Characterization of Hypervirulent Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* Among Urinary Tract Infections: The First Report from Iran

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Introduction: This study was conducted to identify the hypermucoviscosity, iron acquisition, and capsule serotypes of *K. pneumoniae* strains isolated from urinary tract infections among community-acquired patients (CA) and assess the frequency of plasmid-mediated quinolone resistance (PMQR) and extended-spectrum beta-lactamases (ESBL) genes between classic and hypervirulent strains.

Materials and Methods: A total of 105 *K. pneumoniae* were isolated from CA-UTI. Demographic data related to the underlying diseases and clinical manifestations were further collected. Antibiotic resistance pattern and molecular characterization were compared among ESBL-positive, ESBL-negative, hypervirulent, and classic isolates.

Results: The results revealed that 52.4% of the isolates were confirmed as ESBL producers and 11 (10.5%) were considered as hypervirulent *K. pneumoniae* (hvKp). Ciprofloxacin and nalidixic acid were the most inactive antibiotics with resistance rates of 68.6% and 64.8%, respectively. Molecular characterization revealed that 7.6% of all the isolates carried *k1* and 66.6% carried *K2* genes. The most frequent ESBL gene was *bla_{SHV}* 63.8%, followed by *bla_{TEM}* 59.0%, and *bla_{CTX-M}* 58.1%. ESBL genes were significantly more in hvKp than in cKp. Moreover, 61 (84.7%), 47 (65.2%), and 16 (22.2%) of isolates harbored *qnrB*, *qnrS*, and *qnrA*. ESBL genes were detected in all hvKps, and *bla_{SHV}* was observed in 90.9% of hvKp (*P* value= 0.048, 95%).

Discussion: This study reported the high frequency of antimicrobial and multidrug resistance among hvKp isolates. Coexistence of PMQR and ESBL genes in hvKp indicates the necessity to enhance the clinical knowledge and management of hvKp infections.

Keywords: hypervirulent *Klebsiella pneumoniae*, urinary tract infections, capsular group, ESBL, *qnr*

Introduction

Klebsiella pneumoniae is a common Gram-negative organism with worldwide distribution. It causes nosocomial and community-acquired infections, liver abscess, and urinary tract infections, mostly in patients with underlying diseases.^{1,2} This organism is among the top three pathogens of international concern documented in the World Priority List of Antibiotic-Resistant Bacteria released by the World Health Organization (WHO) in 2018.³ Over the past two decades, *K. pneumoniae* has been the second most prevalent etiological agent of community-acquired (CA) urinary tract

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infections (UTI).⁴ There are mainly two pathotypes that pose a threat to public health: hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp). The most common subtype of the *K. pneumoniae* strains is classic *K. pneumoniae* (cKp); unlike this subtype, the new “hypervirulent” *K. pneumoniae* (hvKp) with hypermucoviscosity has emerged as a clinically important infectious agent causing extremely invasive infections such as liver abscesses in both healthy and immunocompromised individuals.^{5,6} These strains can cause serious CA infections in healthy individuals, along with the reported global dissemination of the growing antibiotic resistance.^{7,8} Therefore, hvKp increases the public health costs, restricts the selection of antibiotics, and causes failure in medical therapy, leading to mortality and increased morbidity.⁹

The hvKp strains are of hypermucoviscous type due to the overproduction of the capsular polysaccharide. Hypermucoviscosity is a crucial in vitro parameter for identifying hvKp.¹⁰ Moreover, several virulence genes such as the regulator of mucoid phenotype A gene (*rmpA*) and aerobactin (*iucA*) have been identified to be associated with the hypervirulent variant of *K. pneumoniae*.¹¹ Furthermore, the capsule, particularly serotypes K1 and K2, has long been known as a major virulence factor in *K. pneumoniae*.¹²

On one hand, fluoroquinolones such as ciprofloxacin and levofloxacin as well as β -lactam antibiotics are commonly recommended for complicated UTI treatment.¹³ On the other hand, there has been a global increase in extended-spectrum β -lactamases (ESBL)-producing Enterobacteriaceae and fluoroquinolone resistance.¹⁴ Plasmid-mediated quinolone resistance (PMQR) determinants have been further reported. PMQR includes Qnr (quinolone resistance) proteins (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) which protect the topoisomerase IV and DNA gyrase from quinolone inhibition, which acetylates aminoglycoside and ciprofloxacin and reduces their activity.¹⁵ PMQR genes are mostly on the same plasmid as ESBL genes.¹⁶ Resistance plasmids harboring genes encoding ESBLs can be transferred by conjugation, which helps disseminate PMQR determinants in different Enterobacteriaceae species.¹⁷

Multidrug-resistant (MDR) *K. pneumoniae* isolates are defined by non-susceptibility to at least one agent in three or more antibiotic categories, which severely limits antimicrobial treatment options.¹⁸ Hence, the coexistence of MDR-ESBLs and PMQR genes is a main concern, particularly in the community status.^{19,20} ESBL producers have been associated with resistance to non- β -lactam antimicrobials, including fluoroquinolones, aminoglycosides, and

sulfonamides, which are often used to treat and prevent diseases. Additionally, plasmid-mediated quinolone resistance (PMQR) genes are associated with ESBL production, and the spread of co-expressing PMQRs and ESBL isolates contributes to the growing concerns regarding MDR isolates.²¹ Also, hvKp strains are of major importance as they are hypervirulent and highly transmissible.¹⁸

