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Distribution of Virulence-Associated and Aminoglycoside Resistance Genes Among Clinical Isolates of *Klebsiella pneumoniae* in the Southeast of Iran, During 2019–2023: A Cross-Sectional Study

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ABSTRACT

Background and Aims: *Klebsiella pneumoniae* (*K. pneumoniae*), included in the World Health Organization's list of critical priority pathogens, is considered a serious threat to public health. The present study aims to investigate the prevalence of virulence-associated and aminoglycoside resistance genes in clinical isolates of *K. pneumoniae*.

Methods: This cross-sectional study was carried out on 88 clinical isolates of *K. pneumoniae* collected from patients at Zabol hospital, Iran. Isolates were identified using conventional microbiology tests and polymerase chain reaction (PCR). Antibiotic susceptibility patterns were ascertained by the disc diffusion method. The prevalence of virulence-associated genes (*K1, K2, K5, iucA*, and *peg-344*) and aminoglycoside resistance (AME) genes (*aac (2′)-Ia*, *aac (3)-IIa*, *aac (3)-Ib*, *aac (6′)-1b*, *ant (2″)-Ia*, and *aph (3″)-Ib*) was investigated by PCR.

Results: The isolates were mostly resistant to kanamycin (73.8%) and streptomycin (69.3%). The most predominant virulence gene was *iucA*, observed in 89.8% of isolates, followed by *peg-344* 55.7% and *K5* 14.8%. The most prevalent resistance gene was *aph (3″)-Ib*, which was detected in 35.2% of isolates, followed by *ant (2″)-Ia* 22.7% and *aac (3)-Ib* 17%. In addition, sixteen different patterns of AME genes were observed.

Conclusion: Most investigated isolates of *K. pneumoniae* were positive for different virulence-associated and AME genes and therefore can play a significant role in life-threatening infections. Meanwhile, resistance rates to aminoglycoside antibiotics were high and it was primarily due to the presence of AME genes such as *aph (3″)-Ib*, ant *(2″)-Ia*, and *aac (3)-Ib*.

1 | Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a significant member of the *Enterobacteriaceae* family that can cause different kinds of infections in humans, including pneumonia, urinary tract infection, liver abscess, meningitis, endocarditis, and wound infection [1].

K. pneumoniae is a well-known member of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. (ESKAPE) pathogens. Over the recent decades, growing concern regarding infection burden associated with ESKAPE pathogens has been reported worldwide, especially in developing countries [2–4]. The ESKAPE pathogens, *K. pneumoniae* in

particular, are usually multi-drug resistant (MDR), displaying resistance to aminoglycosides, beta-lactams, and fluoroquinolones antibiotics [5].

In Germany (Central Europe), *K. pneumoniae* has developed resistance to the vast majority of antibiotics, including cephalosporins, carbapenems, and aminoglycosides, in the past two decades. Moreover, extended-spectrum beta-lactamase (ESBL)-producing, as well as *aac (6′)-I* and *aph (2″)* positive *K. pneumoniae* have been reported in different federal states of Germany [4]. Likewise, in Tanzania (East Africa), MDR *K. pneumoniae* was reported to be the major Gram-negative ESKAPE pathogen responsible for bloodstream infections (BSI) in hospitalized patients [2].

Usually, high mortality rates of *K. pneumoniae* infection are attributed to the presence of virulence-associated genes [6]. To accomplish infection, *K. pneumoniae* must successfully conquer different barriers of the host immune system. To achieve this, *K. pneumoniae* uses different virulence-associated genes, including, but not limited to, pili (type 1 and 3) that promote bacterial adhesion to epithelial cells and capsules (inhibit phagocytosis and hamper lysis by complement and antimicrobial peptides [1, 6].

Two different circulating pathotypes of *K. pneumoniae* have been reported in the world including classical *K. pneumoniae* (cKP) and hypervirulent *K. pneumoniae* (hvKp). These two pathotypes differ in their virulence-associated genes, antibiotic resistance patterns, and epidemiology. Infections of hvKp are often associated with higher mortality rates than cKP. Noteworthy, hvKp is recognized by some biomarkers (virulence-associated genes) such as *iucA* (siderophore biosynthesis) *and Peg-344* (metabolite transporter) [7, 8].

