Original Article

Prevalence of Extended-Spectrum β-lactamase and Integron Gene Carriage in Multidrug-Resistant *Klebsiella* Species Isolated from Outpatients in Yazd, Iran

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Received: 23 June 2018
Revised: 04 September 2018
Accepted: 29 September 2018

What's Known

- The presence of integron-mediated, multidrug-resistant, extended-spectrum β-lactamase producing *Klebsiella* in the community has significant implications in the spread of these opportunistic pathogens between the community and health centers worldwide. Most studies have reported the prevalence of these resistance determinants in clinical isolates, and studies on community isolates are few in Iran.

What's New

- This is the first study showing that 8.4% and 7.6% of the Iranian *Klebsiella* outpatient isolates were multidrug-resistant and extended-spectrum β-lactamase producers, respectively.
- The presence of multiple β-lactamase genes and class 1 integron in *85.7%* of these isolates shows the need for molecular characterization of community isolates in Iran.

Abstract

Background: Community-acquired infections by multidrug-resistant (MDR), extended-spectrum β-lactamase (ESBL) producing *Klebsiella* species (*Klebsiella* spp.), is of major concern worldwide. We determined antibiotic resistance, production of extended-spectrum β-lactamases (ESBLs), and carbapenemases, as well as the presence of classes 1, 2, and 3 integrons in outpatient isolates of *Klebsiella* collected from Yazd central laboratory, Yazd, Iran.

Methods: We collected 250 *Klebsiella* isolates from Yazd central laboratory between August 2015 and October 2017. Antibiotic susceptibility was determined against 18 antibiotics by disc diffusion, and multidrug-resistant isolates were tested for ESBL production by the phenotypic confirmatory test according to CLSI 2017 protocols. The amplification of β-lactamase genes bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{OXA-48}, $bla_{KPC,}$ and $bla_{NDM,}$ classes 1, 2, and 3 integrase genes, was carried out using specific primers and polymerase chain reaction (PCR).

Results: Of the 250 *Klebsiella* outpatient isolates, 3.6% were *K. oxytoca* and the rest were *K. pneumoniae.* Disc diffusion showed that 21 (8.4%) isolates were MDR, 19 (90.4%) of which were ESBL producers including one *K. oxytoca.* The most prevalent β-lactamase gene was bla_{SHV} followed by bla_{TEM} and bla_{CTX-M}, but bla_{OXA-48}, $bla_{KPC,}$ and bla_{NDM} were not detected. Class 1 integron was detected in 18 out of 21 MDR isolates (85.7%), but classes 2 and 3 were not observed. Two isolates were resistant to carbapenems and harbored $bla_{SHV,}$ bla_{TEM}, and bla_{CTX-M}, as well as class 1 integron.

Conclusion: ESBL production and the presence of multiple β-lactamase genes in MDR community isolates of *Klebsiella* spp. can have significant implications in terms of the spread of these opportunistic pathogens.

Please cite this article as: Malekjamshidi MR, Zandi H, Eftekhar F. Prevalence of Extended-Spectrum β-lactamase and Integron Gene Carriage in Multidrug-Resistant *Klebsiella* Species Isolated from Outpatients in Yazd, Iran. Iran J Med Sci.

Keywords • *Klebsiella* • Outpatients • Integrons • Drug resistance, microbial

Introduction

Klebsiella spp. isolates are important opportunistic pathogens and the cause of nosocomial as well as community-acquired