To our knowledge, there is a paucity of information concerning the frequency of hvKp in CA-UTI and their antimicrobial susceptibility. Therefore, the objective of this study was to identify the hypermucoviscosity, iron acquisition, and capsule serotypes of *K. pneumoniae* strains isolated from CA-UTI; the frequency of PMQR (*qnrA*, *qnrB* and *qnrS*) and ESBL genes (*bla_{SHV}*, *bla_{TEM}*, *bla_{CTX-M}*) in ESBL and non-ESBL producer isolates was further assessed. In addition, cKp and hvKp strains were compared in terms of antimicrobial susceptibility and molecular characterizations.

Materials and Methods

A cross-sectional study was conducted at Milad Advanced Medical laboratory, Isfahan, Iran, between April and December 2019. All patient data accessed complied with the relevant data protection and privacy regulations. This study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.388), but no human participants were included. Urine samples were collected from outpatients who were suspected of UTI and had not consumed any antibiotics for the past two weeks. Samples were collected from the clean-catch midstream urine of adults and children aged less than two years using a sterile urine bag or a suprapubic catheter for sample collection. Demographic data and information related to underlying diseases and clinical manifestations were further collected.⁴ Urine samples were transferred to the microbiology laboratory at Isfahan University of medical science, then cultivated on blood agar and EMB media (Merck, Germany) for 24 hours. As described previously, a minimum colony count of 10^5 CFU/mL was considered as positive urine culture for midstream urine.²² Cases of mixed infection were excluded where they might reflect contamination with normal flora of the periurethral, vaginal, and perianal regions.²³ All isolates were primarily screened by their pigment production, colony morphology, and Gram-staining techniques. Isolates were then identified by conventional biochemical tests and standard culture methods. In this regard, citrate and urea positive, oxidase, indole,

methyl red, and motility negative strains were identified as *K. pneumoniae*.

String Test

Isolates were considered positive for the hypermucoviscosity phenotype after subculture on blood agar at 37°C overnight if an inoculation loop touching the surface of the colony generated a viscous string of 5 mm or more when pulled away from the plate.²⁴

Antimicrobial Susceptibility Testing

The antibiotic susceptibility profile was determined on Muller–Hinton agar (Merck, Germany) plates using the disk diffusion method (the modified Kirby–Bauer assay) as described by the Clinical and Laboratory Standards Institute (CLSI 2019).²⁵ Quality control strains were included to monitor the test performance. *K. pneumoniae* clinical isolates were tested for their susceptibility to the following antimicrobial agents: amoxicillin-clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefixime (5 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), trimethoprim–sulfamethoxazole (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), levofloxacin (5 µg), ofloxacin (5 µg), and piperacillin-tazobactam (100/10 µg).^{25,26} All disks were obtained from ROSCO Company, Denmark.

Screening of ESBL Producers

The preliminary screening test to detect ESBL activity was performed by the disk diffusion method according to the CLSI guidelines (2019). The inhibition zone size of ≤22 mm for ceftazidime (30 µg) and ≤27 mm for cefotaxime (30 µg) indicated ESBL production.²⁷ The phenotypic confirmatory test for ESBL was carried on by combination disk method using cefotaxime (30 µg) and ceftazidime (30 µg) alone and in combination with clavulanic acid (10 µg).^{28,29} ESBL activities were identified by ≥5 mm increase in zone diameter around the disk with the antibiotic combined with clavulanic acid.^{30,31} In brief, Muller Hinton agar plates were swabbed with 0.5 mL of overnight bacterial culture; after 10 minutes the antibiotic disk was placed on the plates and incubated for 24. Afterwards, the inhibition zone was measured and reported in diameter.³² *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the quality control strains.^{25,33}

Molecular Detection of Capsular Types and Virulence Genes

Genomic DNA was extracted from all *K. pneumoniae* isolates as described previously.³⁴ Polymerase chain reaction (PCR) for virulence-associated genes (such as *rmpA* and *iucA*) was carried out among the string test positive isolates. Moreover, using *K1* and *K2* specifying primers, all isolates were tested for the presence of *K. pneumoniae* capsular types.⁵

Molecular Detection of ESBL and PMQR Genes

ESBL genes (*bla_{SHV}*, *bla_{TEM}*, *bla_{CTX-M}*) were detected in all isolated uropathogens.³⁵ In addition, isolates that were phenotypically resistant to at least one fluoroquinolone were screened for *qnrA*, *qnrB*, and *qnrS* genes. PCR primers were previously described.¹⁰ Amplification was conducted as follows: initial denaturation at 95°C for five minutes, followed by 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for five minutes. The PCR products were analyzed by electrophoresis with 1% agarose gel in 1X Tris–Acetate–EDTA buffer. The gels were stained with stain load dye (CinnaGen Co, Iran) and the PCR products were visualized under UV light.^{36,37}

