which included oxidase and catalase, growth on SIM (sulfide, indole, motility), Simon citrate, MR-VP (methyl red – Voges Proskauer), lysine iron agar, Kligler agar, phenylalanine agar, urea agar, malonate, blood agar, and MacConkey agar. *K. pneumoniae* isolates were stored in tryptic soy broth (TSB, Merck) containing 20% glycerol at -70°C for further characterization.

K. pneumoniae strains were sub-cultured overnight in Luria-Bertani broth (Merck) and genomic DNA was extracted from typical colonies of *K. pneumoniae* using DNA extraction kit (Fermentas, Leon-Rot, Germany) according to the manufacturer's instruction. All of the positive colonies were confirmed another time using PCR-based amplification of the *16SrRNA* gene. PCR was performed according to the method described previously.¹¹

Antibiotic susceptibility pattern

Antibiotic susceptibility pattern of *K. pneumoniae* isolates was performed according to the Kirby-Bauer disk diffusion method.¹² Mueller-Hinton agar (Merck) medium was used for this purpose. Principles of the Clinical and Laboratory Standards Institute (CLSI) were used for this purpose.¹² Antibiotic resistance pattern of the *K. pneumoniae* strains was assessed against 15 commonly used antibiotic agents including amoxicillin/clavulanic acid (20/10 µg), ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), piperacillin/tazobactam (110/10 µg), ceftazidime (30 µg), cefuroxime (30 µg), aztreonam (30 µg), imipenem (10 µg), tobramycin (10 µg), amikacin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), and co-trimoxazole (30 µg) (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, UK). All of the inoculated plates were aerobically incubated at 37°C for 18–24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by the CLSI (2014).¹² *K. pneumoniae* ATCC 4352 was used as a quality control organism.

PCR amplification of antibiotic resistance genes and virulence markers

The frequency of *rmpA*, *fimH-1*, *mrkD*, *traT*, *entB*, *Irp-1*, and *IucC* virulence genes, *OmpK35*, *OmpK36*, *mdtK*, *tolC* and *AcrAB* antibiotic resistance genes and K1 and K2 capsule serotypes were assessed using PCR.¹³ A set of primers introduced in a previous study were used for this purpose.¹³ A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. PCR grade water and *K. pneumoniae* standard strains (ATCC 18883, ATCC 43816, ATCC 4352 and ATCC 10031) were used as negative and positive controls, respectively. Ten microliters of PCR product were exposed to electrophoresis in a 2% agarose gel in 1× TBE buffer at 80 V for 30 min, stained with SYBR Green. The UVI doc

gel documentation systems (Grade GB004, Jencons PLC, London, UK) was applied for analysis of images.

Statistical analysis

Statistics were subjected to Microsoft Office Excel (version 15; Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed by means of the SPSS 21.0 statistical software (IBM Corporation, Armonk, NY, USA). The chi-square test and Fisher's exact two-tailed test were applied to measure any significant relationship. *P*-value <0.05 was considered as the statistically significant level.

Ethics statement

The study was approved by the Ethical Council of Research of the Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. (Consent Ref Number BMSU-2017). Verification of this research project and the licenses related to the sampling process were approved by the Prof Reza Ranjbar (Approval Ref Number 2017/45). All clinical samples were taken from volunteer hospitalized patients. Written informed consent was also obtained from all of the study patients or their parents.

Results

Table 1 characterizes the prevalence of *K. pneumoniae* strains in different types of hospital-acquired infections. One hundred and fifteen of 260 (44.22%) hospital-acquired infections harbored *K. pneumoniae*. All positive strains were also approved using the *16SrRNA*-based PCR amplification. Urine samples (63.75%) had the highest prevalence of *K. pneumoniae*, while wound (33.33%) had the lowest. Statistically significant difference was seen between types of samples and prevalence of *K. pneumoniae* (*P*<0.05).

Table 2 characterizes the antibiotic resistance pattern of *K. pneumoniae* strains isolated from different types of

Table 1 Prevalence of *K. pneumoniae* strains isolated from different types of hospital-acquired infections

Type of samples	No. samples collected	Prevalence of <i>K. pneumoniae</i> (%)
Urine	80	51 (63.75)
Wound	45	15 (33.33)
Blood	64	24 (37.50)
Sputum	71	25 (35.21)
Total	260	115 (44.22)

Abbreviations: *K. pneumoniae*, *Kelebsiella pneumoniae*; NO, number.