Aminoglycosides such as gentamicin and amikacin are a class of antibiotics that in combination with carbapenems (imipenem and meropenem) are usually used to treat infections caused by multi-drug resistant *K. pneumoniae*. However, various antibiotic resistance mechanisms including aminoglycoside nucleotide transferase enzymes (ANT), aminoglycoside phosphotransferase enzymes (APH), and aminoglycoside acetyltransferase enzymes (AAC), as well as mutations in the target site and efflux pumps overexpression have reduced the effectiveness of these antibiotics [9, 10].

Given the substantial impact of *K. pneumoniae* on poor patients' outcomes this study aimed to investigate the prevalence of virulence-associated genes and aminoglycoside resistance (AME) genes in clinical isolates of *K. pneumoniae*.

2 | Materials and Methods

2.1 | Isolation of *K. pneumoniae*

This cross-sectional study was carried out on 88 clinical isolates of *K. pneumoniae* collected from patients at Zabol hospital (31°05′32.40″ N 61°32′22.79″ E), the only referral hospital in the region with 263 approved beds, providing healthcare services for around four hundred thousand people, Iran, during 2019 to 2023. This study was approved by the Ethics Committee of ZBMU (IR. ZBMU. REC.1402.015).

The primary identification of isolates was performed based on conventional microbiology tests including growth on MacConkey agar (HiMedia), Gram staining, oxidase, catalase, Methyl red, Indole, Voges Proskauer, and citrate, as well as hydrogen Sulfide (H2S) production and motility on SIM medium (HiMedia) [11]. Finally, the confirmation of isolates was accomplished by Polymerase Chain Reaction (PCR) and previously explained procedures [10]. The PCR was performed by species-specific primers amplifying internal transcribed spacer (16s-23-s rRNA) region [10]. Each PCR reaction mixture was prepared by using 12.5 μL 2× ready-to-use master mix (Ampliqon, Denmark), 1.5 μL of forward and reverse primers (100 pmol), 2 μL of extracted DNA template, and 7.5 μL nuclease-free sterile distilled water.

2.2 | Virulence-Associated Genes Detection

Based on primer sequences and PCR conditions shown in Table 1 following virulence-associated genes were detected, capsular serotypes 1, 2, and 5 (*K1*, *K2*, and *K5*), aerobactin siderophore biosynthesis (*iucA*) and metabolite transporter (*peg-344*) [7]. For this purpose, genomic DNA was extracted using the boiling method [12]. Briefly, two colonies (24 h) of *K. pneumoniae* that were cultivated in Nutrient Agar (HiMedia) were completely dissolved in 400 μL nuclease-free water. The provided suspension was boiled at 96°C for 10 min. The supernatant, after centrifugation, was stored at −80°C for further analysis.

The PCR (Eppendorf thermal cycler, Hamburg, Germany) reactions assay were carried out at a final volume of 30 μL made up of 15 μL 2× ready-to-use master mix (Ampliqon, Denmark), 1 μL of forward and reverse primers (100 pmol), 3 μL of extracted DNA template and 10 μL nuclease-free sterile distilled water. For PCR product separation and visualization, agarose gel electrophoresis 1% (w/v) (voltage 8 V/cm for 20 min) and sybr safe DNA stain were used, respectively.

2.3 | Aminoglycoside Resistance (AME) Genes Detection

Phenotypic detection of resistance against aminoglycosides (HiMedia, India) including gentamycin (10 μg), amikacin (30 μg), tobramycin (10 μg), kanamycin (30 μg), and streptomycin (10 μg) was performed by disc diffusion method and CLSI (Clinical and Laboratory Standards Institute) guidelines [13]. The genes encoding AME including *aac (2′)-Ia*, *aac (3)-IIa*, *aac (3)-Ib*, *aac (6′)-1b*, *ant (2″)-Ia*, and *aph (3″)-Ib* were detected by PCR (Eppendorf, Germany) [14, 15]. The sequence of used primers and PCR amplification conditions are listed in Table 1. Each PCR reaction mixture (at final volume 25 μL) was prepared by using 12.5 μL 2× ready-to-use master mix (Ampliqon, Denmark), 1 μL of forward and reverse primers (100 pmol), 3 μL

TABLE 1 | PCR programs and sequence of used primers in this study.