Statistical Analysis

The relationship among antibiotic resistance, ESBL production, and PMQR determinants was evaluated by the Chi-square test or Fisher's exact test. P-values ≤0.05 were considered as statistically significant. The data were analyzed using the Statistical Package for Windows v.19.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical Characteristics

Over the eight-month study period, 105 *K. pneumoniae* were isolated from the urine samples of CA patients in Isfahan. The mean age of patients was 46.50 years (1–81 years), and they comprised 32 (30.5%) males and 73 (69.5%) females. According to the demographic data of the patients, most had underlying diseases, including diabetes mellitus (33.5%), kidney transplant (13.3%), chronic renal insufficiency (20.9%), and immunosuppression (11.4%).

Out of the 105 positive urine cultures, 55 (52.4%) were confirmed as ESBL producer based on the combination disk method. MDR phenotype was detected in 59 out of

105 (56.1%) isolates. Of these 105 isolates, 94 (89.5%) were considered as cKp. Therefore, 11 isolates (10.5%) were string test positive and had two virulence genes (*iucA* and *rmpA*), hence considered as hvKp. As shown in Table 1, among these hvKp isolates, nine were females (81.8%), five (45.4%) were identified as MDR, and seven (63.6%) produced ESBL phenotype. Patients suffering from hvKp isolates had risk factors such as diabetes (7/11 (63.6%)), kidney transplant (3/11 (27.2%)), and immunosuppression (2/11 (18.1%)). Clinical and microbiological characteristics of the hvKp isolates are shown in Table 1.

Antimicrobial Resistance

The results revealed that ciprofloxacin and nalidixic acid were the most inactive antibiotics with resistance rates of 68.6% and 64.8%, respectively. However, the most effective antibiotics were meropenem, imipenem, amikacin, tobramycin, and piperacillin-tazobactam with resistance rates of 6.7%, 7.6% and 9.5%, respectively. There was no significant association among antimicrobial resistance, age, and gender ($P>0.05$). According to statistical analysis, the resistance rates of cefixime, cefotaxime, ciprofloxacin, ofloxacin, levofloxacin, and nalidixic acid were significantly higher in ESBL-positive than in ESBL-negative strains ($P\leq 0.05$). The resistance rates to most tested antimicrobial agents were lower in hvKp than in cKp isolates. Nonetheless, cefixime, trimethoprim-sulfamethoxazole, nitrofurantoin, and carbapenems had higher resistance rates. Table 2 depicts the complete results of antibiotic resistance patterns and compares the ESBL-negative and ESBL-positive isolates.

Molecular Analysis

Capsular Types

In this study, 8/105 (7.6%) isolates carried *k1* capsule gene, all belonging to hvKp ($P<0.0001$). Furthermore, 70/105 (66.6%) carried *K2* capsule gene, with only three (27.3%) associated with hvKp strain ($P=0.005$, Table 3). Twenty-seven isolates did not have *k1* and *K2* genes.

Detection of ESBL Genes

Although ESBLs were phenotypically detected in 55 (52.4%) of the isolates, electrophoresis analysis revealed that all isolates had at least one ESBL gene. Table 3 represents the frequency of these genes among ESBL-positive and negative isolates. The most frequent ESBL gene in the tested isolates was *bla_{SHV}* 67 (63.8%), followed by *bla_{TEM}* 62 (59.0%) and *bla_{CTX-M}* 61 (58.1%).

ESBL genes were more observed in hvKp than in cKp. According to the table, *bla_{SHV}* was found in 90.9% of hvKp group and 60.6% of cKp group ($P=0.048$).

Detection of PMQR Genes

In the current study, ESBL-producing isolates had a high level of resistance to all tested quinolones compared with non-ESBL-producing ones. In addition, 72 isolates that were resistant to at least one quinolone antibiotics were tested for *qnr* genes. The most common *qnr* gene was *qnrB* 61 (84.7%), followed by *qnrS* 47 (65.2%) and *qnrA* 16 (22.2%). Table 4 describes the distribution of *qnr* genes among ESBL-positive, ESBL-negative, hvkp, and ckp strains.

Results revealed that *qnr* subtypes could be present alone or in association with *bla_{CTX-M}*, *bla_{TEM}*, and *bla_{SHV}* genes. The simultaneous presence of two or three *qnr* genes was also detected. *qnrBS* combinations were most frequently associated with *bla_{CTX-M}* (54.11%), and 59.7% (43/72) of the isolates had both *qnrB* and *bla_{SHV}* genes. Statistical analysis showed that unlike ESBL genes, *qnr* genes were mostly present in cKp isolates ($P\text{-value}<0.05$ (Table 4)).