Table 2 Antibiotic resistance pattern of *K. pneumoniae* strains isolated from different types of hospital-acquired infections

Type of samples (No. <i>K. pneumoniae</i> strains)	Antibiotic resistance pattern (%)														
	Amp*	Amc	Pit	Cfz	Cfr	Az	Imp	Amk	T30	G10	Tob	Cip	Nal	Cot	C30
Urine (51)	51 (100)	51 (100)	47 (92.15)	47 (92.15)	51 (100)	35 (68.62)	27 (52.94)	24 (47.05)	24 (47.05)	39 (76.47)	39 (76.47)	43 (84.31)	47 (92.15)	43 (84.3)	27 (52.94)
Wound (15)	15 (100)	15 (100)	11 (73.33)	15 (100)	15 (100)	7 (46.66)	8 (53.33)	4 (26.66)	4 (26.66)	15 (100)	15 (100)	15 (100)	11 (73.33)	7 (46.66)	3 (20)
Blood (24)	24 (100)	24 (100)	24 (100)	24 (100)	24 (100)	14 (58.33)	24 (100)	19 (79.16)	19 (79.16)	24 (100)	24 (100)	24 (100)	19 (79.16)	19 (79.16)	10 (41.66)
Sputum (25)	25 (100)	20 (80)	15 (60)	25 (100)	25 (100)	25 (100)	10 (40)	15 (60)	15 (60)	15 (60)	15 (60)	10 (40)	15 (60)	20 (80)	10 (40)
Total (115)	115 (100)	110 (95.65)	97 (84.34)	111 (96.52)	115 (100)	86 (74.78)	69 (60)	62 (53.91)	62 (53.91)	83 (72.17)	93 (80.86)	92 (80)	92 (80)	89 (77.39)	50 (43.47)

Abbreviations: Amp, ampicillin (10 µg); Amc, amoxicillin/clavulanic acid (20/10 µg); Pit, piperacillin/tazobactam (110/10 µg); Cfz, ceftazidime (30 µg); Cfr, cefuroxime (30 µg); Az, aztreonam (30 µg); Imp, imipenem (10 µg); Amk, amikacin (30 µg); T30, tetracycline (30 µg); G10, gentamicin (10 µg); Tob, tobramycin (10 µg); Cip, ciprofloxacin (5 µg); Nal, nalidixic acid (30 µg); Cot, co-trimoxazole (30 µg); C30, chloramphenicol (30 µg); K, *penumonia*, *Klebsiella pneumoniae*.

Table 3 Distribution of virulence genes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections

Type of samples (No. <i>K. pneumoniae</i> strains)	Distribution of virulence genes (%)						
	<i>rmpA</i>	<i>fimH-I</i>	<i>mrkD</i>	<i>traT</i>	<i>entB</i>	<i>jrpl</i>	<i>iuCc</i>
Urine (51)	12 (23.52)	43 (84.31)	47 (92.15)	47 (92.15)	47 (92.15)	8 (15.68)	12 (23.52)
Wound (15)	4 (26.66)	15 (100)	11 (73.33)	15 (100)	7 (46.66)	4 (26.66)	7 46.66()
Blood (24)	–	24 (100)	24 (100)	24 (100)	24 (100)	14 (58.33)	10 (29.41)
Sputum (25)	–	25 (100)	15 (60)	20 (80)	15 (60)	10 (40)	10 (40)
Total (115)	16 (13.91)	107 (93.04)	97 (84.34)	106 (92.17)	93 (80.86)	36 (31.30)	39 (33.91)

Abbreviation: *K. pneumoniae*, *Klebsiella pneumoniae*.

hospital-acquired infections. *K. pneumoniae* strains harbored the highest prevalence of resistance against ampicillin (100%), cefuroxime (100%), amoxicillin/clavulanic acid (95.65%), ceftazidime (95.52%), piperacillin/tazobactam (84.34%), tobramycin (80.80%), ciprofloxacin (80%), nalidixic acid (80%), co-trimoxazole (77.39%) and aztreonam (74.78%) antibiotic agents. *K. pneumoniae* strains harbored the lowest prevalence of resistance against chloramphenicol (43.47%), tetracycline (53.91%) and amikacin (56.52%). Statistically significant difference was seen between types of samples and the prevalence of antibiotic resistance ($P<0.05$).

