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Molecular Typing and Occurrence of *Extended-Spectrum Beta-Lactamases (ESBLs)* Genes among *Klebsiella oxytoca* Isolated from Selected Hospitals in Khorramabad, Iran

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Abstract:

Introduction: Resistance of *Klebsiella spp* to various antibiotic families, such as beta-lactams, has increased due to the acquisition of plasmids carrying genes for Extended-Spectrum Beta-Lactamases (ESBLs). Today, ESBLs have become a major problem in healthcare settings.

Objective: This study aimed to determine the molecular fingerprinting and frequency of bla_{TEM}, bla_{SHV},bla_{CTX-M}, bla_{GES}, bla_{PER}, and bla_{VEB} genes among *Klebsiella oxytoca* from clinical samples collected in selected hospitals in Khorramabad, Iran.

Methods: The present study was a cross-sectional study of Klebsiella oxytoca isolated from clinical specimens collected at selected hospitals in Khorramabad in 2019. After phenotypic identification of the isolates studied, antibiotic susceptibility patterns and beta-lactamase screening were performed using the disk diffusion method; genes encoding resistance were detected by PCR, and molecular fingerprinting was performed by PFGE.

Results: In this study, 32 *K. oxytoca* were isolated. The highest resistance rates were observed with ampicillin (93.8%) and cefotaxime (65.6%), and the lowest resistance rates were observed with colistin (0%), imipenem (15.6%), and amikacin (18.8%). 56.6% of isolates were ESBL- producing. Nineteen (59.4%), 2 (6.3%), and 16 (50%) isolates carried the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes, respectively. In addition, bla_{GES}, bla_{PER}, and *blaVEB* genes were not found in any of the tested isolates. Molecular typing results by the PFGE method showed that the isolates were very heterogeneous, and the 32 tested isolates were clustered in 18 pulse types.

Discussion: In the present study, *K. oxytoca* isolates exhibited concerning resistance to certain antibiotics, particularly cephalosporins. The high prevalence of ESBLs and bla_{TEM} and bla_{CTX-M} genes highlights the importance of continuous monitoring of these factors. On the other hand, the absence of bla_{GES}, bla_{PER}, *and* bla_{VEB} genes and the high genetic heterogeneity of bacterial strains in PFGE indicate high strain diversity and the possibility of multiple sources of contamination.

Conclusion: Overall, the results of this study showed that the isolation and examination of *K. oxytoca* isolates in terms of diversity and molecular susceptibility profiling are important.

Keywords: *Klebsiella oxytoca*, Extended-spectrum beta-lactamases (ESBLs), Pulsed-field gel electrophoresis (PFGE), Molecular typing, Drug resistance, Iran.

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1. INTRODUCTION

Klebsiella oxytoca is a Gram-negative, nonmotile, encapsulated bacillus in the family Enterobacteriaceae. The genus *Klebsiella* generally includes two opportunistic pathogenic species, *K. pneumoniae* and *K. oxytoca* [1]. *K. oxytoca* is unique in nature and is isolated from skin, mucous membranes, oropharynx, and intestines of healthy humans and animals [1, 2]. Human infections caused by *K. oxytoca* are often associated with contamination from disinfectants, or parenteral fluid vials, humidifiers, and ventilators. The bacterium can be transmitted person-to-person, patient-to-patient, and staff-to-patient [3, 4].