Discussion

To our knowledge, this is the first report on the prevalence of hvKp isolates among UTI samples in Iran. Over the recent years, hvKp has emerged as a global health issue and a potential “superbug” for clinical settings, causing a wide variety of infections among community patients.³⁸ In the current study, using a positive string test, 10.5% of *K. pneumoniae* were identified as hypervirulent, which was confirmed by the presence of the *rmpA* and *iucA* genes. Lin et al reported lower amount of hvkp in UTI samples (27.8%).⁴ In the present study, hvKp strain caused UTI mostly among old female patients. Diabetes mellitus was the most prevalent underlying disease 7/11 (63.6%). A previous study implied that patients with diabetes ran a higher risk for symptomatic UTI.³⁹ Other contributing underlying diseases were chronic renal insufficiency, kidney transplant, and immunosuppression. These results are similar to the previous findings on UTI in China.⁴

Consistent with previous reports, the rate of resistance to common antibiotics in hvKp strains was lower than that in the cKp group with the exception of cefixime, trimethoprim-sulfamethoxazole, nitrofurantoin, and carbapenems.⁴⁰ It is widely accepted that carbapenemase-producing hvKp (CR-hvKp) strains cause various life-threatening infections, especially in critical patients.^{41,42}

Table 1 Clinical and Microbiological Characteristics of hvKp Isolates

Strains Name Clinical Characteristics	Kp3	Kp8	Kp22	Kp27	Kp42	Kp50	Kp51	Kp71	Kp83	Kp97	Kp104
Age	78	63	72	45	52	37	45	1	70	60	43
Gender	Female	Female	Male	Female	Female	Female	Female	Female	Female	Female	Male
Risk factor	Diabetes/ Kidney transplant	Immunosuppress/ Chronic renal insufficiency	Diabetes	Kidney transplant	Diabetes	Diabetes\Immunosuppress	Diabetes	–	Diabetes	Diabetes	Kidney transplant
MDR	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Negative
ESBL	Positive	Positive	Positive	positive	Positive	Negative	Negative	Negative	Positive	Positive	Negative
Quinolone resistance	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Negative
String test (mm)	100	60	70	60	70	90	80	70	50	70	150
Virulence associated genes	<i>rmpA</i> <i>iucA</i> <i>K2</i> <i>tem</i> <i>ctx-M</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>Shv</i> <i>Tem</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K2</i> <i>Shv</i> <i>ctx-M</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>Shv</i> <i>ctx-M</i> <i>qnrA</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K2</i> <i>Shv</i> <i>tem</i> <i>ctx-M</i> <i>qnrA</i> <i>qnrB</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>Tem</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>tem</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>ctx-M</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>tem</i> <i>qnrA</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>tem</i> <i>qnrA</i> <i>qnrB</i> <i>qnrS</i>	<i>rmpA</i> <i>iucA</i> <i>K1</i> <i>Shv</i> <i>tem</i> <i>ctx-M</i> <i>qnrB</i> <i>qnrS</i>
Antibiotic resistance	CIP NAL CTX	CFM SXT CIP GEN IMI MERO PTZ	CFM SXT CIP	CFM SXT GEN CIP NAL NIT CTX	CFM SXT NAL NIT CTX	NAL	–	CTX	AMC CFM FEP SXT IMI CIP CTX	CFM FEP SXT IMI CIP NAL LEVO OFLA NIT CTX	NAL

Abbreviations: AMC, amoxicillin-clavulanic acid; CFM, cefixime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; OFLA, ofloxacin; LEVO, levofloxacin; CIP, ciprofloxacin; IMI, imipenem; MERO, meropenem; GEN, gentamycin; NAL, nalidixic acid; PTZ, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin.

Table 2 Percentage of Antimicrobial Resistance of *K. pneumoniae*

Total n=105			ESBL Positive n=55	ESBL Negative n=50	P value	ckp n=94	hvkp n=11	P value
R (%)			R (%)	R (%)		R (%)	R (%)	
Cephalosporins	Cefixime	55(52.4)	36(65.5)	18(36.0)	0.010	48(51.01)	6(54.5)	0.829
	Cefotaxime	64(61.0)	41(74.5)	23(46.0)	0.005	58(61.7)	6(54.5)	0.645
	Ceftazidime	50(47.6)	40(72.7)	10(20.0)	≥.01	46(48.9)	4(36.4)	0.827
	Cefepime	27(25.7)	21(38.2)	6(12.0)	0.009	25(26.6)	2(18.2)	0.618
	Amoxicillin-clavulanic	22(21.0)	16(29.1)	6(12.0)	0.032	21(22.3)	1(9.1)	0.307
Carbapenemase	Imipenem	7(6.7)	6(10.9)	1(2.0)	0.040	5(5.3)	2(18.2)	0.235
	Meropenem	7(6.7)	5(9.1)	2(4.0)	0.214	6(6.4)	1(9.1)	0.168
Beta-lactam inhibitor	Piperacillin-tazobactam	10(9.5)	8(14.5)	2(4.0)	0.107	9(9.6)	1(9.1)	0.780
30s	Gentamycin	24(22.9)	21(38.2)	3(6.0)	≥.01	22(23.4)	2(18.2)	0.560
	Amikacin	8(7.6)	7(12.7)	1(2.0)	0.041	8(8.5)	0(0.0)	0.524
	Tobramycin	8(7.6)	8(14.5)	0(0.0)	0.012	8(8.5)	0(0.0)	0.562
Fluoroquinolone	Ciprofloxacin	72(68.6)	46(83.6)	25(50.0)	≥.01	65(69.1)	6(54.5)	0.337
	Ofloxacin	30(28.6)	23(41.8)	7(14.0)	0.007	29(30.9)	1(9.1)	0.153
	Levofloxacin	30(28.6)	23(41.8)	7(14.0)	0.005	29(30.9)	1(9.1)	0.153
Quinolone	Nalidixic acid	68(64.8)	40(72.7)	23(46.0)	0.005	58(61.7)	5(45.5)	0.444
Sulfonamides	Trimethoprim-sulfamethoxazole	55(52.4)	32(58.2)	23(46.0)	0.441	49(52.1)	6(54.5)	0.508
Nitrofurans	Nitrofurantoin	25(23.8)	14(25.5)	11(22.0)	0.414	22(23.4)	3(27.3)	0.458