Table 3 characterizes the distribution of virulence genes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections. *FimH-I* (93.04%), *traT* (92.17%), *mrkD* (84.34%), and *entB* (80.86%) were the most commonly detected virulence genes amongst the *K. pneumoniae* strains. Prevalence of *rmpA* (13.91%) and *jrpl* (31.30%) was lower than other detected virulence genes. Statistically significant difference was seen between types of samples and the prevalence of virulence genes ($P<0.05$).

Table 4 characterizes the distribution of antibiotic resistance genes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections. *AcrAB*

(96.52%) and *tolC* (85.21%) were the most commonly detected antibiotic resistance genes of the *K. pneumoniae* strains isolated from different types of hospital-acquired infections. *MdtK* (29.54%) had the lowest prevalence amongst all detected antibiotic resistance genes. Prevalence of *ompK35* and *ompK36* virulence genes were 75.65% and 79.13%, respectively. Statistically significant difference was seen between types of samples and the prevalence of antibiotic resistance genes ($P<0.05$).

Table 5 characterizes the distribution of capsule serotypes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections. Prevalence of K1 and K2-positive serotypes of the *K. pneumoniae* strains isolated from different types of hospital-acquired infections were 27.82% and 6.96%, respectively. Serotypes of 65.21% of the studied *K. pneumoniae* strains were not related to K1 and K2 serotypes.

Discussion

K. pneumoniae is an important cause of multidrug-resistant infections worldwide.^{13,14} Epidemiological studies have highlighted the emergence of multidrug-resistant and virulent *K. pneumoniae* strains isolated from hospital-acquired infections.^{13,14} Antibiotic resistant and virulent bacteria caused more severe diseases for longer periods of time.

Table 4 Distribution of antibiotic resistance genes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections

Type of samples (No. <i>K. pneumoniae</i> strains)	Distribution of antibiotic resistance genes (%)				
	<i>ompK35</i>	<i>ompK36</i>	<i>mdtK</i>	<i>tolC</i>	<i>acrAB</i>
Urine (51)	43 (84.31)	47 (92.15)	8 (15.68)	43 (84.31)	51 (100)
Wound (15)	15 (100)	15 (100)	11 (73.33)	11 (73.33)	11 (73.33)
Blood (24)	19 (79.16)	15 (62.50)	10 (41.66)	24 (100)	24 (100)
Sputum (25)	10 (40)	15 (60)	5 (20)	20 (80)	25 (100)
Total (115)	87 (75.65)	91 (79.13)	34 (29.54)	98 (85.21)	111 (96.52)

Abbreviation: *K. pneumoniae*, *Klebsiella pneumoniae*.

Table 5 Distribution of capsule serotypes amongst the *K. pneumoniae* strains isolated from different types of hospital-acquired infections

Type of samples (No. <i>K. pneumoniae</i> strains)	Distribution of antibiotic resistance genes (%)		
	K1	K2	Non K1/K2
Urine (51)	12 (23.52)	4 (7.84)	35 (68.62)
Wound (15)	–	4 (26.66)	11 (73.33)
Blood (24)	10 (41.66)	–	14 (58.33)
Sputum (25)	10 (40)	–	15 (60)
Total (115)	32 (27.82)	8 (6.95)	75 (65.21)

Abbreviation: *K. pneumoniae*, *Klebsiella pneumoniae*.

The present research was done to assess the prevalence, distribution of virulence genes and genotypic and phenotypic analyses of antibiotic resistance amongst the *K. pneumoniae* strains isolated from hospital-acquired infections. Prevalence of *K. pneumoniae* in different types of human hospital infections was 44.22%. Furthermore, the prevalence of *K. pneumoniae* strains in urine, wound, blood, and sputum samples were 63.75%, 33.33%, 37.50%, and 35.21%, respectively. Lev et al¹⁵ reported that the total prevalence of *K. pneumoniae* in hospital-acquired infections in hospitals of Moscow was 16.66%. They characterized that prevalence of *K. pneumoniae* in respiratory, urine, wound, cerebrospinal fluid, blood, and rectal swab specimens were 57%, 30%, 5%, 4%, 3%, and 1%, respectively. Akter et al¹⁶ reported that prevalence of *K. pneumoniae* strains in hospital-acquired infections of Bangladesh hospitals was 19.72%. They characterized that the most prevalent infection caused by *K. pneumoniae* isolates was UTIs (27–36%), followed by swab (8–10%) or pus (3–6%). Similar results were found earlier by Riaz et al (Pakistan),¹⁷ Lina et al (Bangladesh),¹⁸ Sarathbabu et al (India),¹⁹ and Heidary et al (Iran).²⁰ Moreover, higher prevalence of *K. pneumoniae* strains in UTIs has been reported from North Africa,²¹ Nepal,²² USA,²³ and Taiwan.²⁴

