Prevalence of *qnrA*, *qnrB*, and *qnrS* genes in *Klebsiella pneumoniae* isolates from patients in hospitals in Sabzevar, Iran: A cross-sectional from February 2022 to October 2023

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) has emerged as a significant opportunistic pathogen responsible for nosocomial infections, including hospital-acquired pneumonia (HAP) and various intra-abdominal infections. The aim of this study was to investigate the prevalence of *qnrA*, *qnrB*, and *qnrS* genes in *K. pneumoniae* isolates from patients in hospitals in Sabzevar, Iran. Of 100 specimens of urine, respiratory secretions, blood, tracheal lavage, pleural fluid and trachea were collected from patients referred to hospitals in Sabzevar, northeastern Iran. Identification of bacteria was performed by gram stain, culture characteristics and biochemical methods. Bacterial susceptibility to quinolone antibiotics using the Kirby-Bauer disk diffusion method according to Clinical Laboratory

Standards Institute (CLSI) guidelines (2023). The extracted DNA was subjected to polymerase chain reaction (PCR) assay targeting three genes of *qnrA*, *qnrB* and *qnrS* using specific primers. The data were analyzed using the chi-squared test and the Fisher's exact test with the IBM SPSS Statistics version 26 software. P values less than 0.05 were also considered statistically significant. 100 non-duplicate *K. pneumoniae* isolates were collected from various clinical specimens, including urine (62%), respiratory tract secretions (6%), trachea (18%), blood (10%), tracheal lavage (3%), and pleural fluid (1%). The highest and lowest resistance were related to ampicillin (92%) and nitrofurantoin (22%), respectively. The *qnrA*, *qnrB* and *qnrS* genes were present in 61%, 56% and 47% of the isolates, respectively. There is a statistically significant association between the presence of *qnr* genes and resistance to fluoroquinolones ($p<0.002$). Based on these results, *qnrA*-producing *K. pneumoniae* strains were isolated from patients. Thus, prescribing appropriate antibiotics and detection of *qnr* genes is required and it can be useful in tracking, treating and knowledge of *K. pneumoniae* infection prevalence rate.

Keywords: *Klebsiella pneumoniae*, antimicrobial susceptibility, *qnr* genes.

1. Introduction

K. pneumoniae is commonly found in the human body as a commensal organism, particularly in the gastrointestinal tract and oropharynx. Despite its presence in these areas, it can become pathogenic and cause serious infections, especially in critically ill and immunocompromised patients (1). It is the second most common cause of bloodstream infections (BSIs) attributed to Gram-negative bacteria, after *Escherichia coli* (2). While BSI can occur as a primary infection with no clear source, it is more often the result of the bacterium spreading into the bloodstream from a known site of infection (1, 3). In addition, *K. pneumoniae* is an important pathogen associated with a wide variety of other infections, the most common of which are pneumonia, meningitis, pyogenic liver abscesses, and urinary tract and wound infections (4). *K. pneumoniae* has become a significant clinical concern over the past 20 years due to its increasing antibiotic resistance and ability to cause serious infections, particularly in critically ill and immunocompromised patients (5). The

emergence of extensively drug-resistant (XDR) and pan-drug-resistant (PDR) strains is a major challenge, as these strains are resistant to all current antimicrobial therapies, largely due to the accumulation of antibiotic resistance genes (6). Quinolones, especially fluoroquinolones, are commonly used to treat Gram-negative bacterial infections. However, resistance to these antibiotics has been increasing, particularly in *Enterobacteriaceae*. The main mechanism of resistance is mutations in genes encoding key enzymes such as topoisomerase IV and DNA gyrase, as well as alterations in efflux pumps and porins (7). In addition, plasmid-mediated quinolone resistance (PMQR) contributes to the problem, involving several genes, including *qnrA, qnrB, qnrC, qnrD, qnrS, qnrVC, aac(6')-Ib-cr, qepAB,* and *oqxA*. The *qnr* genes produce proteins that interact with DNA gyrase and topoisomerase IV, thereby inhibiting quinolone activity (8). The second mechanism of resistance is the acetylation of fluoroquinolones by a variant of the gene associated with aminoglycoside acetyltransferase, *AAC(6')-Ib* (9). The third *PMQR* mechanism involves quinolone efflux pumps (*QepAB* and *OqxAB*), which confer resistance to hydrophilic quinolones, particularly ciprofloxacin, enrofloxacin and norfloxacin (10). The link between quinolone resistance and resistance to other antimicrobials, particularly aminoglycosides and beta-lactams, poses a significant challenge in the management of these infections (10). In vitro studies indicate that isolates with *PMQR* genes can develop high levels of resistance after antibiotic exposure. Therefore, monitoring for *PMQR* genes in *Enterobacteriaceae* is essential. Data on the prevalence of *qnrA, qnrB,* and *qnrS PMQR* genes among clinical isolates of *K. pneumoniae* from human samples in Sabzevar, Iran are limited. Therefore, the aim of this study was to investigate the prevalence of these genes in *K. pneumoniae* isolates from patients in a hospital in Sabzevar, Iran.