Table 3 Frequency of Different ESBL Genes and Capsular Serotype, Among ESBL Positive, ESBL Negative, hvkp and ckp

Genes	Total=105	ESBL Positive	ESBL Negative	P value	Within hvkp (%)	Within ckp (%)	P value
<i>bla_{SHV}</i>	67(63.8)	42(62.7)	25(37.3)	0.005	10(90.9)	57(60.6)	0.048
<i>bla_{TEM}</i>	62 (59.0)	33(53.2)	29(46.8)	0.835	7(63.6)	55(58.5)	0.744
<i>bla_{CTX-M}</i>	61 (58.1)	38(62.3)	23(37.7)	0.017	7(63.6)	54(57.4)	0.694
<i>K1</i>	8 (7.6)	4(50.0)	4 (50.0)	0.888	6(54.5)	2(2.1)	≥.01
<i>K2</i>	70(66.6)	35(50.0)	35 (50.0)	0.253	3(27.3)	66(70.2)	0.005

Empirical use of beta-lactam drugs for CA-UTI ensues increased rates of resistance and outbreaks. In the present study, 52.4% of isolates were ESBL producers, which is higher than that obtained in Taiwan (20.7%) and South Korea (30.0%).^{43,44} In Turkey (2019), on the other hand, 47% of *Klebsiella spp.* were ESBL producers.⁴⁵ These differences might be attributed to the excessive use of antibiotics and differences in risk factors. Unfortunately, phenotypic ESBLs were more in hvkp strains (7 (63.6%)) than in ckp (48 (51.1%)). In a study carried out in China in 2010, only 17% of hvkp were ESBL producers.⁴⁰ In the current study, all hvKps carried ESBL genes, implying the compatibility of plasmids containing these genes with hvKp strains.⁴⁶

Additionally, MDR-hvKp was identified in 5 out of 11 (45.4%) hvKp isolates. This shows the resistance to at least

one agent in three or more antimicrobial categories, which is higher than a study conducted in China (2019), where 20% MDR-hvKp was detected.⁶ Taken together, these data reveal that MDR-hvKp isolates are emerging and antimicrobial resistance is growing among these strains, which is similar to previous studies in China.⁶ Therefore, new clinical and medical interventions concerning these strains are needed.⁴⁰

Serotypes *K1* and *K2* have been generally considered as predominant virulent strains of *K. pneumoniae*, causing high resistance to phagocytosis.⁴⁷ In this research, 54.5% of hvKp possessed *k1* gene (p value<0.001) whereas 27.3% had *K2* gene (p value= 0.005). This is in accordance with a study carried out in the US in 2018 (55% *k1* vs 20% *k2*) and another performed in China in 2016 (33.3% *k1* vs 17.2 *k2*).^{46,48}

Table 4 Frequency of Different *qnr* Genes, Among ESBL Positive, ESBL Negative, hvkp and ckp Strains

Genes	Total= 72	ESBL Positive	ESBL Negative	P value	hvkp (%)	ckp (%)	P value
<i>qnrA</i>	16 (22.2)	6/12(50.0)	10/12(90.9)	0.033	3/7(42.9)	13/16(81.3)	0.066
<i>qnrB</i>	61 (84.7)	34/35(97.1)	27/27(100.0)	0.376	9/10(90.0)	52/52(100.0)	0.022
<i>qnrS</i>	47 (65.2)	26/28(92.9)	21/22(95.5)	0.701	7/10(70.0)	40/40(100.0)	≥.01

The resistance rates of the antibiotics to ESBL-positive strains between CA-UTIs were significantly higher than those of ESBL-negative strains ($P < 0.05$) (Table 2). Based on these results, ESBL-producing *Klebsiella* spp. are not merely a nosocomial problem, rather they are a growing issue in CA-UTIs.