We found that *K. pneumoniae* strains harbored the highest prevalence of resistance against ampicillin, cefuroxime, amoxicillin/clavulanic acid, ceftazidime, piperacillin/tazobactam, tobramycin, ciprofloxacin, nalidixic acid, co-trimoxazole, and aztreonam antibiotic agents. High prevalence of antibiotic-resistant *K. pneumoniae* strains was also reported from the cases of hospital-acquired infections in Canada,²⁵ Nepal,²⁵ Australia,²⁶ China²⁷, and Pakistan.²⁸ Higher fatality rate was also reported for infections caused by antibiotic-resistant *K. pneumoniae*.²⁹ Prevalence of antibiotic-resistant *K. pneumoniae* strains in Asian and

Latin American countries had a range between 40% and 55%.^{30,31} In a study conducted in Saudi Arabia,³² *K. pneumoniae* strains isolated from hospital-acquired infections exhibited high prevalence of resistance against ceftriaxone (58.93%), meropenem (5.60%), gentamicin (11.10%), piperacillin (11.10%), amikacin (6.70%), and ciprofloxacin (6.70%) antibiotic agents, which was similar to our findings. Mekki et al³³ reported the higher prevalence of resistance of *K. pneumoniae* strains against cotrimoxazole (100%), gentamicin (100%), nalidixic acid (100%), cefuroxime (100%), ciprofloxacin (97.37%), nitrofurantoin (97.37%), and amikacin (39.47%). Seibert et al³⁴ reported that *K. pneumoniae* strains isolated from hospital infections harbored the high prevalence of resistance against amikacin (8.50%), gentamicin (42.60%), ceftriaxone (66%), cefepime (55.60%), imipenem (80%), and meropenem (83%).

K. pneumoniae strains isolated from the clinical samples of the present study harbored high prevalence of *fimH-1*, *traT*, *mrkD*, *entB*, *rmpA*, and *jrp1* virulence genes. This phenomenon characterizes the high pathogenicity of *K. pneumoniae* strains. These virulence genes were also predominant in the *K. pneumoniae* strains isolated from hospital-acquired infections in China,³⁵ UK,³⁶ France,³⁷ and Brazil.³⁸ Type 1 fimbriae is the most frequent adhesive factor in *K. pneumoniae* strains. Presence of this gene can lead to UTIs. Type 1 fimbrial adhesion can also arbitrate the binding of *K. pneumoniae* strains to tissue cells of the urinary and respiratory tracts. *MrkD* protein is a vital factor in binding bacterial pathogens to the collagen molecules of the mammalian cells.¹³ The majority of *K. pneumoniae* clinical isolates usually express type 1 fimbrial adhesins.¹³ In the current investigation, the two genes coding for these adhesive structures (*fimH-1* and *MrkD*) were detected in all types of

infections. The plasmidic *traT* gene encodes an outer membrane protein involved in bacterial conjugation and blocks the complement-mediated cascade, and act as an invasion.¹³ We noticed the *traT* gene in 92.17% of *K. pneumoniae* isolates. The incidence of *traT* gene in our isolates was relatively high as it was frequently associated with the K1 capsule serotype.¹³ The majority of Enterobacteriaceae strains contain genes encoding iron uptake systems, including aerobactin or enterochelin. These siderophores have double roles as they can also prevent T cell proliferation in addition to their role in enhancing iron uptake.^{39–41} The iron siderophores aerobactin synthase gene (*IucC*), enterobactin biosynthesis gene (*entB*), and yersinibactin biosynthesis gene (*Irp-1*) were detected in 33.91%, 31.30%, and 80.86% of the *K. pneumoniae* isolates, respectively. Highly pathogenic *Yersinia* strains have high-pathogenicity island (HPI) that comprise the gene *Irp-1*. This HPI is also predominant in *Klebsiella* and other enterobacteria, including *K. pneumoniae*, *K. oxytoca*, *E. coli*, *Citrobacter* species, and *Enterobacter* species.^{39–41}