2. Methods and materials

2.1. Bacterial Isolation from Clinical Samples

The present study was conducted from February 2022 to October 2023. In this cross-sectional study, a total of 100 specimens of urine, respiratory secretions, blood, tracheal lavage, pleural fluid

and trachea were collected from patients referred to Emdad Hospital (n=1), Zargarian Laboratory (n=7), Rooyesh Laboratory (n=7), Heshmatiyeh Hospital (n=6), Mobini Hospital (n=8) and Wasei Hospital (n=71) in Sabzevar, northeastern Iran. Identification of bacteria was performed by gram stain, culture characteristics and biochemical methods. Positive control strain was *K. pneumoniae* ATCC700603. Trypticase soy broth containing 20% glycerol was used for preservation of bacteria at -70°C.

2.2. Antimicrobial Susceptibility Testing

Bacterial susceptibility to quinolone antibiotics (nalidixic acid (30 μg), ciprofloxacin (5 μg) and ofloxacin (5 μg)) and other antibiotics (trimethoprim-sulfamethoxazole (1.25/23.75 μg), ceftriaxone (30 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), cefazolin (30 μg), ampicillin (10 μg), nitrofurantoin, cefotaxime (30 μg), ceftizoxime (30 μg), cephalothin (30 μg), ceftazidime (30 μg), and cefoxitin) using the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines (11). The discs were ordered from MAST co. England. *E. coli* ATCC25922 and *K. pneumoniae* ATCC700603 were used for antibiotic susceptibility quality control.

2.3. DNA Extraction and PCR Assay

Total DNA extraction was performed by the boiling method: in brief, colonies suspected of being *A. baumannii* were suspended in 500 μL TE buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0), boiled at 95 °C for 10 min and centrifuged at 14,000 × g for 5 min. The supernatants were collected as DNA templates and stored at -20 °C for the PCR. The extracted DNA was subjected to PCR assay targeting three genes of *qnrA*, *qnrB* and *qnrS* using specific primers (Table 1). Reaction mixtures were prepared in a total volume of 20 μL containing 10 μL PCR master mix plus, 1 μL template DNA and 5ng genomic DNA, 0.5 μL of each primer (0.5 μM) and 8 μL water. PCR amplification was performed in a thermocycler as follows: 95°C for 5 minutes and 30 cycles of 50 seconds at 94°C, 45 seconds at a specific annealing temperature for each primer, and 40 seconds at 72°C. A final extension step of 5 minutes at 72°C was performed. The analysis of the PCR products

was checked on 1.5% gel electrophoresis with safe DNA stain and the results were visualized under the gel document system.

Table 1: Characteristics of the primers used in this study.

Primer	Sequence (5'-3')	Pre-Denaturation, 94ºC	Denaturation, 94ºC	Annealing, 40 sec	Extension, 72ºC	Product Size (bp)
qnrA	TCAGCAAGAGGATTTCTCA GGCAGCACTATTACTCCCA	7 min	50 **sec**	57ºC	1 min	516
qnrB	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	7 min	50 **sec**	57ºC	1 min	469
qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	7 min	50 **sec**	57ºC	1 min	388

2.4. Statistical Analysis

The data were analyzed using the chi-squared test and the Fisher's exact test with the IBM SPSS Statistics version 26 software. P values less than 0.05 were also considered statistically significant.