infections including pneumonia, urinary tract and wound infections, gastro intestinal diseases, and septicemia.[1-3] A wide range of β-lactam antibiotics are commonly used for the treatment of *Klebsiella* related infections. However, the frequent use of antibacterial agents has led to the emergence of resistance mostly due to the extended-spectrum β-lactmases (ESBLs) production by the organism worldwide.[4, 5] ESBLs hydrolyze extended spectrum β-lactam antibiotics and aztreonam.[6] Furthermore, ESBL producing *K. pneumoniae* are often resistant to non-β-lactam antibiotics such as aminoglycosides and fluoroquinolones, leading to the emergence of multidrug-resistant (MDR) strains.[6, 7] The major groups of ESBLs, commonly detected among both community and hospital-acquired isolates of *Klebsiella* spp., belong to SHV, TEM, and CTX-M classes.[4, 7, 8] Another class of antibiotics, carbapenems, has also been extensively used to treat ESBL producing *Klebsiella* related infections. However, carbapenemase-producing isolates with reduced susceptibility or resistance to carbapenems have restricted the use of these antibiotics.[9] Functional carbapenemases include *K. pneumoniae* carbapenemase (KPC), Metallo-β-lactamases, and oxacillinase.[10-12] Of these, KPC-producing *K. pneumoniae* is most frequently associated with high mortality rates.[13] The genes encoding ESBL resistance along with a number of other antibiotic resistance determinants are often found on class I integrons and are usually carried by plasmids.[14, 15] The presence of multiple resistance determinants on mobile genetic elements allows for the spread of the organism in large populations and can cause serious community and/or hospital-acquired infections.[16] Considering that the majority of studies have been performed on nosocomial *Klebsiella* isolates, we studied the antibiotic resistance profiles of community isolates of *Klebsiella* followed by the detection of ESBL and carbapenemase production, as well as the presence of class 1, 2 and 3 integrons in MDR isolates collected from outpatient specimens in Yazd central laboratory, Yazd, Iran.

Materials and Methods

Isolation and Identification of Bacteria

In this study, a total of 250 *Klebsiella* isolates were collected from outpatients (age range of 23 to 87 years old) at the central laboratory in Yazd, Iran, between August 2015 and October 2017. Conventional biochemical tests were used to confirm the identity of the isolates which were then maintained in Tryptic Soy Broth (TSB; Merck, Germany), containing 4% glycerol (Merck, Germany) at −70 °C.

Antibacterial Susceptibility

Susceptibility against 18 antibiotics was performed by disc diffusion according to the 2017 CLSI guidelines using commercially available discs (Mast, UK) including amoxicillin (AMX, 10 µg), cefalotin (CF, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), gentamicin (GM, 10 µg), nalidixic acid (NA, 30 µg), kanamycin (KM, 30 µg), amikacin (AN, 30 µg), trimethoprim/sulfamethoxazole (SXT, 23.75/1.25 µg), ciprofloxacin (CIP, 30 µg), ofloxacin (OFX, 5 µg), norfloxacin (NOR, 10 µg), nitrofurantoin (NF, 300 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), ertapenem (ETP 10µg), tetracycline (TE, 30 µg), and chloramphenicol (CM, 30 µg).[17] *K. pneumoniae* ATCC 10031 was used as the susceptible control. MDR was recorded when the isolates were resistant to at least three classes of antibiotics.

Screening for ESBL Production

ESBL production was detected by the phenotypic confirmatory test (PCT) using ceftazidime (30 µg) and cefotaxime (30 µg) alone or in combination with clavulanic acid (10 µg) (Mast, UK), as recommended by the CLSI 2017 guidelines.[17] An increase of ≥5 mm in the zone diameter of the antibiotic in combination with clavulanic acid, compared to the antibiotic alone, was recorded as ESBL production. *K. pneumoniae* ATCC 10031 was used as the susceptible control for ESBL production.

Determination of Minimum Inhibitory Concentrations

Minimum inhibitory concentrations (MICs) for imipenem were measured for imipenem-resistant and intermediately resistant isolates by the Epsilometer test (E-test, Liofilchem, Italy) according to the CLSI 2017 guidelines.[17]

Phenotypic Detection of Carbapenemase Activity

Carbapenemase production was detected by the Modified Hodge Test (MHT).[17] Briefly, a 1:10 dilution of an overnight grown *E. coli* ATCC 25922 culture (turbidity adjusted to McFarland no. 0.5) was used to make a bacterial lawn on the surface of a Mueller Hinton agar (Merck, Germany) plate and a meropenem or ertapenem disc (10 µg) was placed in the center of the test area. Carbapenem-resistant test isolates were then streaked from the edge of the disc to the edge of the plate. After overnight incubation at 37 °C, carbapenemase production was confirmed if a cloverleaf-like distortion of the inhibition zone was observed.

DNA Extraction and Amplification

Bacterial genomic DNA was extracted directly by boiling. Briefly, a loopful of bacteria grown overnight on MacConkey agar (Merck, Germany) was resuspended in 500 µl of sterile double distilled water, boiled for 10 minutes, and centrifuged at 10,000 g for 10 minutes. The supernatant was used as DNA template for PCR amplifications.