The capsular serotypes K1 and K2 are associated with the main virulent strains of *K. pneumoniae*.⁴² Feizabadi et al have shown that K1 and K2 serotypes were detected in 11.2% and 14.6% of *K. pneumoniae* strains isolated from hospital-acquired infections, respectively.⁴³ K1 and K2 serotypes were detected in 27.82% and 6.96% of the *K. pneumoniae* isolates of our study, respectively. Higher distribution of the K1 capsular serotype was also reported from *K. pneumoniae* strains isolated from hospital-acquired infections in Japan⁴⁴ and Australia.⁴⁵

The final section of our research was conducted on the distribution of some of the most important antibiotic resistance genes of the *K. pneumoniae* strains. *AcrAB*, *tolC*, *MdtK*, *ompK35*, and *ompK36* were predominant amongst the *K. pneumoniae* strains isolated from hospital-acquired infections. Antibiotic efflux pumps act as one of the most important mechanisms of antimicrobial resistance in *K. pneumoniae*.^{46–48} The increased efflux of the antimicrobial agent leads to the reduction of its intracellular concentration, which can increase bacterial survival. The *AcrAB* efflux pump was more frequent than *mdtK*. The presence of the efflux pump system (*AcrAB-TolC*) was significantly associated with the antibiotic resistance pattern.^{46–48} Furthermore, most of the *K. pneumoniae* strains were missing either the *AcrAB* efflux pump or the *tolC* outer membrane protein or both. It has been reported that loss of *ompK35* and *ompK36* led to an increase in

carbapenem, ciprofloxacin, and chloramphenicol resistance.^{46–48} Similarly, in our study, resistant *K. pneumoniae* strains harbored high distribution of *ompK35* and *ompK36* resistance genes. Both *OmpK35* and *OmpK36* also play a role in *K. pneumoniae* virulence and infection.^{46–48} Deletion of *OmpK36* and *OmpK35* can lead to the reduction in virulence of highly virulent strains and can increase their susceptibility to neutrophil phagocytosis. In our investigation, both *OmpK35* and *OmpK36* genes were simultaneously detected in the majority of *K. pneumoniae* isolates and especially those of urine and wound samples. Their distributions were variable though in sputum and blood samples. A direct correlation between efflux pumps and virulence of pathogenic bacteria was reported by Padilla et al (2010).⁴⁹ Several genes essential for intracellular invasion and survival were downregulated in mutant strains lacking *acrAB-tolC* efflux pumps.^{46–49}

Conclusion

We identified a high prevalence of *K. pneumoniae* strains with a considerable distribution of putative virulence factors, antibiotic resistance genes, and capsular serotypes, and a high prevalence of antibiotic resistance of the *K. pneumoniae* strains isolated from different hospital-acquired infections. *K. pneumoniae* strains exhibit high prevalence of resistance against ampicillin, cefuroxime, amoxicillin/clavulanic acid, ceftazidime, piperacillin/tazobactam, tobramycin, ciprofloxacin, nalidixic acid, co-trimoxazole, and aztreonam antibiotic agents, high distribution of *fimH-1* (93.04%), *traT* (92.17%), *mrkD* (84.34%), and *entB* (80.86%) virulence genes, considerable prevalence of *acrAB*, *tolC*, *mdtK*, *ompK35* and *ompK36* antibiotic resistance genes, and finally significant incidence of K1 capsular serotype. High prevalence of resistance against several types of antibiotic agents and the simultaneous presence of some virulence factors and resistance genes pose an important public health issue regarding the worse condition of hospital infections. However, further studies are required to find other epidemiological aspects of the *K. pneumoniae* strains and a correlation between virulence genes and antibiotic resistance properties of the *K. pneumoniae* strains.

Abbreviations

Amp, ampicillin; AMX, amoxicillin; rmpA, regulators of mucoid phenotype A; IucC, aerobactin (iron siderophore) synthase; entB, enterobactin; irp-1, yersinibactin; traT, serum resistance-associated outer membrane lipoprotein;

CLR, clarithromycin; CLSI, Clinical Laboratory Standards Institute.

Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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