3. Result

In this cross-sectional study, 100 non-duplicate *K. pneumoniae* isolates were collected from various clinical specimens, including urine (n=62;62%), respiratory tract secretions (n=6), trachea (n=18; 18%), blood (n=10; 10%), tracheal lavage (n=3; 3%), and pleural fluid (n=1; 1%)(Table 2). The mean age of the patients was 62.2±3.25 years. In addition, 25 isolates were from outpatients and 75 isolates were from inpatients. According to antibiotic susceptibility testing, the highest resistance rate of the isolates was to ampicillin (92%), followed by cephalothin (n=68; 68%), cefazolin (n=64; 64%), ceftriaxone, meropenem, nalidixic acid and trimethoprim-sulfamethoxazole (n=62; 62%), cefoxitin and ofloxacin (n=57; 57%), ceftizoxime (n=55; 55%), ciprofloxacin (n=60; 60%), cefotaxime (n=52; 52%), gentamicin (n=43; 43%), ceftazidime (n=42; 42%), amikacin (n=39;

39%), imipenem (n=38; 38%) and nitrofurantoin (n=22; 22%). There was no statistically significant association of resistance to different antibiotics with sex and age ($p > 0.739$). Table 2 described the characterizations of *K. pneumoniae* isolates from clinical samples. The *qnrA, qnrB* and *qnrS* genes were present in 61% (n=61), 56% (n=56) and 47% (n=47) of the isolates, respectively. The *qnrA, qnrB and qnrS* genes were present simultaneously in 16 (16%) isolates, while 9 (9%) isolates had none of the genes. Also, 21 (21%) isolates had both *qnrA* and *qnrB* genes while 11 (11%) isolates had both *qnrA* and *qnrS* genes. Table 3 indicated the relationship between *qnr(s)* genes and fluoroquinolone resistance. There is a statistically significant association between the presence of *qnr* genes and resistance to fluoroquinolones ($p<0.002$). In addition, of 59 norfloxacin-resistant isolates, 56 (56%) isolates had at least one of the *qnr* genes. Also, out of 57 ofloxacin resistant isolates, 53 (53%) isolates had at least one of the *qnr* genes. Of 60 nalidixic acid-resistant isolates, 60 (60%) isolates had at least one of the *qnr* genes. In addition, of 60 ciprofloxacin-resistant isolates, 57 (57%) isolates had at least one of the *qnr* genes.

Table 2: Characterizations of *K. pneumoniae* isolates from clinical samples

Strain NO.	**Sample**	**Ward**	***qnrA***	***qnrB***	***qnrS***	**CIP**	**NAL**	**OFL**	**NOR**
1	Peural effusion	ICU	Pos	Pos	Neg	R	R	R	R
2	Urine	Internal	Pos	Neg	Neg	R	R	S	R
3	BAL	Internal	Pos	Neg	Neg	S	S	S	S
4	Urine	Children	Pos	Neg	Pos	S	R	R	R
5	Trachea	ICU	Neg	Neg	Pos	S	S	S	S
6	Blood	Emergency	Pos	Neg	Neg	S	R	S	S
7	Trachea	ICU	Pos	Neg	Pos	R	R	S	R
8	Urine	Out	Pos	Neg	Neg	S	S	S	S
9	Urine	Out	Pos	Pos	Neg	S	R	S	S
10	Urine	Neurological	Pos	Neg	Neg	R	R	R	R
11	Urine	Internal	Pos	Neg	Neg	R	S	R	R
12	Trachea	ICU	Pos	Neg	Neg	R	R	R	R