Primers used for the amplification of β-lactamase genes (*bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX}$), carbapenemases (*bla*$_{OXA-48}$, *bla*$_{KPC}$ and *bla*$_{NDM}$), integron classes 1, 2 and 3 genes are shown in table 1.[18-21] Thermocycler (Applied Biosystems, USA) conditions are also shown in table 1. Each PCR reaction mixture (25 µl) contained 1.5 µl DNA template, 1.5 mM $MgCl_2$, 0.25 mM of dNTP mix (Cinnagen, Iran), 1 unit of DFS-Taq DNA polymerase (Bioron, Germany), and 20 pmol of each primer (Faza Biotech, Iran). PCR amplification products were run on 1.5% agarose gels and visualized using Gel Documentation System (ATP, Iran). PCR products were sequenced (Pishgam, Tehran, Iran) and accession numbers were obtained from Genbank. Positive controls for PCR experiments were chosen from the verified sequenced PCR products.

Results

Of the 250 *Klebsiella* outpatient isolates, nine (3.6%) were *K. oxytoca* and the rest were identified as *K. pneumoniae.* Among these, 245 (98%) were obtained from urine and five (2%) were from wound and sputum specimens. Disc diffusion results showed that resistance rates to the test antibiotics ranged from 0.4% to 12.8% (table 2). Multidrug-resistance (resistance to at least three antibiotic classes including β-lactams) was observed in 21 (8.4%) isolates (table 3). Two isolates were intermediately resistant to imipenem; one of which was also intermediately resistant to ertapenem and the other was resistant to meropenem. Among the 21 MDR isolates (table 3), one was *K. oxytoca* and the rest were *K. pneumoniae.* MIC measurements for imipenem confirmed the disc diffusion results (MICs of 2 to 3 mg/L).

Phenotypic confirmatory test results (table 3) showed that 19 of the 21 isolates (90.4%) were ESBL producers, 17 of which were from urine, one was from sputum and one from a wound specimen. All ESBL producing MDR isolates recovered from urine were from female patients over 60 years of age. Sequence analyses of the PCR products obtained from ESBL producing isolates by the PCT confirmed the identity of genes. Figure 1 shows the PCR amplification products of β-lactamase and int1 genes in outpatient isolates of *Klebsiella.* PCR results revealed that all 21 MDR isolates carried at least one β-lactamase gene, 18 of which were positive for class 1 integron. Classes 2 and 3 integrons were not detected. As it can be observed from table 3, 19 isolates (90.5%) harbored the *bla*$_{SHV}$ gene, 17 (80.9%) had *bla*$_{TEM}$, and *16* (76.2%) carried the *bla*$_{CTX-M}$ gene. The two isolates which contained both *bla*$_{SHV}$ and *bla*$_{TEM}$ were negative for ESBL production by the phenotypic methods and did not carry class 1 integron.

The MHT results showed that the two isolates were carbapenemase producers, both of which harbored ESBL genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, and *bla*$_{TEM}$ genes) as well as class 1 integron (Kp 11 and Kp 142). The other carbapenemase genes (*bla*$_{KPC}$, *bla*$_{OXA-48}$, and *bla*$_{NDM}$) were not detected.

Table 1: Primers and temperatures used for the detection of β-lactamases, carbapenemases, and integrase genes

Gene	Primer Sequence	PCR Product (bp)	Thermocycler conditions				Ref
			Denaturation	Annealing	Extension	Cycles	
bla$_{TEM}$-F *bla*$_{TEM}$-R	GAGTATCAACATTTCCGTGTC TAATCAGTGAGGCACCTTCTC	889	94° 1 min	45° 1 min	72° 1 min	32	18
bla$_{CTX-M}$-F *bla*$_{CTX-M}$-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550	94° 1 min	63° 1 min	72° 1 min	32	18
bla$_{SHV}$-F *bla*$_{SHV}$-R	ATGCGTTATATTCGCCTGTG AGCGTTGCCAGTGCTCGATC	862	94° 1 min	58° 1 min	72° 1 min	32	19
bla$_{KPC}$-F *bla*$_{KPC}$ -R	TGTTGCTGAAGGAGTTGGGC ACGACGGCATAGTCATTTGC	340	95° 1 min	56° 1 min	72° 1 min	35	20
bla$_{OXA-48}$-F *bla*$_{OXA-48}$-R	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	585	95° 1 min	56° 1 min	72° 1 min	35	20
bla$_{NDM}$-F *bla*$_{NDM}$-R	TAAAATACCTTGAGCGGGC AAATGGAAACTGGCGACC	439	95° 1 min	52° 1 min	72° 1 min	35	20
Int1-F Int1-R	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280	94° 1 min	60° 1 min	72° 1 min	35	21
Int2-F Int2-R	TTATTGCTGGGATTAGGC ACGGCTACCCTCTGTTATC	233	94° 1 min	60° 1 min	72° 1 min	35	21
Int3-F Int3-R	AGTGGGTGGCGAATGAGTG TGTTCTTGTATCGGCAGGTG	600	94° 1 min	60° 1 min	72° 1 min	35	21