The PCR revealed the prevalence of ESBL genes in the current study. Nevertheless, we found that *bla_{SHV}* was the most prevalent ESBL gene, followed by *bla_{TEM}* and *bla_{CTX-M}*. In a similar investigation done in Iraq (2019), 64.7% *bla_{TEM}*, 35.2% *bla_{SHV}*, and 41.1% *bla_{CTX-M}* genes existed in the isolates of *K. pneumoniae*.⁴⁹

ESBL producer strains are mostly resistant to common antibiotics such as fluoroquinolones.⁵⁰ Moreover, plasmid-mediated quinolone resistance might facilitate the spread and increased frequency of quinolone-resistant strains. A high proportion of isolates (68%) in this study were resistant to fluoroquinolones. According to the findings, resistance to the tested fluoroquinolones in ESBL-producing isolates was significantly higher than in non-ESBL-producing isolates, which is similar to another study performed in the Asia-Pacific region.⁵¹ In the current research, 46 (83.6%) of ESBL-positive *K. pneumoniae* isolates were resistant to ciprofloxacin, and their prevalence was higher than that of Meshkat (37.5%) in 2017.⁵²

So far, *qnr* genes have been widely detected in different parts of the world.⁵³ The frequencies found in the current research were higher than those reported in a Moroccan community in 2014, where the prevalence of *qnr* gene between 50% *Klebsiella* and 18.7% *E. Coli* was 23% *qnrB*, 10% *qnrA*, and 3% *qnrS*.⁵⁴ PMQR are plasmid-mediated and can be easily transmitted across the Enterobacteriaceae family members, thereby facilitating the epidemic spread of quinolone resistance through horizontal gene transfer.⁵⁵ Coexistence of PMQR and ESBL genes was identified in four out of 11 hvKp isolates. Although ESBL genes were mostly observed in hvKp than in cKp, *qnr* genes mostly existed in cKp strains ($P < 0.05$). These results imply that hvKp strains are capable of acquiring various types of antibiotic resistance mechanisms via the transmission of mobile

genetic elements. In addition to the disconcerting antimicrobial resistance pattern of hvKp strains, these isolates underscore the need for specific surveillance programs.

Conclusions

In this study, the frequency of hvkp was lower than similar studies in other regions; however, the higher rates of resistance to antimicrobial agents, particularly carbapenems, and the high rates of MDR emergence indicate that these strains have emerged as major issues in health-care centers. Moreover, the coexistence of PMQR and ESBL genes severely affects the clinical outcomes due to the complications in therapy, hence the necessity to enhance the clinical knowledge and management of hvKp infections. It is also important to take appropriate supportive measures regarding empirical antibiotic therapy so as to control the spread of resistant strains.

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Disclosure

The authors report no conflicts of interest in this work.

References

- Melot B, Colot J, Guerrier G. Bacteremic community-acquired infections due to *Klebsiella pneumoniae*: clinical and microbiological presentation in New Caledonia, 2008–2013. *Int J Infect Dis*. 2015;41:29–31. doi:10.1016/j.ijid.2015.10.013
- Ghanavati R, Darban-Sarokhalil D, Navab-Moghadam F, Kazemian H, Irajian G, Razavi S. First report of coexistence of AmpC beta-lactamase genes in *Klebsiella pneumoniae* strains isolated from burn patients. *Acta Microbiol Immunol Hung*. 2017;64(4):455–462. doi:10.1556/030.64.2017.028
- Shrivastava SR, Shrivastava PS, Ramasamy J. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *J Med Soc*. 2018;32(1):76.
- Lin W-H, Wang M-C, Tseng C-C, et al. Clinical and microbiological characteristics of *Klebsiella pneumoniae* isolates causing community-acquired urinary tract infections. *Infection*. 2010;38(6):459–464.