Gene	Sequence (5′...3′)	Thermocycler program	Reference
iucA	GCTTATTTCTCCCCAACCC TCAGCCCTTTAGCGACAAG	Initial denaturation 95℃ for 5 min, denaturation 94℃ for 1 min, annealing 59℃ 50 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	7
peg-344	AAAGGACAGAAAGCCAGTG CAATGACGAGGGGGATAATC	Initial denaturation 95℃ for 5 min, denaturation 94℃ for 1 min, annealing 54℃ for 50 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	7
K1	GTAGGTATTGCAAGCCATGC GCCCAGGTTAATGAATCCGT	Initial denaturation 96℃ for 5 min, denaturation 96℃ for 1 min, annealing 56℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension72℃ for 10 min	16
K2	GGAGCCATTTGAATTCGGTG TCCCTAGCACTGGCTTAAGT	Initial denaturation 96℃ for 5 min, denaturation 96℃ for 1 min, annealing 55℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension72℃ for 10 min	16
K5	GCCACCTCTAAGCATATAGC CGCACCAGTAATTCCAACAG	Initial denaturation 96℃ for 5 min, denaturation 96℃ for 1 min, annealing 56℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	16
aac (2′)-Ia	AGAAGCGCTTTACGATTTATTA GACTCCGCCTTCTTCTTCAA	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 54℃ for 30 s, extension 72℃ for 50 s (30 cycles), final extension 72℃ for 10 min	14
aac (3)-Ib	GCAGTCGCCCTAAAACAAA GGATCGTCACCGTAGTCTGC	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 56℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	14
aac (6′)-1b	TATGAGTGGCTAAATCGAT CCCGCTTTCTCGTAGCA	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 50℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	15
ant (2″)-Ia	ACGCCGTGGGTCGATGTTTGATGT CTTTTCCGCCCCGAGTGAGGTG	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 64℃ for 30 s, extension 72℃ for 50 s (30 cycles), final extension 72℃ for 10 min	14
aac (3)-IIa	GGCAATAACGGAGGCGCTTCAAAA TTCCAGGCATCGGCATCTCATACG	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 62℃ for 30 s, extension 72℃ for 55 s (30 cycles), final extension 72℃ for 10 min	14
aph (3″)-Ib	GTGGCTTGCCCCGAGGTCATCA CCAAGTCAGAGGGTCCAATC	Initial denaturation 95℃ for 5 min, denaturation 95℃ for 1 min, annealing 58℃ for 30 s, extension 72℃ for 50 s (30 cycles), final extension 72℃ for 10 min	14

of extracted DNA template, and 7.5 μL nuclease-free sterile distilled water.

2.4 | Statistical Analysis

Statistical analysis performed by SPSS software (V16, Chicago). $p < 0.05$ was considered statistically significant.

3 | Results

3.1 | Isolates

In this study, 88 nonduplicate strains of *K. pneumoniae* were collected from clinical specimens including, urine ($n = 52$, 59.1%), sputum ($n = 17$, 19.3%), blood ($n = 10$, 11.4%), and wound ($n = 9$, 10.2%). Out of 88 patients, 46 patients (52.3%) were male.

3.2 | Virulence-Associated Genes

Results of virulence-associated genes detection revealed that the most predominant virulence gene was *iucA*, which was observed in 89.8% (79/88) isolates, followed by *peg-344* 55.7% (49/88), *K5* 14.8% (13/88), *K1* 10.2% (9/88) and *K2* 9.1% (8/88), of these isolates, respectively. In addition, 58 isolates (65.9%) did not belong to serotypes K1, K2, and K5. Some isolates had only one virulence gene and some others harbored a combination of two or more genes (Table 2). In addition, thirteen different patterns of virulence genes were observed (Table 2). The most predominant combination was *iucA*+ *peg-344*, which was detected in 37.5% (33/88) isolates, followed by *iucA*+ *peg-344* + *K5* 6.8% (6/88) and *iucA* + *k1* 5.7% (5/88), respectively.

3.3 | Antibiotic Resistance and AME Genes

Out of 88 tested isolates, 67 (76.1%) isolates exhibited resistance to at least one of the tested aminoglycosides. Resistance rates to

TABLE 2 | Combination of virulence-associated genes among 88 *K. pneumoniae* isolates.

Patterns	Combination	*N* (%)
1	*iucA+ peg-344*	33 (37.5%)
2	*iucA*	22 (25%)
3	*iucA+ peg-344 + K5*	6 (6.8%)
4	*iucA + K5*	5 (5.7%)
5	*iucA + K1*	5 (5.7%)
6	*iucA + K2*	4 (4.5%)
7	*iucA+ peg-344 + K1*	3 (3.4%)
8	*peg-344 + K5*	2 (2.3%)
9	*peg-344 + K2*	2 (2.3%)
10	*K2*	1 (1.1%)
11	*peg-344*	1 (1.1%)
12	*peg-344 + K1*	1 (1.1%)
13	*iucA+ peg-344 + K2*	1 (1.1%)
14	Not detected	2 (2.3%)
	Total	88 (100%)

TABLE 3 | Coexistence of AME genes among aminoglycoside-resistant *K. pneumoniae* isolates.