Table 2: Antibacterial susceptibility of 250 community isolates of *Klebsiella* spp., measured by disc diffusion

Antibiotic	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
Amoxicillin	250 (100)	0 (0)	0 (0)
Cefalotin	27 (10.8)	1 (0.4)	222 (88.8)
Ceftazidime	10 (4)	9 (3.6)	231 (92.4)
Cefotaxime	12 (4.8)	7 (2.8)	231 (92.4)
Imipenem	0 (0)	2 (0.8)	248 (99.2)
Meropenem	1 (0.4)	0 (0)	249 (99.6)
Ertapenem	0 (0)	2 (0.8)	248 (99.2)
Gentamicin	11 (4.4)	1 (0.4)	238 (95.2)
Amikacin	3 (1.2)	1 (0.4)	246 (98.4)
Kanamycin	10 (4)	2 (0.8)	238 (95.2)
Nalidixic acid	19 (7.6)	6 (2.4)	225 (90)
Ciprofloxacin	12 (4.8)	5 (2)	233 (93.2)
Ofloxacin	12 (4.8)	2 (0.8)	236 (94.4)
Norfloxacin	10 (4)	2 (0.8)	238 (95.2)
Nitrofurantoin	7 (2.8)	0 (0)	243 (97.2)
Chloramphenicol	4 (1.6)	1 (0.4)	245 (98)
Tetracycline	18 (7.2)	4 (1.6)	228 (91.2)
Trimethoprim-Sulfamethoxazole	32 (12.8)	0 (0)	218 (87.2)

Table 3: Characterization of β-lactamase-producing of *Klebsiella* spp. outpatient isolates

Isolate No.	Phenotypic confirmatory test (PCT)	β-lactamase genes	Class 1 Integron	Antibiotic resistance profile (disc diffusion)
Kp 9	+	CTX, TEM, SHV	-	AMX, CF, CTX, CM, NA, TE, SXT CIP^{I}, OFL^{I}
Kp 11	+	CTX, TEM, SHV	+	AMX, CF, CTX, CAZ, IMP^{I}, MEM, GM, AK, KM^{I}, NA, TE, SXT, CIP^{I}, OFL^{I}
Kp 24	+	CTX, TEM, SHV	+	AMX, CF, CTX, CAZ, GM, AK^{I}, KM, TE^{I}, SXT,
Kp 31	+	TEM, SHV	+	AMX, CF, CAZ, CTX^{I}, CIP^{I}, TE, SXT
Kp 42	+	CTX, TEM, SHV	+	AMX, CF, CAZ, CTX, GM, AK, KM, NA, CIP, OFL, NF, TE, SXT
Ko 55	+	CTX, TEM, SHV	+	AMX, CF, CAZ, CTX, CIP, OFL, NA, KM, NF, TE, SXT
Kp 63	+	CTX, TEM, SHV	+	AMX, CF, CAZ^{I}, CTX^{I}, CIP^{I}, OFL^{I}, NF, TE, SXT
Kp 70	+	TEM, SHV	+	AMX, CF, CAZ, CTX^{I}, GM, KM^{I}, CIP, OFL, NF, NA,TE, SXT
Kp 84	+	CTX, TEM, SHV	+	AMX, CF, CTX, GM, TE^{I}
Kp 93	+	TEM, SHV	+	AMX, CF, CAZ^{I}, NA, TE, SXT
Kp 98	-	TEM, SHV	-	AMX, CF, CAZ^{I}, NA^{I}, TE, SXT
Kp 118	+	CTX, TEM, SHV	+	AMX, CF, CAZ^{I}, CTX, KM, SXT
Kp 137	-	TEM, SHV	-	AMX, CF, CTX^{I}, NOR, CM, SXT
Kp 142	+	CTX, TEM, SHV	+	AMX, CF, CTX^{I}, CAZ, ETP^{I}, IMP^{I}, CIP, OFL, NA, KM, NF, CM, TE, SXT
Kp 163	+	CTX, SHV	+	AMX, CF, CAZ^{I}, CTX, KM, CM, SXT
Kp 173	+	CTX, TEM, SHV	+	AMX, CF, CTX, GM, KM, SXT
Kp 192	+	CTX	+	AMX, CF, CAZ^{I}, CTX^{I}, CIP, OFL, NA, NF, SXT
Kp 213	+	CTX, TEM, SHV	+	AMX, CF, CAZ^{I}, CTX^{I}, NA^{I}, SXT
Kp 229	+	CTX	+	AMX, CF, CAZ^{I}, CTX^{I}, GM, CIP, OFL, NA, NF, SXT
Kp 230	+	CTX, SHV	+	AMX, CF, CAZ^{I}, CTX, SXT
Kp 234	+	CTX, TEM, SHV	+	AMX, CF, CAZ, CTX, CIP, OFL, NA, GM, KM, NF, TE, SXT

