The Face of Hypervirulent *Klebsiella Pneumoniae* (hvKp) Isolated from Clinical samples of Two Iranian Teaching Hospitals

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Research

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Abstract

Hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged as a pathogen of global concern. In this study, both phenotypic and genotypic tests were used to detect hvKp. Antimicrobial resistance profiles and clonal relatedness of clinical isolates were also determined. We found that 62.6% of the isolates were tellurite resistant and among them *iucA* or *iutA* or *peg344* as hvKp molecular markers, were positive. The *bla*_{SHV} (81.4%), followed by *bla*_{CTX-M15} (75.5%) and *bla*_{TEM} (67.6%), *bla*_{OXA-48} (33.7%), *bla*_{NDM-1} (32.3%) were detected, while *bla*_{KPC-1} was not present in any hvKp isolates. It was found that the majority of hvKp isolates belonged to capsular serotype K20 and *ompK36* group C, which is related to CG23 (e.g. ST23). A high percentage of multidrug-resistant hvKp (MDR-hvKp) and high resistance to imipenem (66%) indicated that there is an urgent problem that should be addressed in the clinical settings.

1. Introduction

Hypervirulent *Klebsiella pneumoniae* (hvKp), an emerging pathotype of *K. pneumoniae* was first reported from Taiwan. It was identified as an important cause of pyogenic liver abscess (1, 2). In hvKp isolates, pLVPK-like plasmids (Large Virulence Plasmid of *K. pneumoniae*) encoding virulence factor genes including capsular polysaccharide synthesis regulators (*rmpA* and *rmpA2*) and iron acquisition systems (*iuc*, *iut*, and *iro* siderophore gene cluster), a metabolic transporter (*peg-344*) and also heavy metal resistance genes (copper, silver, lead, and tellurite), have been identified (3). Therefore, most hvKp isolates are able to reduce tellurite and form a black colony due to the presence of the major virulence plasmids containing a tellurite resistance gene (4). The pLVPK-like plasmids may carry all virulence factor genes or have lost some of them (5, 6). On the other hand, acquisition of antibiotic resistance plasmids or insertion of resistant mobile genetic elements into the hvKp plasmid turns them into superbugs that can be termed hyper-resistant hvKp strains (7–9). Some *K. pneumoniae* clones are characterized as high-risk clones that play an important role in the spread of antibiotic-resistant strains (10, 11).

The association of the porin *ompK36* with clonal relatedness of *K. pneumoniae* isolates has been described in several studies (12, 13). Four different genotypes for *ompK36* porin (A to D) in *K. pneumoniae* were defined and the correlation of different variants of *ompK36* with specific sequence types (STs) was illustrated (10, 12, 13). The objectives of the current study were to investigate the phenotypic and genotypic identification of hvKp isolates, prevalence of virulence factors, antibiotic resistance and molecular epidemiology of hvKp strains obtained from two Iranian educational hospitals.

2. Materials And Methods

2.1. Bacterial isolation and identification

In this cross-sectional study, we collected a total of 477 non-repetitive *K. pneumoniae* as clinical isolates from two educational hospitals in Tehran over a period of time from June 2019 to December 2020. All bacterial isolates were identified using standard biochemical laboratory methods and then the isolates were stored in a freezer at -70°C in nutrient broth containing 20% glycerol until further studies.

2.2. HvKp phenotypic identification

2.2.1. Tellurite resistance

We used tellurite agar culture as a rapid screening test in this study. The isolates that formed black colonies on this tellurite-containing selective medium were selected as presumptive hypervirulent strains for further study. For this purpose, 0.1 g of potassium tellurite powder was first dissolved in 10 ml of sterile distilled water and filtered using membrane filters of pore size 0.45 µm. Then we added 300 µl of the potassium tellurite solution to 100 ml of Mueller-Hinton agar medium, which was autoclaved and cooled to 45–50°C. Finally, we dispensed into sterile plates. Colonies were examined after overnight incubation at 37°C (This study).

2.2.2. String Test

Hypermucoviscous phenotype of the hvKp isolates was examined by the string test, and the positive test was confirmed via the formation of a 5-mm mucoviscous filament by stretching of bacterial colonies on a blood agar after 24 h of incubation at 37°C (14).