13	Trachea	ICU	Pos	Neg	Neg	R	R	S	R
14	Trachea	ICU	Pos	Neg	Neg	R	R	R	R
15	Blood	Neurological	Neg	Neg	Neg	S	R	S	S
16	Urine	Out	Neg	Neg	Neg	S	S	R	R
17	Urine	Out	Pos	Neg	Neg	S	R	S	S
18	Urine	Out	Neg	Neg	Neg	R	S	R	R
19	Urine	VIPinfectious	Neg	Neg	Pos	R	S	R	R
20	Urine	Out	Neg	Neg	Pos	R	R	R	R
21	Urine	VIPinfectious	Pos	Pos	Pos	R	S	R	R
22	Urine	Internal	Pos	Pos	Pos	R	R	R	R
23	Blood	Internal	Pos	Neg	Neg	S	R	S	S
24	Urine	Infectious	Pos	Neg	Pos	R	S	R	R
25	Urine	Out	Pos	Neg	Pos	S	S	S	S
26	Blood	Internal	Pos	Pos	Neg	S	R	S	S
27	Urine	Infectious	Neg	Neg	Pos	R	R	R	R
28	Blood	Internal	Pos	Pos	Pos	S	S	S	S
29	Urine	Out	Pos	Pos	Pos	S	R	S	S
30	Urine	Out	Neg	Pos	Pos	S	S	S	S
31	Urine	Internal	Neg	Pos	Pos	R	R	R	R
32	Urine	Out	Pos	Pos	Neg	R	R	R	R
33	Trachea	ICU	Pos	Pos	Neg	R	R	R	R
34	Blood	Internal	Pos	Pos	Neg	S	R	S	S
35	Urine	Out	Neg	Pos	Neg	S	S	S	S
36	Urine	Neonatal	Neg	Pos	Neg	S	R	S	S
37	Urine	Out	Pos	Pos	Neg	S	R	S	S
38	Trachea	ICU	Pos	Pos	Neg	R	R	R	R
39	Trachea	Emergency	Pos	Pos	Neg	S	R	S	S
40	Urine	Children	Pos	Pos	Neg	R	S	R	R
41	Urine	Out	Pos	Pos	Neg	S	R	S	S
42	Urine	Internal	Pos	Neg	Pos	R	R	R	R
43	Urine	Emergency	Pos	Neg	Pos	S	R	S	S
44	Urine	Internal	Pos	Pos	Pos	R	R	R	R
45	Urine	Emergency	Pos	Pos	Neg	S	S	S	S
46	Urine	Oncology and surgery	Pos	Neg	Neg	R	R	R	R

47	Blood	Emergency	Pos	Pos	Pos	S	R	S	S
48	Urine	Internal	Pos	Pos	Neg	R	R	R	R
49	Urine	Emergency	Neg	Pos	Neg	R	S	R	R
50	Urine	Out	Neg	Pos	Neg	R	R	R	R
51	Urine	Neurological	Pos	Pos	Pos	S	S	S	S
52	Trachea	ICU	Pos	Neg	Pos	R	R	R	R
53	Urine	Emergency	Pos	Pos	Pos	S	S	S	S
54	Urine	Neurological	Neg	Pos	Neg	R	R	R	R
55	Urine	ICU	Neg	Pos	Neg	R	S	R	R
56	Urine	Neurological	Pos	Pos	Neg	R	R	R	R
57	Urine	ICU	Neg	Pos	Neg	S	R	S	S
58	Bal	ICU	Pos	Pos	Neg	R	R	R	R
59	Urine	Internal	Pos	Neg	Pos	R	R	R	R
60	Trachea	Internal	Pos	Pos	Pos	R	S	R	R
61	Urine	Out	Neg	Pos	Pos	S	R	S	S
62	Trachea	Infectious	Pos	Pos	Pos	R	S	R	R
63	Urine	Out	Neg	Pos	Pos	R	S	R	R
64	Urine	Vipinfectious	Pos	Pos	Neg	R	R	R	R
65	Urine	Out	Pos	Pos	Pos	R	S	R	R
66	Blood	Internal	Pos	Neg	Neg	R	S	R	R
67	Trachea	ICU	Pos	Neg	Pos	S	R	S	S
68	BAl	Infectious	Pos	Pos	Neg	R	R	R	R
69	Urine	Out	Neg	Pos	Neg	S	S	S	S
70	Urine	Out	Neg	Neg	Pos	R	R	R	R
71	Urine	Internal	Pos	Neg	Pos	S	S	R	S
72	Urine	Internal	Pos	Pos	Pos	S	S	S	S
73	Urine	Emergency	Pos	Pos	Neg	S	R	S	R
74	Trachea	ICU	Pos	Pos	Pos	R	R	R	R
75	Trachea	ICU	Neg	Neg	Pos	R	S	R	R
76	Blood	Oncology and surgery	Pos	Neg	Pos	S	R	S	S
77	Urine	ICU	Pos	Pos	Pos	R	S	R	R
78	Blood	Emergency	Neg	Pos	Neg	S	R	S	S
79	Urine	Neurological	Neg	Pos	Pos	S	S	S	R
80	Urine	Out	Neg	Pos	Pos	S	R	S	S