5. Prokesh BC, TeKippe M, Kim J, Raj P, TeKippe EM, Greenberg DE. Primary osteomyelitis caused by hypervirulent *Klebsiella pneumoniae*. *Lancet Infect Dis*. 2016;16(9):e190–e5. doi:10.1016/S1473-3099(16)30021-4
6. Liu C, Guo J. Hypervirulent *Klebsiella pneumoniae* (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. *Ann Clin Microbiol Antimicrob*. 2019;18(1):4. doi:10.1186/s12941-018-0302-9
7. Zhang Y, Zeng J, Liu W, et al. Emergence of a hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolate from clinical infections in China. *J Infect*. 2015;71(5):553–560.
8. Zhan L, Wang S, Guo Y, et al. Outbreak by hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a tertiary hospital in China. *Front Cell Infect Microbiol*. 2017;7:182.
9. Patel PK, Russo TA, Karchmer AW, editors. Hypervirulent *Klebsiella pneumoniae*. In: *Open Forum Infectious Diseases*. Oxford University Press; 2014.
10. Liu YM, Zhang YY, Zhang W, Shen H, Li H, Cao B. Clinical and molecular characteristics of emerging hypervirulent *Klebsiella pneumoniae* bloodstream infections in mainland China. *Antimicrob Agents Chemother*. 2014;58:5379–5385. doi:10.1128/AAC.02523-14
11. Ye M, Tu J, Jiang J, et al. Clinical and genomic analysis of liver abscess-causing *Klebsiella pneumoniae* identifies new liver abscess-associated virulence genes. *Front Cell Infect Microbiol*. 2016;6(165). doi:10.3389/fcimb.2016.00165
12. Zhao Y, Zhang X, Torres VL, et al. An outbreak of carbapenem-resistant and hypervirulent *Klebsiella pneumoniae* in an intensive care unit of a major teaching hospital in Wenzhou, China. *Front Public Health*. 2019;7:229.
13. Bajaj P, Singh NS, Viridi JS. *Escherichia coli* β -lactamases: what really matters. *Front Microbiol*. 2016;7:417.
14. Ni Q, Tian Y, Zhang L, et al. Prevalence and quinolone resistance of fecal carriage of extended-spectrum β -lactamase-producing *Escherichia coli* in 6 communities and 2 physical examination center populations in Shanghai, China. *Diagn Microbiol Infect Dis*. 2016;86(4):428–433.
15. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev*. 2009;22(4):664–689.
16. Garcia-Fulgueiras V, Bado I, Mota MI, et al. Extended-spectrum β -lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay. *J Antimicrob Chemother*. 2011;66(8):1725–1729. doi:10.1093/jac/dkr222
17. Rodríguez-Martínez JM, Velasco C, Pascual A, Cano ME. Plasmid-mediated quinolone resistance: an update. *J Infect Chemother*. 2011;17(2):149–182. doi:10.1007/s10156-010-0120-2
18. Magiorakos A-P, Srinivasan A, Carey R, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–281.
19. Mansouri S, Abbasi S. Prevalence of multiple drug resistant clinical isolates of extended-spectrum beta-lactamase producing Enterobacteriaceae in Southeast Iran. *Iran J Med Sci*. 2015;35(2):101–108.
20. Di Tella D, Tamburro M, Guerrizio G, et al. Molecular epidemiological insights into Colistin-resistant and Carbapenemases-producing clinical *Klebsiella pneumoniae* isolates. *Infect Drug Resist*. 2019;12:3783.
21. Liu X, Liu H, Wang L, et al. Molecular characterization of extended-spectrum β -lactamase-producing multidrug resistant *Escherichia coli* from swine in Northwest China. *Front Microbiol*. 2018;9:1756.
22. CDC/HICPAC. *Urinary Tract Infection (UTI) Event for Long-Term Care Facilities*. Atlanta, GA, USA: CDC; 2009.
23. Sathiananthamoorthy S, Malone-Lee J, Gill K, et al. Reassessment of routine midstream culture in diagnosis of urinary tract infection. *J Clin Microbiol*. 2019;57(3):e01452–18.
24. Cubero M, Marti S, M D, Gonzlez-Daz A, Berbel D, Ardanuy C. Hypervirulent *Klebsiella pneumoniae* serotype K1 clinical isolates form robust biofilms at the air-liquid interface. *PLoS One*. 2019;14(9):e0222628.
25. Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing; Twenty-ninth informational supplement CdM-SCaLSI, Wayne, PA.
26. Shoja S, Ansari M, Faridi F, et al. Identification of carbapenem-resistant *Klebsiella pneumoniae* with emphasis on New Delhi metallo-beta-lactamase-1 (bla NDM-1) in Bandar Abbas, South of Iran. *Microb Drug Resist*. 2018;24(4):447–454.
27. Rajivgandhi G, Maruthupandy M, Veeramani T, Quero F, Li WJ. Anti-ESBL investigation of chitosan/silver nanocomposites against carbapenem resistant *Pseudomonas aeruginosa*. *Int J Biol Macromol*. 2019;132:1221–1234.
28. Rajivgandhi G, Maruthupandy M, Ramachandran G, Priyanga M, Manoharan N. Detection of ESBL genes from ciprofloxacin resistant Gram negative bacteria isolated from urinary tract infections (UTIs). *Front Lab Med*. 2018;2(1):5–13.
29. Rajivgandhi G, Vijayan R, Maruthupandy M, Vaseeharan B, Manoharan N. Antibiofilm effect of *Nocardiosis* sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs. *Microb Pathog*. 2018;118:190–198.
30. Rajivgandhi G, Ramachandran G, Maruthupandy M, Senthil R, Vaseeharan B, Manoharan N. Molecular characterization and antibacterial investigation of marine endophytic actinomycetes *Nocardiosis* sp. GRG 2 (KT 235641) compound against isolated ESBL producing bacteria. *Microb Pathog*. 2018.
31. Rajivgandhi G, Maruthupandy M, Muneeswaran T, et al. Biologically synthesized copper oxide nanoparticles enhanced intracellular damage in ciprofloxacin resistant ESBL producing bacteria. *Microb Pathog*. 2019;127:267–276.
32. Maruthupandy M, Muneeswaran T, Song J-M, Manoharan N. Biologically synthesized zinc oxide nanoparticles as nanoantibiotics against ESBLs producing gram negative bacteria. *Microb Pathog*. 2018;121:224–231. doi:10.1016/j.micpath.2018.05.041
33. Halaji M, Shahidi S, Atapour A, et al. Characterization of extended-spectrum β -lactamase-producing uropathogenic *Escherichia coli* among Iranian kidney transplant patients. *Infect Drug Resist*. 2020;13:1429.
34. Chen W-P, Kuo -T-T. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Res*. 1993;21(9):2260.
35. Rajivgandhi G, Maruthupandy M, Manoharan N. Detection of TEM and CTX-M genes from ciprofloxacin resistant *Proteus mirabilis* and *Escherichia coli* isolated from urinary tract infections (UTIs). *Microb Pathog*. 2018;121:123–130.
36. Dallenne C, Decr D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β -lactamases in Enterobacteriaceae. *J Antimicrob Chemother*. 2010;65(3):490–495. doi:10.1093/jac/dkp498
37. Chen X, Zhang W, Pan W, et al. Prevalence of qnr, aac(6′)-Ib-cr, qepA, and oqxAB in *Escherichia coli* isolates from humans, animals, and the environment. *Antimicrob Agents Chemother*. 2012;56(6):3423–3427. doi:10.1128/AAC.06191-11
38. Lee C-H, Chuah S-K, Tai W-C, Chang -C-C, Chen F-J. Delay in human neutrophil constitutive apoptosis after infection with *Klebsiella pneumoniae* serotype K1. *Front Cell Infect Microbiol*. 2017;7:87.
39. Stamm WE, Hooton TM, Johnson JR, et al. Urinary tract infections: from pathogenesis to treatment. *J Infect Dis*. 1989;159(3):400–406.
40. Li W, Sun G, Yu Y, et al. Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in China. *Clin Infect Dis*. 2014;58(2):225–232.
41. Zhang R, Lin D, Chan EW-C, et al. Emergence of carbapenem-resistant serotype K1 hypervirulent *Klebsiella pneumoniae* strains in China. *Antimicrob Agents Chemother*. 2016;60(1):709–711.