2.3. Molecular Characteristics

2.3.1. DNA extraction and identification

Plasmid DNA extraction Mini Kit (FAVORGEN Biotech Corporation, Taiwan) has been used for the detection of genes carried on plasmids. In addition, the boiling method was used for isolation of genomic DNA (15). All amplification reactions for PCR assays were prepared in a total volume of 25 µl. The list of primer sequences, PCR product sizes, and PCR conditions is shown in Table 1. Finally, all PCR amplification products were sequenced and then matched against the GenBank database using BLAST tool (<http://www.ncbi.nlm.nih.gov/blast/>).

2.3.2. HvKp molecular identification

All tellurite-resistant *K. pneumoniae* were screened for the presence of the aerobactin (*iucA*), its receptor (*iutA*) genes and *peg344*. The isolates containing the *iucA* or *iutA* or *peg344* genes were considered as hvKps (16).

2.4. Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion

method. according to the clinical and laboratory standards institute (CLSI) guidelines (CLSI 2018-M100-S28) by the following antibiotic discs including amikacin (AK), gentamicin (GN), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), imipenem (IMI), meropenem (MRP), cefepime (FEP), ciprofloxacin (CIP), ampicillin (AMP) and aztreonam (AZM). Minimum inhibitory concentration (MICs) of imipenem and ceftazidime were determined by broth dilution method. *Escherichia coli* ATCC 25922 was used as the quality control strain for antimicrobial susceptibility testing.

2.5. Capsular genotyping and detection of virulence genes, and antimicrobial resistance genes

The hvKp capsular serotypes K1, K2, K5, K20, K54, and K57 were identified using PCR method. The hvKp virulence genes including salmochelin siderophore (*iroB*), mucoviscosity-associated gene (*magA*), *Klebsiella* ferric uptake (*kfu*), yersiniabactin (*ybt*), allantoin metabolism gene (*allS*), and *rmpA* were detected by specific primers listed in Table 1. In addition, PCR assays were carried out for detection of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M15}, *bla*_{KPC-1}, *bla*_{NDM-1}, and *bla*_{OXA-48} genes for all hvKp isolates.

2.6. Determination of clonal relatedness using *ompK36* typing

All hvKp isolates were subjected to *ompK36* typing by the PCR-based method described by Yan *et al.*, using four primer pairs (12).

Table 1
Primers, product size, and annealing temperature used for this study

Primer name	Primer sequence (5' → 3')	Amplicon size (bp)	Annealing temperature (°C)	REF.
<i>iucA</i>	F: AATCAATGGCTATTCCCGCTG R: CGCTTCACTTCTTTCACTGACAGG	239	62	(16)
<i>peg 344</i>	F: GCGGGAAGGACAGAAAGCCAGTG R: GAGGGAAGATGAGAAATACGAGC	332	56	This study
<i>iutA</i>	F: GCCGCTAGGTTGGTGATGT R: CTCTGGTCGTGCTGGTTGA	949	61	This study
<i>iroB</i>	F: GTGTTGGATTCCGCCAGTGA R: TTCCGCCGCTACCTCTTCA	366	61	This study
<i>magA</i>	F: GGTGCTCTTTACATCATTGC R: GCAATGGCCATTTGCGTTAG	1282	51	(17)
<i>rmpA</i>	F: GAGTATTGGTTGACAGCAGGAT R: AGCCGTGGATAATGGTTTACAA	250	53	This study
<i>kfu</i>	F: ATAGTAGGCGAGCACCGAGA R: AGAACCTTCCTCGCTGAACA	520	60	(18)
<i>allS</i>	F: CCGAAACATTACGCACCTTT R: ATCACGAAGAGCCAGGTCAC	508	60	(18)
<i>ybt</i>	F: GACGGAAACAGCACGGTAAA R: GAGCATAATAAGGCGAAAGA	242	60	(17)
<i>bla_{CTX-M15}</i>	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	590	53	This study
<i>bla_{TEM}</i>	F: GAGTATTCAACATTTCCGTGTC R: TAATCAGTGAGGCACCTATCTC	800	54	(19)
<i>bla_{SHV}</i>	F: AAGATCCACTATCGCCAGCAG R: ATTCAGTTCCGTTTCCCAGCGG	200	60	(19)
<i>bla_{OXA-48}</i>	F-GCGTGTTAAGGATGAACAC R-CATCAAGTTCAACCCAACCG	745	60	(19)
<i>bla_{KPC-1}</i>	F: CGTCTAGTTCTGCTGTCTTG R:CTTGTCATCCTTGTTAGGCG	798	55	(20)
<i>bla_{NDM-1}</i>	F: GGTGTTGGCGATCTGGTTTTTC R: CGGAATGGCTCATCACGATC	621	54	(20)
Ompk36 group A	F: GAAGGCGCTCTGTCTCCTA R: TGCCATCATAGATGTCATAGG	97	60	(12)