81	Urine	Out	Neg	Neg	Pos	R	S	R	R
82	Urine	Out	Neg	Neg	Neg	S	R	S	S
83	Urine	Internal	Neg	Pos	Neg	R	R	R	R
84	Urine	Out	Neg	Pos	Pos	R	S	R	R
85	Urine	ICU	Neg	Pos	Pos	S	R	S	S
86	Urine	Internal	Neg	Neg	Pos	R	R	R	R
87	Urine	Out	neg	neg	neg	R	S	R	S
88	Trachea	ICU	Pos	Pos	Pos	R	R	R	R
89	Urine	Out	Neg	Neg	Neg	R	S	S	R
90	Urine	Infectious vip	Pos	Pos	Neg	R	R	R	R
91	Trachea	Infectious	Pos	Pos	Pos	R	R	R	R
92	Trachea	Infectious	Neg	neg	pos	R	R	S	R
93	Trachea	ICU internal	Neg	Neg	Neg	R	S	R	S
94	Trachea	Internal	Neg	Neg	Neg	R	R	R	R
95	Trachea	ICU internal	Neg	Neg	Pos	R	R	R	S
96	Wound	ICU	Neg	Neg	Pos	R	R	S	S
97	Trachea	Infectious	Pos	Pos	Neg	R	S	R	R
98	Urine	Emergency	Neg	Pos	Pos	R	R	R	R
99	Trachea	Infectious	Pos	Pos	Neg	R	S	R	S
100	Urine	Internal	Neg	Neg	Neg	S	S	S	S

Table 3: The relationship between *qnr(s)* genes and fluoroquinolone resistance.

Gene	***qnrA***		***qnrB***		***qnrS***		***qnr(s)***	
Antibiotic	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
CIP (S)	15(15%)	24(24%)	16(16%)	28(28%)	22(22%)	31(31%)	10(10%)	0
CIP (R)	25(%)	36(36%)	24(24%)	32(32%)	18(%)	29(29%)	28(28%)	57(57%)
P value	0.485		0.543		0.839		0.002	
NAL(S)	18(18%)	21(21%)	17(17%)	27(27%)	18(18%)	35(35%)	10(%)	0
NAL(R)	20(20%)	41(41%)	21(21%)	35(35%)	20(20%)	27(27%)	25(25%)	60(60%)
P value	0.209		0.536		0.414		0.006	
OFL(S)	17(17%)	22(22%)	19(19%)	25(25%)	24(24%)	29(29%)	10(10%)	0
OFL(R)	26(26%)	35(35%)	24(24%)	32(32%)	19(19%)	28(28%)	32(32%)	53(53%)
P value	0554		0.567		0.688		0.001	

NOR(S)	17(17%)	22(22%)	18(18%)	26(26%)	23(23%)	30(30%)	10(%)	0
NOR(R)	24(24%)	37(37%)	23(23%)	33(33%)	18(18%)	29(29%)	29(29%)	56(56%)
P value	0.683		0.575		0.685		0.002	