First-line drugs for the treatment of *Klebsiella spp* infections are mainly cephalosporins [5]. However, in case of resistance to cephalosporins, aminoglycosides such as gentamicin, fluoroquinolones such as ciprofloxacin, or carbapenems are used [3]. Today, reports of resistance to various antibiotic families, including beta-lactams [6, 7], are emerging. *Klebsiella spp* has become resistant to various antibiotics, including extended-spectrum β-lactams, due to the acquisition of plasmids carrying genes for extended-spectrum β-lactamases (ESBLs). In *Klebsiella spp.*, ESBLs can hydrolyze extended-spectrum cephalosporins, monobactams, and penicillins [8]. The distribution of β-lactamases, which are often carried by plasmids, is widespread worldwide; these plasmids also carry genes for resistance to aminoglycosides, sulfonamides, and other antibiotics [9]. In recent decades, ESBLs have become a serious problem in healthcare systems. Although the first β-lactam-resistant isolates were observed in hospital-acquired infections, they have now also spread in communities. In different geographical areas, including cities across Iran, ESBLs in the TEM, SHV, and CTX-M families have been identified. According to the results of studies conducted in Europe, the frequency of beta-lactamase-positive isolates is also higher than that of methicillin-resistant staphylococci [10, 11]. *TEM, SHV*, and *CTX-M* genes are widespread in Gram-negative bacilli of the Enterobacteriaceae family, especially in *Escherichia coli* and *K. pneumoniae* [12, 13]. The frequency of β-lactamase-producing isolates among Gram-negative bacilli in Asia is 39%, and the global average has been reported to be 16.3% [14-16]. Enterobacteriaceae strains isolated from healthcare facilities were producers of extended-spectrum β-lactamases. Epidemiological data from various parts of the world, including Africa, Asia, and the Middle East, have reported a high frequency of ESBL-positive isolates in clinical *Klebsiella spp*. isolates. In some studies, 50–70% of K. oxytoca isolates were reported to be ESBL producers. Such reports indicate a significant expansion of ESBL-carrying isolates, and the evaluation and detection of ESBL-carrying *K. oxytoca* strains could be important for infection control [17]. Given that β-lactamase enzymes can confer resistance to a wide range of cephalosporins, the presence of these enzymes delays the treatment of infections caused by β-lactam-resistant bacteria [18]. On the other hand, in epidemiological studies, molecular typing methods such as Pulsed-Field Gel Electrophoresis (PFGE) have received great attention because, in addition to determining the source of infections, they could be used to detect genetic diversity in bacteria, including changes in antibiotic resistance genes [19]. Therefore, the present study aimed to perform molecular fingerprinting and determine the occurrence of bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{GES}, bla_{PER}, and bla_{VEB} genes among *K. oxytoca* isolated from clinical samples from selected hospitals in Khorramabad city in 2019.

2. METHODS

2.1. Sample Collection

The present study was cross-sectional. The study population included all *K. oxytoca* isolates collected from clinical samples at Shahid Rahimi and Shohadaye Ashayer hospitals in Khorramabad, Iran, in 2019, as part of a census. Given that *K. oxytoca* isolates were received from the laboratory without the patient's name and with a code, there was no need for written consent from the patients.

Information on the samples, including patient age and gender, hospitalization department, and sample type, was recorded. Isolates were collected from various clinical specimens, including urine, blood, wounds, secretions, and body fluids. After culturing the samples on blood agar and MacConkey agar media, the colonies were diagnosed by biochemical and microbial tests, including Gram staining, catalase, oxidase, citrate consumption, motility, indole production, MR, VP, and sugar consumption in TSI medium [20].

2.2. Inclusion Criteria

The inclusion criteria are as follows:

- All clinical samples collected in 2019 from patients hospitalized or referred to Shahid Rahimi and Shohadaye Ashayer hospitals in Khorramabad city.

- Isolates that grew on Blood agar and MacConkey agar after cultivation.
- Samples that were identified as *K. oxytoca* based on biochemical and microbiological tests, including Gram stain, catalase, oxidase, citrate consumption, motility, indole production, MR and VP tests, and sugar fermentation in TSI medium.
- Clinical samples, including urine, blood, wound, secretions, and other body fluids.
- Samples for which the patients' demographic information (age, gender) and the relevant hospitalization ward were recorded.

2.3. Exclusion Criteria

Hospital-acquired bacteria that are contaminated with other bacteria.

2.4. Determination of Antibiotic Resistance Patterns and Detection of Extended-spectrum Beta-lactamase (ESBL)-producing Isolates

Antibiotic susceptibility pattern was determined by disk diffusion on Mueller-Hinton Agar (Merck, Germany) according to CLSI standard guidelines 2020 [21]. Ceftazidime, cefotaxime, ciprofloxacin, cefepime, aztreonam, imipenem, amikacin, gentamicin, nalidixic acid, and co-trimoxazole disks (Rosco, Denmark) were used in this study. To screen for ESBL-producing *K. oxytoca*, a half-McFarland bacterial suspension was cultured on Mueller-Hinton Agar. Then, 3 disks of ceftazidime, cefotaxime, and cefpodoxime alone and 3 discs in combination with clavulanic acid (ceftazidime/clavulanic acid, cefotaxime/clavulanic acid, and cefpodoxime/clavulanic acid) were placed on the culture medium. After incubation for 18-24 hours at 37°C, the diameters of the growth inhibition zones were measured. If the difference in the diameter of the zone of inhibition around at least one of the combined disks (containing clavulanic acid) compared to the single disk (without clavulanic acid) was more than 5 mm, the isolate was ESBLs-positive phenotype [22].