Primer name	Primer sequence (5' →3')	Amplicon size (bp)	Annealing temperature (°C)	REF.
Ompk36 group B	F: CGGTCGTGGCGCGCAGAAA R: GGTTGTTCTGA TCGTCGGTA	125	64	(12)
Ompk36 group C	F: CAACAACGGTCGT GGTTGGA R: CCCAGTGCCGGAACACTATT	144	62	(12)
Ompk36 group D	F: GAAGGTACTTCTCCGACCAA R: AATCAGATTCTCCGTTGCCG	283	62	(12)

3. Results

3.1. Phenotypic tests

In this study, out of 477 *K. pneumoniae* isolates, 163 (34.2%) were able to grow on tellurite-containing selective medium and were considered tellurite-resistant strains, so they were selected for the molecular identification test. In addition, 62 out of the 477 *K. pneumoniae* isolates (13%) were reported with positive string test and hypermucoviscous phenotype.

3.2. Molecular identification of hvKp

On molecular identification (16), we found the *iucA* or *iutA* or *peg344* as hvKp molecular markers in 21.4% (102/477) of total *K. pneumoniae* and 62.6% (102/163) of tellurite-resistant isolates. Thus, 45 isolates had only the *iucA*, 6 isolates had only the *iutA*, and 48 strains had both the *iucA* and *iutA* genes, all three genes (*iucA*, *iutA* and *peg344*) were detected simultaneously in only three hvKp isolates. Also 48% (49/102) hvKp isolates were string positive. (Table 2)

Table 2
Epidemiology, phenotypic and molecular characteristics of the hvKp isolates included in this study.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	Internal	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Blood	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Emergency	Urine	Pos	<i>iucA</i> , <i>kfu</i> , <i>ybt</i>	ND	B	ND	<i>bla</i> _{SHV}	ND ND
A	ICU	Tracheal	Neg	<i>iucA</i>	K20	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
A	Surgery	Abscess	Pos	<i>iucA</i> , <i>ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
A	Surgery	Abscess	Pos	<i>iucA</i>	ND	C	ND	<i>bla</i> _{SHV}	ND ND
A	Surgery	Abdominal Secretions	Pos	<i>peg-344</i> , <i>iucA</i> , <i>iutA</i> , <i>iro</i> , <i>ybt</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	16 × 2
A	ICU	Tracheal	Neg	<i>iucA</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
A	ICU	Urine	Pos	<i>iucA</i> , <i>kfu</i>	ND	B	Susceptible	ND	ND ND
A	ICU	Blood	Neg	<i>iucA</i> , <i>ybt</i>	ND	A	CTX	<i>bla</i> _{SHV}	ND ND
A	Orthopedics	Synovial fluid	Neg	<i>iucA</i> , <i>kfu</i>	ND	B	Susceptible	ND	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	Out-patient	Urine	Pos	<i>iucA, ybt</i>	ND	C	GN, CAZ	<i>bla</i> _{TEM} and <i>bla</i> _{SHV}	ND ND
A	ICU	Abscess	Neg	<i>iucA, iutA</i>	K20	C	CTX, GN, IMI, CRO, CAZ	<i>bla</i> _{TEM}	ND ND
A	Internal	Urine	Neg	<i>iucA</i>	ND	A	ND	<i>bla</i> _{TEM} and <i>bla</i> _{SHV}	ND ND
A	ICU	Urine	Pos	<i>iucA, kfu, ybt</i>	K2	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Internal	Urine	Neg	<i>iucA, ybt</i>	ND	D	ND	<i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Out-patient	Urine	Pos	<i>iucA, ybt</i>	ND	C	Susceptible	ND	ND ND