Pattern	Combination	No. of isolates (%)
1	*aph (3″)-Ib*	15 (17%)
2	*ant (2″)-Ia*	10 (11.4%)
3	*aac (3)-Ib*	8 (9.1%)
4	*aac (3)-Ib+ aph (3″)-Ib*	5 (5.7%)
5	*ant (2″)-Ia+ aph (3″)-Ib*	4 (4.5%)
6	*aac (3)-IIa*	4 (4.5%)
7	*aac (3)-IIa+ aph (3″)-Ib*	2 (2.3%)
8	*aac (6′)-1b*	2 (2.3%)
9	*aac (6′)-1b+ aph (3″)-Ib*	2 (2.3%)
10	*aac (6′)-1b+ ant (2″)-Ia*	2 (2.3%)
11	*aac (6′)-1b+ ant (2″)-Ia +aph (3″)-Ib*	1 (1.1%)
12	*aac (3)-IIa+ ant (2″)-Ia*	1 (1.1%)
13	*aac (3)-IIa +aac (6′)-1b*	1 (1.1%)
14	*aac (3)-IIa +aac (6′)-1b+ ant (2″)-Ia*	1 (1.1%)
15	*aac (3)-Ib+ ant (2″)-Ia+ aph (3″)-Ib*	1 (1.1%)
16	*aac (3)-Ib+ aac (3)-IIa+ aph (3″)-Ib*	1 (1.1%)
	Total	60 (68.2%)

used aminoglycoside antibiotics were 65 (73.8%) for kanamycin, 61 (69.3%) for streptomycin, 55 (62.5%) for tobramycin, 48 (54.5%) for gentamicin, and 17 (19.3%) for amikacin. In total, 60 isolates (68.2%) were at least positive for one AME genes. Some isolates had only one gene and some others harbored a combination of two or more genes. In addition, sixteen different patterns of AME genes were observed (Table 3). The most predominant resistance gene was *aph (3″)-Ib*, which was detected in 35.2% (31/88) isolates, followed by *ant (2″)-Ia* 22.7% (20/88), *aac (3)-Ib* 17% (15/88), *aac (3)-IIa* 11.3% (10/88) and *aac (6′)-1b* 10.2% (9/88), of these isolates, respectively. In this work *aac (2′)-Ia* was not detected.

3.4 | Association Between Virulence-Associated Genes and AME

Based on statistical analysis, as shown in Table 4, a significant association was observed between *K2* and *aac (3)-IIa*. In addition, considering the positive value of *Phi* (0.26, $p = 0.01$), it can be concluded that there was a weak positive association between *aac (3)-IIa* and *K2*.

4 | Discussion

K. pneumoniae (a well-known member of the ESKAPE group of pathogens) was reported to be abundant among human and nonhuman (animals, environment, and foods) resources and can be transmitted to humans by direct contact. For example, based on phylogenetic analysis, a high association between humans and animals (sheep, cows, and chickens) *K. pneumoniae* has been observed, indicating transmission from animals to humans or from humans to animals. Therefore, constant monitoring of antibiotic resistance mechanisms and molecular characteristics will help reduce the spread of infections [17, 18].

To the best of our knowledge, this is the first report on the prevalence of virulence-associated and AME genes in *K. pneumonia* clinical isolates collected from Zabol hospital, Iran. In this work, 88 strains of *K. pneumoniae* were used. The PCR results showed that *iucA* and *peg-344* were the most prevalent virulence-associated genes, with 89.8% (79/88) and 55.7% (49/88) of positive isolates, respectively.

It has been reported that two different pathotypes of *K. pneumonia* are being isolated from patients worldwide, classical *K. pneumonia* (cKp) and hypervirulent *K. pneumonia* (hvKp) [19, 20]. Severe and extremely invasive infections of *K. pneumonia* such as liver abscesses, tissue-invasive infections, endophthalmitis, osteomyelitis, and pneumonia in both healthy and immunocompromised individuals usually attribute to hvKp strains [19, 20]. These two pathotypes differ in epidemiology and antibiotic resistance patterns [20–22]. Different molecular biomarkers, including *rmpA* (mediate capsule synthesis), *peg-344* (metabolic transporter), *iucABCD* (mediate siderophore production), *iroB* (salmochelins), siderophore yersiniabactin-related genes (*ybtA, ybtE, ybtP*), and allantoin utilization genes (*allA-D* and *allR-S*), have been reported for hvKp identification [2, 7].