42. Ripabelli G, Sammarco ML, Scutellà M, Felice V, Tamburro M. Carbapenem-resistant KPC-and TEM-producing *Escherichia coli* ST131 isolated from a hospitalized patient with urinary tract infection: first isolation in Molise region, Central Italy, July 2018. *Microb Drug Resist*. 2020;26(1):38–45.
43. Kao C-Y, Udval U, Huang Y-T, et al. Molecular characterization of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella* spp. isolates in Mongolia. *J Microbiol Immunol Infect*. 2016;49(5):692–700.
44. Yang Y-S, Ku C-H, Lin J-C, et al. Impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* on the outcome of community-onset bacteremic urinary tract infections. *J Microbiol Immunol Infect*. 2010;43(3):194–199.
45. Koksai E, Tulek N, Sonmezer MC, et al. Investigation of risk factors for community-acquired urinary tract infections caused by extended-spectrum beta-lactamase *Escherichia coli* and *Klebsiella* species. *Investig Clin Urol*. 2019;60(1):46–53.
46. Zhang Y, Zhao C, Wang Q, et al. High prevalence of hypervirulent *klebsiella pneumoniae* infection in china: geographic distribution, clinical characteristics, and antimicrobial resistance. *Antimicrob Agents Chemother*. 2016;60:6115–6120. doi:10.1128/AAC.01127-16
47. Lin J-C, Chang F-Y, Fung C-P, et al. High prevalence of phagocytic-resistant capsular serotypes of *Klebsiella pneumoniae* in liver abscess. *Microbes Infect*. 2004;6(13):1191–1198.
48. Russo TA, Olson R, Fang C-T, et al. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. *J Clin Microbiol*. 2018;56(9):e00776–18.
49. Pishtiwan AH, Khadija KM. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated from thalassemia patients in Erbil, Iraq. *Mediterr J Hematol Infect Dis*. 2019;11(1).
50. Yadav KK, Khadka R, Pant AD, Shah B. Multidrug resistant Enterobacteriaceae and extended spectrum beta-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. *Antimicrob Resist Infect Control*. 2015;4:42.
51. Lu P-L, Liu Y-C, Toh H-S, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the study for monitoring antimicrobial resistance trends. *Int J Antimicrob Agents*. 2012;40:S37–S43. doi:10.1016/S0924-8579(12)70008-0
52. Izadi N, Nasab MN, Mood EH, Meshkat Z. The frequency of qnr genes in extended-spectrum β -lactamases and non-ESBLs *Klebsiella pneumoniae* species isolated from patients in Mashhad, Iran. *Iran J Pathol*. 2017;12(4):377.
53. Salah FD, Soubeiga ST, Ouattara AK, et al. Distribution of quinolone resistance gene (qnr) in ESBL-producing *Escherichia coli* and *Klebsiella* spp. in Lomé, Togo. *Antimicrob Resist Infect Control*. 2019;8(1):104.
54. Jamali L, Haouzane F, Bouchajou M. Prevalence of plasmid mediated quinolone resistance genes among enterobacteria isolates in Moroccan community. *Int J Innov Sci Res J*. 2014;11:387–399.
55. Jacoby G, Strahilevitz J, Hooper D. Plasmid-mediated quinolone resistance. *Microbiol Spectr*. 2014;2. doi:10.1128/microbiolspec.PLAS-0006-2013

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