A	Internal	Urine	Pos	<i>iucA, kfu, ybt</i>	ND	C	ND	<i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	Out-patient	Urine	Neg	<i>iucA, iutA, ybt, rmpA</i>	ND	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{NDM-1}	ND ND
A	Out-patient	Urine	Pos	<i>iucA, kfu</i>	ND	A	ND	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	Out-patient	Urine	Pos	<i>iucA, ybt</i>	K20	B	ND	<i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	Internal	Urine	Neg	<i>iucA, kfu</i>	ND	C	ND	<i>bla</i> _{SHV}	ND ND
A	Internal	Sputum	Pos	<i>peg-344, iucA, iutA, ybt, rmpA</i>	K20	C	CAZ	ND	ND ND
A	Surgery	Abdominal Secretions	Pos	<i>iucA</i>	ND	A	ND	<i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	Emergency	Urine	Pos	<i>iucA, kfu, ybt</i>	K2	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Out-patient	Urine	Pos	<i>iucA, kfu, ybt</i>	K2	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Surgery	Urine	Pos	<i>iucA, kfu, ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
A	Emergency	Urine	Pos	<i>iucA, kfu</i>	ND	A	ND	<i>bla</i> _{SHV}	ND ND
A	ICU	Blood	Pos	<i>iucA, kfu</i>	ND	D	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{SHV} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
A	Orthopedics	Wound	Neg	<i>iucA, ybt</i>	ND	D	Susceptible	ND	ND ND
A	Graft	Urine	Neg	<i>iucA, kfu, rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Out-patient	Urine	Pos	<i>iucA, kfu</i>	ND	C	CTX, GN, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	Graft	Urine	Neg	<i>iucA, ybt, allS, rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} , <i>bla</i> _{NDM-1}	16 4
A	ICU	Abscess	Neg	<i>iucA</i>	ND	C	Susceptible	ND	ND ND
A	ICU	Urine	Neg	<i>iucA</i>	ND	C	CTX, FEP, CAZ	<i>bla</i> _{SHV}	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	ICU	Blood	Neg	<i>iucA</i>	ND	D	ND	<i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Abscess	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	ND	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{NDM-1}	64 ¼4
A	Internal	Tracheal	Neg	<i>iucA</i> , <i>ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i>	ND	C	CTX, GN, FEP, CRO, AK, CAZ, CIP	<i>bla</i> _{SHV}	ND ND
A	Surgery	Wound	Neg	<i>iucA</i> , <i>kfu</i> , <i>ybt</i>	ND	C	CTX, CRO, CIP	<i>bla</i> _{SHV}	ND ND
A	Internal	Abscess	Neg	<i>iucA</i> , <i>ybt</i>	ND	D	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Surgery	Abscess	Neg	<i>iucA</i> , <i>ybt</i>	ND	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Emergency	Urine	Neg	<i>iucA</i> , <i>kfu</i> , <i>ybt</i>	ND	C	Susceptible	ND	ND ND
A	Internal	Sputum	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i>	K20	C	CTX, GN, FEP, CRO, CAZ	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	Out-patient	Urine	Neg	<i>iucA</i> , <i>kfu</i> , <i>ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP	<i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Abscess	Pos	<i>iucA</i> , <i>ybt</i>	ND	D	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	¼16 8