2.5. DNA Extraction and PCR

The SinnaPure DNA kit (SinaClon, Iran) was used to extract bacterial DNA (chromosomal and plasmid). All steps of DNA extraction were performed according to the kit instructions. In this study, the multiplex PCR method was used to detect the bla_{TEM} and $bla_{\text{CTX-M}}$ genes. However, other genes were examined by simple PCR. The primer sequences and amplification temperature programs for the studied genes are shown in Tables **1** and **2**, respectively. The PCR products were electrophoresed on 1% agarose, and the gels were finally visualized using a Gel Doc (BioDoc Analyse, Biometra). *Pesedomonas aeroginosa* strain containing bla_{GES}, bla_{PER}, $bla_{\text{VEB,}}$ and *K. pneumoniae* ATCC 7881 were used as positive gene controls.

Table 1. Primer sequences of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{GES}, bla_{PER}, bla_{VEB} genes.

Primers	Sequence of Primers	Size of Amplicon (bp)	References
bla_{TEM}	5′-TCGCCGCATACACTATTCTCAGAATGA-3′ 5′-ACGCTCACCGGCTCCAGATTTAT-3′	445	[23]
bla_{SHV}	5′-TTAGCGTTCCCAGTGCTC-3′ 5′-GGTTATGCGTTATATTCGCC-3′	865	[24]
$bla_{\text{CTX-m}}$	5′-ATGTGCAGYACCAGTAARGTKATGGC-3′ 5′-TGGGT RAA RTA RGTSACCAGAA YCAGCGG-3′	593	[24]
bla_{PER}	5′-GCTCCGATAATGAAAGCGT-3′ 5′-TTCGGCTTGACTCGGCTGA-3′	520	[25]
bla_{VEB}	5′-CATTTCCCGATGCAAAGCGT-3′ 5′-CGAAGTTTCTTTGGACTCTG-3′	648	[26]
bla_{GES}	5′-AGTCGGCTAGACCGGAAAG-3′ 5′-TTTGTCCGTGCTCAGGAT-3′	399	[26]

Note: *K is G or T, R is A or G, S is G or C, Y is C or T.

Table 2. Time and temperature schedule of replication of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{GES}, bla_{PER}, bla_{VEB} genes.

	bla_{TEM}, $bla_{\text{CTX-M}}$ (Multiplex)		bla_{SHV}		bla_{PER}		bla_{GES}		bla_{VEB}	
	Temp	Time	Temp	Time	Temp	Time	Temp	Time	Temp	Time
Initial denaturation	94Cº	10 min	94Cº	10 min	94Cº	10 min	94Cº	10 min	94Cº	10 min
Denaturation	94Cº	40 s	94Cº	1 min	94Cº	40 s	94Cº	40 s	94Cº	40 s
Annealing	60Cº	40 s	58Cº	1 min	60Cº	40 s	60Cº	40 s	60Cº	40 s
Extension	72Cº	1 min	72Cº	1 min	72Cº	1 min	72Cº	1 min	72Cº	1 min
No. of cycles	30 cycles		32 cycles		30 cycles		30 cycles		30 cycles	
Final extension	72Cº	7 min	72Cº	10 min.	72Cº	7 min	72Cº	7 min	72Cº	5 min