Ko: *Klebsiella oxytoca*; Kp: *Klebsiella pneumoniae;* AMX: Amoxicillin; CF: Cefalotin; CAZ: Ceftazidime; CTX: Cefotaxim; GM: Gentamicin; AN: Amikacin; KM: Kanamycin; NA: Nalidixic acid; CP: Ciprofloxacin; OFX: Ofloxacin; NOR: Norfloxacin; NF: Nitrofurantoin; IMP: Imipenem; MEM: Meropenem; CM: Chloramphenicol; TE: Tetracycline; SXT: Trimethoprim-Sulfamethoxazole; I: Intermediate

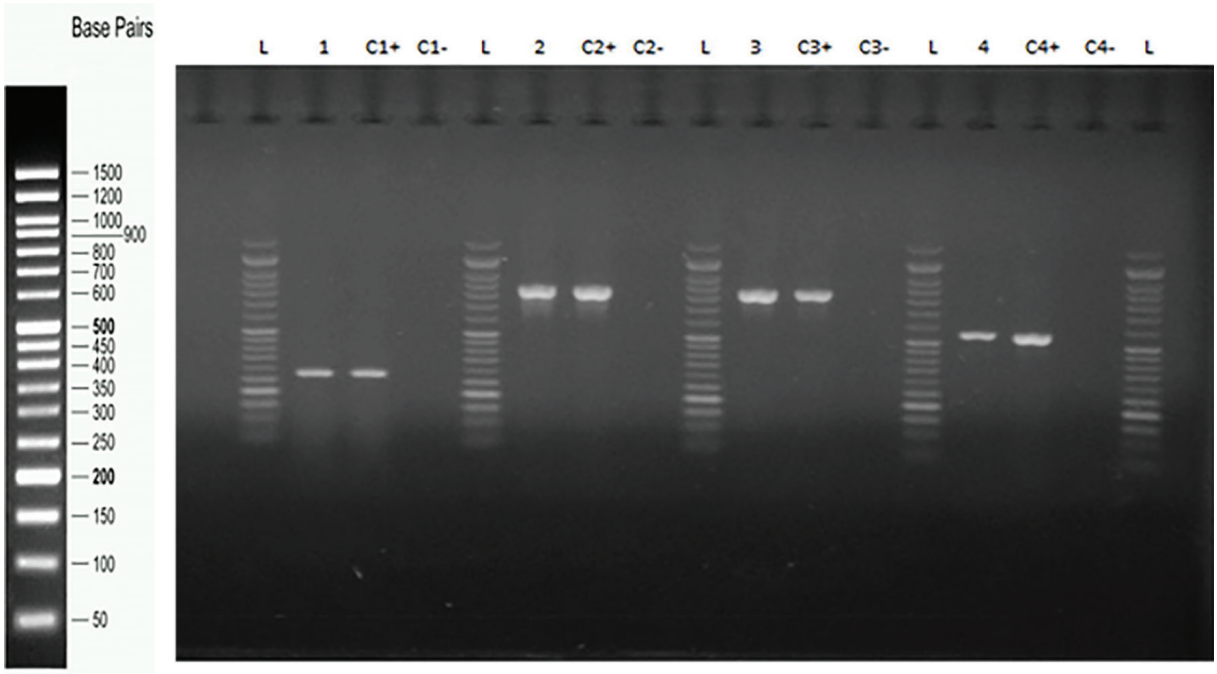

Figure 1: The figure displays the PCR amplification of ESBL and *int1* genes in outpatient isolates of *Klebsiella* spp. Lane 1, *int*1 (280 bp), C1+ (KP234, Genbank Accession No. MH369808). Lane 2, bla_{TEM} (889 bp), C2+ (KP24, Genbank Accession No. MH369809). Lane 3, bla_{SHV} (862 bp), C3+ (KP70, Genbank Accession No. MH487650). Lane 4, $bla_{CTX-M,}$ (550 bp), C4+ (KP229, Genbank Accession No. MH469722). L, 50 bp DNA ladder. C-, negative control.

Discussion

The emergence of ESBL producing *K. pneumoniae* among the community isolates is a major concern and an important cause of failure in antibiotic therapy. In this research, 21/250 (8.4%) of the *Klebsiella* spp. isolated from outpatients were MDR, among which 19 (90.5%) were ESBL producers including one *K. oxytoca.* In a study from Saudi Arabia, among 955 outpatient isolates, 30% and 2% were *K. pneumoniae* and *K. oxytoca*, respectively, with similar rates of ESBL production. They also reported that ESBL producing isolates were mostly obtained from urine specimens of subjects over the age of 60.[22] In the present study, almost all of ESBL producing MDR strains were urinary isolates obtained from female patients over 60 years old, showing a significant relationship between the patients age, multidrug resistance, and ESBL production in *Klebsiella*-related infections. These results suggest that elderly patients may have been repeatedly exposed to previous uses of antibiotics including the broad-spectrum β-lactams.[22] Due to the high frequency of *K. pneumoniae*-related nosocomial and community-acquired infections, almost all research has been conducted on this organism.[4, 16, 23]