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162 **4. Discussion**

163 *K. pneumoniae* is a Gram-negative, non-motile, facultative anaerobic bacterium. *K. pneumoniae*
164 frequently colonizes various mucosal surfaces, including the upper respiratory tract and the
165 intestines, where colonization rates vary greatly among individuals based on habitat and exposure to
166 disease. This bacterium is one of the most important causes of nosocomial infections globally (10).
167 *Klebsiella* species cause infections in several sites, including the lungs, urinary tract, bloodstream,
168 wound or surgical sites, and brain. These infections are more likely to occur in people with pre-
169 existing conditions. The discrimination between colonization and infection has always been obscure
170 for clinicians and researchers, making subsequent intervention strategies a stubborn problem.
171 Nonetheless, several factors can be considered to discriminate between colonization and infection
172 (4). In this study, urine had the highest concentration of *K. pneumoniae* by 62%. Similarly, in the
173 study by Beyene et al., 50% of *K. pneumoniae* cases were isolated from the urine of patients (12). In
174 the study by Kot et al., 87.1% of *K. pneumoniae* were isolated from urine (5). Jalal et al. isolated the
175 most *K. pneumoniae* from blood, followed by sputum (13). Selden et al. found evidence from
176 sources of gastrointestinal infection that *K. pneumoniae* infections often have the same serotypes as
177 the bacteria inhabiting the intestinal tract (14). More recent studies have confirmed the association
178 between *K. pneumoniae* colonization and strains obtained from infection sites (15). Chang et al.
179 suggest that because of the widespread presence of *K. pneumoniae* in humans, gastrointestinal
180 colonization serves as a major reservoir for transmission and infection to other sites (4). In this
181 study, 25 isolates were obtained from outpatients and 75 from inpatients. The ICU ward showed the

highest rate of isolates with 21 cases. And subgroup analysis of the antibiotic resistance patterns in ICU ward compared to non-ICU inpatients showed that strains isolated in the ICU ward were more resistant than those isolated in other wards. Similar to our study, the prevalence of *Klebsiella* in inpatients was higher in the study by Jalal et al. (13). In the study by Hafiz et al., the ICU ward had the highest prevalence of *K. pneumoniae* among inpatients. Hafiz et al. stated that ICUs are considered as a setting where antibiotic resistance is developed, strengthened, and spread (1). Jalal et al. also mentioned the weakening of the immune system due to aging and prolonged hospitalization, especially in the ICU, as a potential risk factor for *K. pneumoniae* infection (13). Thus, it can be concluded that patients admitted to the ICU are mainly more susceptible to infection due to a attenuated immune system (16). Pathogens use several mechanisms to develop antibiotic resistance, including beta-lactamase production, biofilm formation, creation of genetic and phenotypic diversity, and loss of sensitive outer membrane proteins, efflux pumps, and integrons. Exposure to antibiotics at sublethal concentrations may lead to the development of resistance among pathogens. In addition to the inappropriate clinical use of antimicrobial agents, human populations are often exposed to a wide range of non-iatrogenic antibacterial drugs in everyday life, including exposure to livestock antibiotics in the meat processing industry, leading to increased drug resistance in pathogens. It appears that the use of quinolones at a higher level in animal and fish farm treatment have played a significant role in the development of this type of resistance (17). The increased use of antibiotics in both clinical and nonclinical settings is associated with an increased number of drug-resistant strains (4). *K. pneumoniae* infection is often treated with beta-lactam antibiotics which are one of the most widely-used resistant antibiotics, thence causing a major crisis in medical clinics in the last two decades (18). Based on the results of this study, the highest and lowest resistance rates of *K. pneumoniae* isolates were against ampicillin (92%) and nitrofurantoin (22%), respectively. In the study by Beyene et al., the highest resistance was against ampicillin and amoxicillin, and the lowest resistance was against nitrofurantoin (4). Jalal et al. reported ampicillin (97.6%) as the most-resisted antibiotic in their study (15). Moreover, in a study