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	Emergency	Blood	Neg	<i>iucA</i> , <i>kfu</i>	ND	B	AK	ND	ND ND
A	ICU	Tracheal	Neg	<i>iucA</i> , <i>ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Blood	Pos	<i>iucA</i> , <i>ybt</i>	ND	D	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Neurology	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Emergency	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Internal	Wound	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	16 2
A	Emergency	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	ND	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	32 4
A	ICU	Bal	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	Surgery	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
A	ICU	Blood	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	16 4
A	ND	ND	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	ND	ND	Pos	<i>iucA</i> , <i>iutA</i> , <i>rmpA</i>	ND	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ND	ND	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	D	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	64 4
A	Emergency	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} and <i>bla</i> _{CTX-M15}	ND ND
A	ICU	CSF	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
A	ICU	Tracheal	Pos	<i>iucA</i> , <i>ybt</i>	K20	D	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ND
A	ICU	Blood	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
B	Infectious	Sputum	Neg	<i>iutA</i> , <i>ybt</i>	ND	C	CTX, GN, FEP, CRO, CAZ, CIP	<i>bla</i> _{CTX-M15}	ND ND
B	ND	ND	Pos	<i>iucA</i> , <i>ybt</i>	ND	D	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	64 8

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
B	ICU	Urine	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 16
B	Infectious	Urine	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	16 4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 4
B	ICU	Tracheal	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	64 4
B	ICU	Urine	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, FEP, CRO, CAZ, CIP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND 2
B	ND	ND	Neg	<i>iucA</i> , <i>kfu</i> , <i>ybt</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{NDM-1}	16 4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 4
B	ND	ND	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, AK, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
B	ICU	Blood	Pos	<i>peg-344</i> , <i>luc</i> , <i>iutA</i> , <i>iro</i> , <i>kfu</i> , <i>ybt</i> , <i>magA</i> , <i>allS</i>	K1	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	≤16 ≤2
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15}	ND 2
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND 2
B	ICU	Urine	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15}	ND 2
B	Surgery	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, CAZ, CIP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
B	ICU	Tracheal	Pos	<i>iutA</i> , <i>iro</i> , <i>rmpA</i>	ND	C	CTX, GN, FEP, CRO, AK, CAZ, CIP	<i>bla</i> _{CTX-M15}	ND ND
B	ICU	Tracheal	Neg	<i>iutA</i>	ND	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} and <i>bla</i> _{SHV}	ND ND
B	Internal	Urine	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND 4
B	Internal	Tracheal	Pos	<i>iucA</i> , <i>iutA</i> , <i>kfu</i> , <i>ybt</i> , <i>rmpA</i>	K20	D	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{OXA-48}	ND ≤4
B	ND	ND	Pos	<i>iucA</i> , <i>iutA</i> , <i>kfu</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, FEP, CRO, AK, CAZ, CIP	<i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
B	ND	ND	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, FEP, CRO, CAZ, CIP	<i>bla</i> _{SHV} and <i>bla</i> _{CTX-M15}	ND ND
B	ICU	Tracheal	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	32 ¼4
B	Infectious	Tracheal	Neg	<i>iutA</i> , <i>ybt</i>	ND	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 ¼4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	32 4
B	ICU	Tracheal	Neg	<i>iutA</i>	K20	A	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} and <i>bla</i> _{NDM-1}	ND ¼4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 ¼4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ¼1

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 16
B	ICU	CSF	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	128 $\pm$ 4
B	ICU	Urine	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 $\pm$ 4
B	ICU	Bal	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 4
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	64 $\pm$ 4
B	ICU	Tracheal	Pos	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND $\pm$ 4
B	Surgery	Blood	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 256

* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; *iuc* and *iutA*, aerobactin genes; *ybt*, yersiniabactin; *rmpA*, regulator of mucoid phenotype; *Kfu*, Klebsiella iron uptake; *magA*, mucoviscosity-associated gene; *iroB*, salmochelin iron uptake systems; *allS*, allantoin metabolism gene; *peg-344*, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.

Hospital	Ward	Source	String test	Virulence factor genes	Capsule serotype	<i>ompK36</i> typing	Antibiotic resistance pattern	Antibiotic resistance genes	MIC (µg/ml) CAZ IMI
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 16
B	Surgery	Wound	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND ND
B	ICU	Tracheal	Pos	<i>iucA</i> , <i>iutA</i> , <i>iro</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, AK, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 8
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 8
B	ICU	Tracheal	Neg	<i>iucA</i> , <i>iutA</i> , <i>ybt</i> , <i>rmpA</i>	K20	C	CTX, GN, IMI, FEP, CRO, CAZ, CIP, MRP, AZM	<i>bla</i> _{SHV} , <i>bla</i> _{CTX-M15} , <i>bla</i> _{OXA-48} and <i>bla</i> _{NDM-1}	ND 8
* M, male; F, female; ICU, Intensive Care Unit; Bal, bronchoalveolar lavage; CSF, cerebrospinal fluid; Pos, positive; Neg, negative; <i>iuc</i> and <i>iutA</i>, aerobactin genes; <i>ybt</i>, yersiniabactin; <i>rmpA</i>, regulator of mucoid phenotype; <i>Kfu</i>, Klebsiella iron uptake; <i>magA</i>, mucoviscosity-associated gene; <i>iroB</i>, salmochelin iron uptake systems; <i>allS</i>, allantoin metabolism gene; <i>peg-344</i>, metabolic transporter; AK, amikacin; GN, gentamicin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; FEP, cefepime; CIP, ciprofloxacin; AZM, aztreonam; MIC, minimum inhibitory concentration; ND, not determined.									

3.3. Demographic data

In this collection, 61.7% (63/102) and 38.2% (39/102) of hvKp strains were isolated from hospital A and B, respectively. Most of hvKp isolates were obtained from urine (33.3%, 34/102), followed by tracheal (27.5%, 28/102), blood (9.8%, 10/102) and abscess (7.8%, 8/102) specimens. Also among hospital wards, most hvKps were obtained from patients admitted to intensive care unit (ICU) (46.1%), internal (11.8%), surgical (10.8%) and emergency (7.8%) wards. Fifty-eight (56.9%) were men and 36 (35.5%) were women, most of whom were over 60 years of age (45.1%). (Table 2).