2.6. Pulsed Field Gel Electrophoresis (PFGE)

All 32 *K. oxytoca* isolates were typed using the PFGE technique according to the previously described procedure by Elahi *et al*. [27]. Briefly, the genomic DNA of the tested isolates and *Salmonella enterica* serovar Braenderup H9812 (as a DNA marker) was embedded in low-melting-point agarose (Thermo Scientific, Lithuania). Digestion was carried out with 20 U *XbaI* enzyme (Fermentas, Lithuania). The fragments were electrophoresed on a CHEF MAPPER apparatus (Bio-Rad, USA) for 22 h at 14°C, with a switch time of 5-35 s, a voltage gradient of 120°, and a field strength of 6 V/cm. Gels were stained with ethidium bromide and visualized by the UVItec Gel Doc system (UVItec, UK). The obtained electrophoretic patterns were compared using BioNumerics software, version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). The Dice coefficient calculated the similarities, and the unweighted pair-group method was used for cluster analysis (UPGMA). The similarity of more than 80% between the DNA patterns of isolates was considered a clone.

2.7. Statistical Analysis

Data were processed using SPSS software (version 25) and statistically analyzed by the Chi-square (X^2) test. In all cases, $p \leq 0.05$ was considered significant.

3. RESULTS

In this study, 32 isolates of *K. oxytoca* were identified using biochemical tests. The isolates were Gram-negative coccobacilli, citrate-positive, indole-positive, VP-positive, catalase-negative, MR-negative, and negative for acid production, glucose and lactose fermentation, and H2S production in TSI medium. Of the 32 *K. oxytoca* isolates, 21 (65.6%) were isolated from women and 11 (34.4%) from men. The majority of subjects belonged to the 41–60 age group (10 subjects, 31.3%). The mean age of the subjects whose samples were included in the study was 46.16 ± 22.18 years. The youngest subject was 10 years old, and the oldest was 84 years old. Most isolates were from urine samples (28, 87.5%), followed by wounds and sputum (2 each, 6.3%). Of the total samples, 28.1% were collected from Shahid Rahimi Hospital and 71.9% from Shohadaye Ashayer Hospital. In addition, most of the samples were from the outpatient ward, accounting for 53.1% (Table **3**).

3.1. Antibiotic Susceptibility Pattern

Among the 32 *K. oxytoca* isolates collected, the resistance rate to antibiotics ceftazidime, cefotaxime, ciprofloxacin, cefepime, aztreonam, imipenem, amikacin, gentamicin, nalidixic acid, co-trimoxazole and colistin was (43.8%) 14, (65.6%) 21, (46.9%) 15, (31.3%) 10, (31.3%) 10, (15.6%) 5, (18.8%) 6, (31.3%) 10, (59.4%) 19, (59.4%) 19 and (0.0%) 0, respectively. Therefore, the highest resistance rate was to ampicillin and cefotaxime, and the lowest was to colistin, imipenem, and amikacin.

3.2. Screening for ESBL-producing *K. oxytoca*

Among 32 *K. oxytoca* strains, 21 (56.6%) were phenotypically ESBL-producing. The distribution of ESBL-positive isolates according to the source of specimens, patients' gender, ward, and hospital is shown in Table **4**. There was no significant relationship between the variables studied and the frequency of ESBL-producing *K. oxytoca* isolates ($p<0.05$).

Table 3. Frequency distribution and demographic characteristics of the tested samples.

Variable	Category	Frequency (%)
Gender	Male	11 (34.4)
	Female	21 (65.6)
Age groups	< 20	6 (18.8)
	21–40	7 (21.9)
	41–60	10 (31.3)
	61–80	7 (21.9)
	> 80	2 (6.3)
Source of samples	Urine	28 (87.5)
	Wound	2 (6.3)
	Sputum	2 (6.3)
Hospitals of sampling	Shohadaye Ashayer	23 (71.9)
	Shahid Rahimi	9 (28.1)
Sampling wards	Outpatient	17 (53.1)
	Women	4 (12.5)
	Urology	2 (6.3)
	ICU	4 (12.5)
	Surgery	2 (6.3)
	Burn	3 (9.4)

Table 4. The distribution of ESBL-positive *K. oxytoca* isolates based on variables.