by Amraie et al. conducted on 195 *Klebsiella* isolates, the highest and lowest resistance rates were against amoxicillin (79.19%) and ciprofloxacin (15.60%), respectively (18). Considering these results, in most studies, isolates were resistant against amoxicillin and ampicillin, implying that these antibiotics cannot be used as empirical treatment. On the other hand, very low levels of resistance to antibiotics such as nitrofurantoin have been reported in previous studies (14). It can also be said that the regional variations of resistance to antibiotics may be partially explained by different local antibiotic practices. Further studies in different geographical areas are required to investigate the susceptibility of organisms to antibiotics due to genetic differences, hospital practices, and environmental factors (18). The impact of excessive or inappropriate use of antibiotics has been shown to lead to the emergence of antibiotic-resistant strains, especially broad-spectrum agents that are administered practically. Reducd frequency of prescriptions for certain antibiotics can lead to reduced resistance. The transmission of resistant isolates between people or the consumption of foods derived from animals that have received antibiotics has also contributed to the spread of antibiotic resistance (18). In this study, the prevalence rate of three genes *qnrA*, *qnrB*, and *qnrS* was investigated. *qnrA* was the most frequent gene isolated from isolates with 61%. Besides, 16% of isolates entailed all three genes and 9% of isolates contained none of the genes. In the study by Nourozi et al., the *qnrB* gene was the most frequent gene isolated from the isolates with 43%, followed by the *qnrS* and *qnrA* genes with 34% and 23%, respectively (19). Further, in the study by Moghadasi et al., the *qnrB* gene showed the highest prevalence with 46.66% (17). In the study by Dehghan Banadkouki et al., the *qnrB* gene was isolated in 21 isolates (45.7%), the *qnrS* gene in 7 isolates (15.2%), and the *qnrA* gene was not detected in any of the isolates (16). In this study, a statistically significant association was observed between the presence of *qnr* genes and resistance to fluoroquinolones ($P<0.05$). Resistance to nalidixic acid was higher in isolates containing the *qnrA* gene compared to other antibiotics (41%). Besides, in the study by Eftekhar et al., isolates containing both *qnrB* and *aac(6')-Ib-cr* genes were significantly more resistant to quinolones ($P<0.05$) compared to isolates containing either gene alone. Eftekhar et al. suggested

that additional resistance mechanisms, such as mutations in the chromosomal genes *gyrA* and *gyrC* or the presence of efflux pumps such as *QepA*, are responsible for the high levels of resistance (20). These findings confirm that although *qnr* genes are not solely involved in quinolone resistance, they do reduce susceptibility to nalidixic acid and fluoroquinolones (20). The *qnr* genes protect quinolone targets in bacteria, and the coding genes are widely distributed in the *Enterobacteriaceae* family. The *qnr* genes are assumed to promote low to moderate quinolone resistance, while strains with mutations in the *gyrA* and *parC* genes or the plasmid-mediated *aac(6')-Ib-cr* and *qnr* genes exhibit high levels of quinolone resistance. Nourozi et al. reported that the purified *qnrB* gene, like the *qnrA* gene, protects DNA gyrase from quinolone action. Resistance to quinolones is also associated with loss of purine, specific efflux pump, or DNA gyrase alterations and a four- to eight-fold increase in expression (19). Amraie et al. further suggested that some genes are mobile among isolates and spread in the environment. A different mechanism of gene transfer, such as horizontal gene transfer between serotypes, could possibly cause the spread of resistance genes (18). In conclusion detection of virulence factors in *K. pneumoniae* isolates, especially combined with antibiotic resistance, is very important as it allows for assessing the possible course and site of infection in the human body and is essential for development of treatment strategy. Hence, today, much attention has been paid to molecular studies of resistance genes in *K. pneumoniae*. Although molecular methods exhibit very high sensitivity and specificity, their application is limited because these methods are not available in every laboratory and require specific skills and standards. Based on these results, *qnrA*-producing *K. pneumoniae* strains were isolated from patients. Thus, prescribing appropriate antibiotics and detection of *qnr* genes is required and it can be useful in tracking, treating and knowledge of *K. pneumoniae* infection prevalence rate.

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Authors' Contribution

Study concept and design: F. K. Acquisition of data: M. R. Analysis and interpretation of data: E. J. Drafting of the manuscript: T. N & R.F. Critical revision of the manuscript for important intellectual content: M. KS & MM. Statistical analysis: S.K Administrative, technical, and material support: M. H & R.F.

Ethics

The study design was approved by the ethics committee of the Sabzevar University of Medical Sciences, Sabzevar, Iran (IR.MEDSAB.REC.1400.133).

Conflict of Interest

The authors certify that they have no conflicts of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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