3.4. Antimicrobial susceptibility testing

In our study, we investigated the antimicrobial susceptibility profile in 90 hvKp isolates. Table 2 shows the resistance profile of each hvKp isolate to the antibiotics used in this study. The highest rate of antibiotic resistance was related to ampicillin (100%), followed by cefotaxime and ceftazidime (90%). The lowest rate of resistance was found in amikacin (34.4%). The resistance

rates to other antibiotics including ceftriaxone, cefepime, ciprofloxacin, gentamicin, meropenem, imipenem and aztreonam were 87.7%, 86.6%, 85.5%, 78.8%, 66.6%, 66.6% and 58.8% respectively. In addition, 76.6% of the isolates (69/90) were resistant to at least three classes of antibiotics and were defined as multidrug resistant (MDR) (21). Finally, the MIC of some resistant hvKp to the antibiotics ceftazidime and imipenem was determined by broth dilution method. All 23 isolates selected for ceftazidime had MIC $\geq$ 16: for 6 isolates MIC $\geq$ 16, 2 isolates MIC = 16, 3 isolates MIC = 32, 11 isolates MIC = 64, one isolate MIC = 128. Of the 39 resistant hvKp isolates, 79.5% had a MIC above the CLSI resistance criteria for imipenem (MIC $\geq$ 4): for 16 isolates MIC $\geq$ 4, 6 isolates MIC = 4, 5 isolates MIC = 8, 3 isolates MIC = 16, one isolate MIC = 256. 4 isolates (10.2%) were classified as intermediate resistant (MIC = 2) and 4 isolates (7.8%) were classified as susceptible (MIC $\leq$ 2).

3.5. Capsular genotyping and detection of virulence genes, and antimicrobial resistance genes

Capsular genotyping (K genotyping) of hvKp isolates showed that capsular serotype K20 was detected in more than half of the hvKp strains (54.9%). K2 and K1 were identified in only 3 (2.9%) and one isolate (1%), respectively, while K5, K54 and K57 were not detected in any of the hvKp isolates. In addition, 42 isolates (41.2%) did not belong to serotypes K1, K2, K5, K20, K54 and K57 as shown in Table 2.

PCR for virulence-associated genes revealed that *ybt* (77.5%) was the most common virulence factor gene after *iucA*. The other virulence factor genes including *ompA*, *iroB*, *magA*, *kfu* and *allS* were detected in 48%, 3.9%, 0.98, 21.6% and 1.96% hvKp isolates, respectively. (Table 2)

The distribution of Extended spectrum beta-lactamases (ESBLs) and carbapenemase genes among hvKp isolates is shown in Table 2. The results showed that 92.2% (94/102) hvKp isolates carried at least one antibiotic resistance gene and only 7.8% (8/102) had no resistance gene. The *bla_{SHV}* was the most common beta-lactamase gene (81.4%), followed by *bla_{CTX-M15}* (75.5%) and *bla_{TEM}* (67.6%). Also, PCR amplification of carbapenemase genes showed that *bla_{OXA-48}* (33.7%) was the dominant genotype of carbapenem-resistant strains, followed by *bla_{NDM-1}* (32.3%), while *bla_{KPC-1}* was not detected in any hvKp isolate. Thus, 56.8% (58/102) of the hvKp isolates carried ESBL and carbapenemase simultaneously and *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M15}*, *bla_{OXA-48}*, *bla_{NDM-1}* was the predominant MDR-hvKp genotype 39.6% (23/58). On the other hand, as shown in Table 2, more than 2 virulence factor genes were detected simultaneously in the majority of resistant hvKp strains and virulence profiles including: *iucA*, *iutA*, *ybt*, *ompA* genes have been reported in 40.4% (38/94) resistant hvKp isolates.

3.6. *ompK36* typing

PCR-based *ompK36* typing revealed that *ompK36* group C was the most common type with 70.6% (72/102) frequency. The prevalence of the other types, including *ompK36* groups A, B, and D, was (14/102) 13.7%, (5/102) 4.9%, and 10.8% (11/102), respectively (Table 2).

3.7. Nucleotide accession numbers

The accession numbers of *bla_{OXA-48}*, *bla_{NDM-1}*, *iutA*, *iucA* and *peg344* are MZ245618, MZ245619, MZ245620, MZ245621 and MZ245622, respectively in GenBank database.