In this study, ESBL-positive isolates were also MDR. However, the non-ESBL isolates were susceptible to almost all classes of antibiotics. For example, all ESBL producers were resistant to trimethoprim-sulfamethoxazole, compared to 4.8% of non-ESBL isolates (data not shown). Our results are in line with those of a study from Japan showing that nosocomial as well as ESBL producing community isolates of *K. pneumoniae* are also multidrug resistant.[24] On the other hand, almost all our ESBL producing MDR isolates were susceptible to carbapenems, suggesting that these antibiotics could still be the drugs of choice for the treatment of infections caused by ESBL producing *Klebsiella* spp. In agreement with our results, Bouchillon and colleagues reported that ESBL producing community *Enterobacteriaceae* isolates were susceptible to carbapenems.[25]

The rate of ESBL production in our outpatient isolates of *K. pneumoniae* was similar to those reported from Taiwan (7.6%), Japan (8.5-9.2%) and the United States.[23-25] However, the high levels of ESBL producing *K. pneumoniae* community isolates have been reported worldwide.[26-31]

The majority of investigations suggest that among ESBL classes produced by *K. pneumoniae* community isolates, three (SHV, TEM, and CTX-M) seem to be the most common types.[4, 7, 8] In the current study, SHV was the most prevalent type of β-lactamase (90.5%), followed closely by TEM (80.9%) and CTX-M (76.2%). The predominance of SHV type ESBL in our community isolates is similar to some other reports from Iran.[26, 32] On one hand, the

majority of the studies have reported CTX-M as the most prevalent ESBL in *K. pneumoniae* community isolates.[16, 23, 29, 31] On the other hand, some Iranian studies have shown TEM as the prevalent ESBL in *K. pneumoniae*.[8, 27] In a recent study from Isfahan, Maleki et al. showed that 25.5% of the outpatient isolates were ESBL producers, 92% of which had MDR phenotype with high rates of bla_{CTX-M} (92%) and bla_{TEM} (76%) gene carriage.[33] Furthermore, we also found that 12 of our 19 ESBL producing *Klebsiella* isolates (63.1%), including one *K. oxyteca*, contained all three β-lactamase genes. As in this research, other investigators have shown the presence of multiple ESBL genes in the community urinary isolates of *K. pneumoniae.*[8, 23, 28, 33] The differences observed in the prevalence of ESBL genes, found in *Klebsiella* isolates from different nosocomial or community settings, could be due to the presence of genetic elements such as integrons which facilitate the dissemination of ESBL genes by horizontal transfer in different geographical regions.