Variable	Category	Positive n (%)	Negative n (%)	Chi-square (X^2)	*p*-value
Age groups	< 20	4 (66.7)	2 (33.3)	1.582	0.850
	21–40	4 (57.1)	3 (42.9)		
	41–60	8 (80.0)	2 (20.0)		
	61–80	4 (57.1)	3 (42.9)		
	> 80	1 (50.0)	1 (50.0)		
Source of samples	Urine	19 (67.9)	9 (32.1)	0.415	0.998
	Wound	1 (50.0)	1 (50.0)		
	Sputum	1 (50.0)	1 (50.0)		
Hospitals of sampling	Shohadaye Ashayer	14 (60.9)	9 (39.1)	0.82	0.441
	Shahid Rahimi	7 (77.8)	2 (22.2)		
Gender	Male	7 (63.6)	4 (36.4)	0.029	0.998
	Female	14 (66.7)	7 (33.3)		
Sampling wards	Outpatient	10 (58.8)	7 (41.2)	4.142	0.605
	Women	3 (75.0)	1 (25.0)		
	Urology	2 (100.0)	0		
	ICU	3 (75.0)	1 (25.0)		
	Surgery	2 (100.0)	–		
	Burn	1 (33.3)	2 (66.7)		

3.3. The Distribution of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{GES}$, *bla*$_{PER}$, and *bla*$_{VEB}$ genes

Based on the results of PCR tests, the frequency of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes was 19 (59.4%), 16 (50%), and 2 (6.3%). Also, the frequency of *bla*$_{GES}$, *bla*$_{PER}$, and *bla*$_{VEB}$ genes was zero. As shown in Table **5**, the association between gender and the frequency of *bla*$_{CTX-M}$ genes was statistically significant (p=0.012). In addition, the association between hospitals and the presence of *bla*$_{SHV}$ (p=0.02) and *bla*$_{CTX-M}$ (p=0.049) genes was statistically significant. Conversely, the difference in the distribution of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes by source of samples, wards, and age groups was not statistically significant.

3.4. Molecular typing by PFGE

PFGE analysis was successfully performed on all 32 *K. oxytoca* isolates. To define clusters (pulse types), isolates with at least 80% similarity in PFGE patterns were grouped into a cluster, in accordance with standard PFGE protocols and previous studies. Based on this criterion, the isolates were divided into 18 distinct clusters, indicating high genetic diversity (Fig. **1**). The largest cluster (cluster no. 10) contained 7 isolates (21.9%; 7 out of 32). Epidemiological investigation showed that all isolates in this cluster were collected from the outpatient ward, and no strong epidemiological links were observed among them. The other 25 isolates (78.1%; 25 of 32) were grouped into 17 clusters with one to three members, further confirming that there is significant genetic diversity among isolates and no clear clonal expansion is observed.

4. DISCUSSION

K. oxytoca is a member of the Enterobacteriaceae family and is reported less frequently than *K. pneumoniae*. In the present study, 32 *K. oxytoca* isolates were obtained from various clinical specimens collected from wards of Shahid Rahimi and Shohadaye Ashayer Teaching Hospitals in Khorramabad over a 1-year period. The prevalence observed in this study is comparable to previous reports from Iran and other countries, including studies conducted in Tehran (1–12.8%), Shahrekord (2.2%), and Burkina Faso (14.06%), confirming the relatively low frequency of *K. oxytoca* compared with *K. pneumoniae* [28–31]. *K. oxytoca* is recognized as an important pathogen across all age groups. In the present study, the highest isolation rate was observed in patients aged 41–60 years. This finding differs from those reported by Validi *et al*., who found the highest prevalence in the 16–30-year age group [28], and by Khodaparast *et al*., who found the highest prevalence in children aged 1–15 years [32]. These discrepancies may be attributed to differences in patient populations, geographic regions, and specimen sources. Cephalosporins, carbapenems, and aminoglycosides are commonly used as first-line agents for the treatment of *Klebsiella* infections [33, 34]. However, increasing resistance to cephalosporins and carbapenems has become a major concern worldwide, largely due to the acquisition of plasmid-mediated resistance genes, particularly those encoding Extended-Spectrum β-Lactamases (ESBLs) [35, 36]. In the present study, none of the isolates showed resistance to colistin. Similarly, Nassari *et al*. reported complete susceptibility of *K. oxytoca* isolates to imipenem and colistin in hospitals in Shiraz [37], findings that are in agreement with ours. In contrast, high resistance rates were observed against amikacin, cefoxitin, ciprofloxacin, and cefepime. These results indicate that many β-lactam antibiotics are no longer effective for treating infections caused by *K. oxytoca* in this region. Similar resistance patterns have been reported by Validi *et al*., who observed variable susceptibility of *K. oxytoca* isolates to gentamicin, carbapenems, cephalosporins, and aztreonam, with the