4. Discussion

Because of the importance of hvKp isolates in human infections, especially in people without underlying disease and immunodeficiency, it is necessary to use an appropriate laboratory method to obtain an accurate diagnosis of these strains. Previously, the string test was used as a phenotypic method to identify hvKp isolates (22, 23). According to our results, only 62 out of 477 *K. pneumoniae* and 49 out of 102 hvKp isolates showed string test positive. Thus, this result indicates that the string test is not a reliable rapid test for hvKp detection, and these results are in agreement with the findings of Russo et al. and Parrott et al. (16, 24). On the other hand, we used tellurite resistance in clinical strains for the first time for phenotypic isolation of hvKps. Of 162 tellurite-resistant isolates, 102 were confirmed as hvKps by the molecular assay, making this method superior to the string test for phenotypic identification of hvKp isolates. To increase the accuracy and sensitivity of hvKp detection from clinical

samples, we also used three key virulence genes as molecular biomarkers previously introduced by Russo et al. We also examined all hvKp isolates for the presence of other virulence factor genes. In general, the frequencies of virulence factor genes, from highest to lowest, *iucA*, *ybt*, *iutA*, *rmpA*, *kfu*, *iroB*, *peg-344*, *allS*, and *magA*, respectively, were reported. Other studies have also shown that aerobactin is produced by more than 90% of hvKp, whereas only 6% of cKp strains can express it (16, 25). In a study by XU et al. the prevalence of *iucA*, *iutA*, *rmpA* and *iro* was reported to be 56.8%, 56.8%, 43.2% and 40.9%, respectively. The prevalence of *iutA* and *rmpA* was similar to our study, but in the present study, the prevalence of *iucA* was higher and *iro* was lower than the results of the study by Xu et al (26). The *ybt* was the second most prevalent virulence factor gene among hvKp isolates in this study. The yersiniabactin gene and its receptor, which is an important virulence factor for the survival of *Klebsiella* strains under severe conditions, can transmit both an integrative conjugative element (ICEKp) and a plasmid (recently reported) (27). Some studies have described the correlation between yersiniabactin-producing hvKps and pulmonary infectious diseases (28, 29). In Iran, a study conducted by Tabrizi et al. Reported that 5 of 53 *K.pneumoniae* strains isolated from ventilator-associated pneumonia were hvKp (30). In the current study, of 33 hvKps isolated from lung-related samples, 27 isolates were *ybt*-positive, confirming the results of previous studies. The *rmpA* was identified as the fourth most virulence factor. Because *rmpA* increases the expression of capsular polysaccharide (CPS), we expected that the *rmpA*-producing hvKp that were isolated would be string test-positive, but this hypothesis was refuted by our results, such that only 36.7% of the *rmpA*-positive isolates were reported as hypercystic phenotype. Studies have shown that other genes besides *rmpA* are involved in capsular gene expression, such as regulation of capsular synthesis B (*rcsB*). Both *rmpA* and *rcsB* genes have been shown to co-occur (31). In addition, the data show that the co-presence of four genes (*iucA*, *ybt*, *iutA*, *rmpA*) was more frequent in hvKp. In addition, other plasmid-born genes such as *iro*, *peg344* were less frequent and were reported only sporadically.

Sequencing and analysis of large virulence plasmids from hvKp strains revealed that virulence-associated genes were mainly found in two regions. The *rmpA2*, *iucABCD* and *iutA* genes are located close to each other, followed by the *rmpA*, *peg344* and *iroBCDN* genes in the second region. Some virulence plasmids carry all virulence genes (e.g. pLVPK, accession number: AY378100.), but others have lost one or more virulence-associated loci, confirming our result (e.g. pVir, accession number: CP029383.2) (5, 32). Despite most Asian countries having introduced K1 and K2 as the most common capsular serotypes (33–36), we identified K20 as the most common capsular type in Iran. This phenomenon suggests that the prevalence of the different serotypes may vary depending on the geographical area. Although there has been no comprehensive study on the hvKp isolates in Iran and little information is available on them, no K1 and K2 were found among the *K. pneumoniae* isolates in the study conducted by Aghamohammad et al (37). Also, in another study, one K1 and 15 K2 were identified among 122 *K. pneumoniae* isolates from Semnan, Iran, which are in agreement with our results (we detected only one K1 and three K2) (38). Another study from Iran conducted by Solgi et al., Reported that the prevalence of K1 and K2 was 45.9% and 13.5% respectively, which was more than the present study (39).

Most hvKps are sensitive to most antibiotics except for intrinsic resistance to ampicillin, similarly in this study all hvKp isolates were ampicillin resistant (40). Studies have shown that hvKps are unlikely to take up DNA from other resistant bacteria due to the large size of the capsule and increased expression of capsule-related genes, therefore antibiotic resistance is less common in hvKps than in cKp isolates (41). However, the prevalence of antibiotic-resistant hvKp isolates is increasing worldwide (42, 43). The current study revealed the high prevalence of MDR-hvKp and high resistance to imipenem (66%). Moreover, the presence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M15}, *bla*_{OXA-48} and *bla*_{NDM-1} was detected simultaneously in 56.8% of hvKp isolates.