The outpatient isolates of this study did not harbor carbapenemase encoding genes bla_{OXA48}, bla_{KPC}, and bla_{NDM} genes, similar to what was reported by van Hoek in the Netherlands.[34] However, other studies have reported the presence of $bla_{KPC,}$ bla_{NDM}, and bla_{oxa-48} among community isolates of *K. pneumoniae.*[11, 35, 36] In this study, two isolates were positive for carbapenemase production by the phenotypic MHT test, neither of which harbored carbapenem resistance genes bla_{OXA-48}, bla_{KPC}, and bla_{NDM}. However, disc diffusion results showed that one isolate (KP11) was resistant to meropenem and intermediately-resistant to imipenem. The other isolate (Kp142) was intermediately-resistant to both imipenem and ertapenem. Resistance to carbapenems may be due to other mechanisms including other carbapenem resistance genes, decreased antibiotic absorption due to the lack of outer membrane porins, and the active excretion of the drug through efflux pumps or other possible mechanisms.[9, 10, 13]

It is well known that multidrug resistance in *Enterobacteriaceae* is often the result of the acquisition of resistance genes by horizontal transfer. In addition, a large number of resistance genes are present on integrons carried by plasmids and transposons. Among the antibiotic resistance integrons, the association of class 1 integron and multidrug resistance is well known.[14] In the present study, a strong association was found between phenotypic ESBL production, β-lactamase gene carriage, and the presence of class 1 integron. We showed that 85.7% of the ESBL producing MDR isolates (including the *K. oxytoca* strain) harbored class I integron. Mahluji and colleagues reported that 18% of *K. pneumoniae* outpatient isolates were MDR and all carried class 1 integron.[37]

The presence of integron in community isolates of *Klebsiella* could be an important factor in the spread of antibacterial resistance genes, not only between bacterial species but also among other Gram-negative enterobacterial pathogens. Interestingly, the two isolates of our study which were negative for class I integron and ESBL production (Kp 98 and Kp 137) carried bla_{TEM} and $bla_{SHV.}$ Dehghan and colleagues found that 32.8% of the outpatient *K. pneumoniae* isolates were ESBL producers, among which CTX-M (58.6%) was predominant followed by TEM (43.1%). They also found a significant association between ESBL production and class 1 integron carriage.[38] In another study from Iran, nosocomial and outpatient MDR isolates of *K. pneumoniae* and *K. oxytoca* also carried class 1 integron.[39]

Because of the lack of extensive information on molecular characteristics of community isolates of *Klebsiella*, a prevalent urinary pathogen, this study was limited to outpatient isolates. Our results are limited and represent the prevalence of MDR and ESBL production in the outpatient isolates obtained from Yazd Central Laboratory and could not be applicable to other geographical regions in Iran. Further surveillance programs are needed from other regions to provide a better picture of the community spread of these multi-resistant organisms in Iran. Whether the community isolates are originated from nosocomial infections or vice versa, the presence of ESBL producing, MDR *Klebsiella* spp., in the community is of great concern and could have significant implications in terms of the spread of these opportunistic pathogens, indicating the need for molecular characterization of community isolates.

Conclusion

The presence of ESBL producing, MDR *Klebsiella* spp., in the community is of great concern and could have significant implications in terms of the spread of these opportunistic pathogens. In order to provide information on the distribution of these organisms in Iran, large-scale studies involving the characterization of the community *Klebsiella* spp isolates from different parts of the country are needed.

Acknowledgment

The authors are grateful to Shahid Beheshti

University in Tehran and Shahid Sadoughi University of Medical Sciences in Yazd, Iran, for their financial support of this study.

Conflict of Interest: None declared.

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