Results of this study revealed that the most prevalent virulence gene was *iucA* followed by *peg-344*, with 89.8% (79/88) and

TABLE 4 | Association between AME genes and virulence-associated genes.

AME genes	*iucA* $n = 79$		*Peg-344* $n = 49$		*K1* $n = 9$		*K2* $n = 8$		*K5* $n = 13$	
	P (%)	*N* (%)	*P* (%)	*N* (%)	*P* (%)	*N* (%)	*P* (%)	*N* (%)	*P* (%)	*N* (%)
aac (3)-IIa $n = 10$	10 (100%)	0 (0%)	6 (60%)	4 (40%)	0 (0%)	10 (100%)	3 (30%)	7 (70%)	1 (10%)	9 (90%)
p value	0.25		0.77		0.25		**0.01***		0.65	
aac (6′)-Ib $n = 9$	8 (88.9%)	1 (11.1%)	6 (66.7%)	3 (33.3%)	1 (11.1%)	8 (88.9%)	1 (11.1%)	8 (88.9%)	1 (11.1%)	8 (88.9%)
p value	0.92		0.48		0.92		0.82		0.74	
ant (2″)-Ia $n = 20$	16 (80%)	4 (20%)	13 (65%)	7 (35%)	2 (10%)	18 (90%)	2 (10%)	18 (90%)	2 (10%)	18 (90%)
p value	0.1		0.34		0.97		0.87		0.49	
aph (3″)-Ib $n = 31$	29 (93.5%)	2 (6.5%)	19 (61.3%)	12 (38.7%)	3 (9.7%)	28 (90.3%)	1 (3.2%)	30 (96.8%)	7 (22.6%)	24 (77.4%)
p value	0.38		0.43		0.9		0.15		0.12	
aac (3)-Ib $n = 15$	12 (80%)	3 (20%)	10 (66.7%)	5 (33.3%)	2 (13.3%)	13 (86.7%)	0 (0%)	15 (100%)	2 (13.3%)	13 (86.7%)
p value	0.17		0.34		0.66		0.17		0.86	

*Statistically significant $p < 0.05$.

55.7% (49/88) of all isolates being positive, respectively. Similarly, in a study conducted in China, the most predominant virulence gene was reported to be *iucA* (56.8%) [23]. Likewise, in other studies such as Matsuda et al., (Japan) and Sanikhani et al., (Iran) the most prevalent gene was reported to be *iucA*, with 64% and 62.6% of isolates being positive, respectively [24, 25].

These findings may be attributed to pLVPK-like plasmids, encoding virulence-associated genes such as *iucABCD* (mediate siderophore production), *rmpA* (mediate capsule synthesis), and *peg-344* (metabolic transporter) [26].

In the present study, the prevalence of three capsular serotypes (K1, K2, and K5) was also investigated. Our results demonstrate that the most predominant capsular serotype was K5 14.8%, followed by K1 10.2% and K2 9.1%, respectively. In a similar study conducted in Iran, capsular serotypes 5 and 2 were the most isolated serotypes, with 4% and 2% of all isolates being positive, respectively [27]. Based on the results of other studies, the prevalence of capsular serotypes varied, for example, Solgi et al., and Taraghian et al., reported that K1 and K2 were the most prevalent serotypes, with 45.9%, 13.5%, and 7.6%, 66.6% of all isolates being positive, respectively [28, 29]. Contrary to most studies in Asia having introduced K1 and K2 as the most common serotypes [16, 20, 30–32], Hasani et al. reported that corpuscular serotypes 54 (29.5%) and 20 (21.3%) were the most commonly identified serotypes among analyzed strains [33].

These differences in virulence-associated genes and capsular serotypes observed among studies may be attributed to genetic recombination, mutation, population under study, sample size, source of isolated *K. pneumoniae*, or even diversity in applied detecting methods [27, 34].