Two pathways have been identified for the emergence of MDR-hvKp strains, the horizontal acquisition of resistance genes by plasmids and mobile genetic elements (MGEs) by hvKp isolates (type I), and another pathway is the acquisition of the virulence-associated plasmid (e.g., pLVPK and pVir) by classical MDR-*K. pneumoniae* strains (MDR-cKp) (type II) (42, 44). Ultimately, both mechanisms lead to the development of MDR-hvKp strains that are resistant to antibiotic treatment in addition to having a very high pathogenicity that poses a serious threat to public health.

The correlation of *OmpK36* porin variants with specific sequence types (STs) of *K. pneumoniae* was first described by Papagiannitsis et al. *K. pneumoniae* isolates can be classified into four groups (designated groups A to D) by *ompK36* genotyping (12, 45). There is a relationship between *ompK36* type and clonal group (CG). The studies have described the correlation between CG15, CG22, CG23, CG34, CG65 to type A, CG292, CG347, CG1612, CG1928 to group B, CG23, CG48, CG42,

CG65, CG86, CG76, CG420, CG107 to group C and CG45, CG268, CG420 to group D (46). Some STs were reported to be associated with hvKp isolates, e.g. ST11 and ST23, that ST11 (CG258) belonged to ompK36 group A and ST23 (CG23) belonged to ompK36 group C (12, 46). In this study, clonal relatedness by ompK36 typing revealed that group C (70.6%) was the most common ompK36 porin type among hvKp isolates. A study in Taiwan showed that ompK36 group C was significantly more abundant among *K. pneumoniae* isolates (46). This study was in agreement with our study in Iran.

5. Conclusion

This study presented a new screening method based on the resistance of hvKp to tellurite, which was superior to the string test in phenotypic identification of hvKp isolates. A high prevalence of MDR-hvKp and a high level of resistance to imipenem (66%) were detected. In addition, coexistence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M15}, *bla*_{OXA-48} and *bla*_{NDM-1} was identified in 56.8% of hvKp isolates. Using the PCR-based ompK36 typing method, which was simpler and less expensive than MLST, we were also able to investigate the clonal relatedness of the strains. It was found that the majority of hvKp isolates belonged to capsular serotype K20 and ompK36 group C, which is related to CG23 (e.g. ST23). It seems that the control of MDR-hvKp is an urgent issue in the healthcare setting.

List Of Abbreviations

K. pneumoniae, *Klebsiella pneumoniae*; **hvKp**, Hypervirulent *Klebsiella pneumoniae*; *rmpA*, regulator of mucoid phenotype; *magA*, mucoviscosity-associated gene; *kfu*, *Klebsiella* ferric uptake; *iroB*, salmochelin iron uptake systems; *ybt*, yersiniabactin; *allS*, allantoin metabolism gene; **AK**, amikacin; **GN**, gentamicin; **CTX**, cefotaxime; **CAZ**, ceftazidime; **CRO**, ceftriaxone; **IMI**, imipenem; **MRP**, meropenem; **FEP**, cefepime; **CIP**, ciprofloxacin; **AMP**, ampicillin; **AZM**, aztreonam; **MIC**, Minimum inhibitory concentration; **ICU**, intensive care unit; **ESBL**, Extended spectrum beta-lactamases; **pLVPK**, Large Virulence Plasmid of *K. pneumoniae*; **MDR**, multidrug resistant; **MDR-cKp**, classical MDR-*K. pneumoniae*; **ST**, sequence types; **Bal**, bronchoalveolar lavage; **CSF**, cerebrospinal fluid.

Declarations

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Conflict of interest

The authors have no conflicts of interest to declare.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Competing interests

The authors declare that they have no competing interests

Ethical statement

This project was done based on ethical guidelines as previously approved by the Pasteur Institute of Iran (IR. B-9427).

Authors' contributions

Hamid Solgi & Mohammad Moeinirad, collected the samples and their data; **Rahimeh Sanikhani**, carried out other phenotypic and genotypic tests; **Rahimeh Sanikhani & Mohammad Moeinirad**, wrote the manuscript and analyzed the data; **Azar Hadadi**, Professor of Infectious Diseases; **Farzad Badmasti & Fereshteh Shahcheraghi**, supervised the project and write and revised the manuscript.

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