Aminoglycoside antibiotics are of paramount importance to treat patients infected with *K. pneumoniae*. Based on phenotypic tests, the isolates were mostly resistant against kanamycin 73.8% and streptomycin 69.3% followed by tobramycin 62.5% and gentamicin 54.5%. Different aminoglycoside resistance mechanisms including efflux pump overexpression, decreased permeability, and modification of 30 s ribosomal subunit have been documented [35, 36]. However, AME genes such as *aac*, *ant*, or *aph* mediate enzymatic modification and inactivation of the aminoglycoside antibiotics and are commonly observed across gram-negative bacteria, particularly *Enterobacteriaceae* family (*K. pneumoniae*) [35–37]. Our results demonstrated that the most prevalent AME genes were *aph (3″)-Ib* 35.2%, followed by *ant (2″)-Ia* 22.7%, *aac (3)-Ib* 17%, *aac (3)-IIa* 11.3%, and *aac (6′)-1b* 10.2%. These findings are lower than study conducted in Ahvaz, in which 44% of investigated *K. pneumoniae* were positive for *ant (2″)-Ia*, 36.5% for *aac (3′)-IIa*, 24.7% for *aac (6′)-Ib*, and 20% for *aph (3′)-Ia* [38]. Likewise, Al-Badawy et al. reported that *acc (6′)-Ib* and *acc (3′)-II* are highly distributed among *K. pneumoniae* isolates, with 88% and 58% of isolates being positive, respectively [39]. Results of independent studies on the distribution of AME genes in Egypt, Spain, Nigeria, Australia, and China revealed that the most prevalent AME genes were *aac (3′)-IIa* and *aac (6′)-Ib* [40–43].

In this work different combination of AME genes was observed, notably, 17 isolates (28.4%) had two AME genes, and 4 isolates (6%) had three AME genes, simultaneously. These findings can explain the high level of resistance against different aminoglycosides at the same time since different AME genes can be carried by the mobile genetic elements such as plasmids and transposons, leading to simultaneous resistance against different aminoglycoside antibiotics [5, 6].

It has been reported that due to the continued overuse of antibiotics, the coexistence of AME genes and other antibiotic resistance genes such as extended-spectrum beta-lactamase (ESBL) on mobile genetic elements (plasmids and transposons) is becoming increasingly common [6, 44]. Noteworthy, these transferable plasmids belonged to different incompatibility groups and usually harbor different resistance genes, conferring resistance to various drugs. Therefore, successful treatment of infections caused by these isolates is becoming more difficult with each passing year [44].

Despite using PCR tests for virulence-associated and aminoglycoside resistance gene detections, the whole-genome sequencing (WGS) technique can be more useful because it can provide comprehensive information about the complete genome of microorganisms. Hence, WGS can play a pivotal role in the detection and prevention of bacterial infections [45–47].

Some limitations must be considered. Although a high level of *iucA* and *peg-344* (biomarkers for hvKp identification) positive isolates were identified, genotypic identification does not necessarily reflect the expression of genes; therefore, further studies are needed. In addition, to accurately estimate the prevalence of hvKp other biomarkers should be evaluated. Quantitative antibiogram methods such as dilution assays and other resistance mechanisms must be investigated to better define the rate of aminoglycoside resistance and the role of AME genes.

5 | Conclusion

The results of this work indicate that the prevalence of aminoglycoside-resistant *K. pneumoniae* in investigated hospital is high and should be considered a serious threat to patients' health. Meanwhile, resistance rates to different aminoglycoside antibiotics are high and it is primarily attributed to the presence of AME genes such as *aph (3″)-Ib*, ant *(2″)-Ia*, and *aac (3)-Ib*. Due to various combinations of AME genes and virulence-associated genes, probably different mobile genetic elements distributed among isolates can create a high risk of rapid spread of these genes.

Author Contributions

Conceptualization: Hamid Vaez. Data collection: Zahra Yazdanpour, Hamid Vaez. Formal analysis: Hamid Vaez. Funding acquisition: Hamid Vaez. Investigation: Hamid Vaez, Zahra Yazdanpour. Methodology: Hamid Vaez, Zahra Yazdanpour. Laboratory procedure: Hamid Vaez, Zahra Yazdanpour. Project administration: Hamid Vaez. Software: Hamid Vaez. Supervision: Hamid Vaez. Writing–original draft: Hamid Vaez.

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Ethics Statement

This study was approved by the Ethics Committee of ZBMU IR. ZBMU. REC.1402.015 (https://ethics.research.ac.ir/ProposalCertificateEn.php?id=340563).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in this published article.

Transparency Statement

All authors have read and approved the final version of the manuscript corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. The lead author Hamid Vaez affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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