highest resistance to ampicillin, amoxicillin, and ticarcillin [28]. Furthermore, studies from Iraq have reported high resistance rates to β-lactams and carbapenems among urinary isolates of *K. oxytoca*, emphasizing the risk of emerging multidrug resistance and the need for continuous surveillance [38]. The variability in antimicrobial resistance patterns across studies and regions reflects differences in antibiotic consumption, infection control practices, sample sources, and the timing of isolation collection. Even within the same geographic region, resistance profiles may change over time, highlighting the importance of periodic monitoring to guide appropriate empirical therapy [39]. In the present study, 56.6% of the isolates were identified as ESBL producers based on phenotypic methods, which is higher than rates reported in several previous studies, including those conducted in India (25%), the Netherlands (2.4%), and Nepal (16.7%) [40–42]. Molecular analysis revealed that bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes were present in 59.4%, 50%, and 6.3% of isolates, respectively, while bla_{GES}, bla_{PER}, and bla_{VEB} were not detected. These findings differ from studies conducted in Iran, Tunisia, Lebanon, and Egypt, where lower frequencies of ESBL genes among *K. oxytoca* isolates were reported [43–47]. The absence of bla_{PER} in our isolates is in line with the findings of Nakhaei Moghaddam *et al*. in Tehran [48]. Recent studies from Iraq and Japan have reported a high burden of resistance genes, including bla_{CTX-M}, bla_{AmpC}, and bla_{SHV}, in both human and animal *K. oxytoca* isolates, indicating the potential for widespread dissemination of resistance genes [49, 50]. Whole-genome sequencing studies from China have also demonstrated high genomic diversity among *K.oxytoca* complex isolates, supporting the heterogeneous nature of this species [51]. PFGE analysis in the present study revealed high genetic diversity, with 32 isolates grouped into 18 clusters at ≥80% similarity, suggesting multiple sources rather than clonal spread. This diversity may be related to patient referral from different healthcare centers within the province.

Table 5. Frequency distribution table of the studied genes by different variables.

		bla_{CTX-M}		bla_{TEM}			bla_{SHV}	
Variable	**Category**	**Positive n (%)**	**Negative n (%)**	**Positive n (%)**	**Negative n (%)**	**Total n (%)**	**Positive n (%)**	**Negative n (%)**
Source of samples	Urine	15 (53.6)	13 (46.4)	16 (57.1)	12 (42.9)	28 (100)	2 (7.1)	26 (92.9)
	Wound	–	2 (100)	1 (50.0)	1 (50.0)	2 (100)	–	2 (100)
	Sputum	1 (50.0)	1 (50.0)	2 (100)	–	2 (100)	–	2 (100)
	Chi-square (X^2)	2.143		1.499			0.305	
	***P*-value**	0.731		0.757			0.999	
Sampling wards	Outpatient	8 (47.1)	9 (52.9)	9 (52.9)	8 (47.1)	17 (100)	1 (5.9)	16 (94.1)
	Women	4 (100)	–	3 (75.0)	1 (25.0)	4 (100)	–	4 (100)
	Urology	–	1 (100)	2 (100)	–	2 (100)	–	2 (100)
	ICU	2 (50.0)	2 (50.0)	2 (50.0)	2 (50.0)	4 (100)	1 (25.0)	3 (75.0)
	Surgery	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	2 (100)	–	2 (100)
	Burn	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)	3 (100)	–	3 (100)
	Chi-square (X^2)	5.365		2.35			3.137	
	***P*-value**	0.449		0.873			0.721	
Hospitals of sampling	Shohadaye Ashayer	9 (39.1)	14 (60.9)	13 (56.5)	10 (43.5)	23 (100)	–	23 (100)
	Shahid Rahimi	7 (77.8)	2 (22.2)	6 (66.7)	3 (33.3)	9 (100)	2 (22.2)	7 (77.8)
	Chi-square (X^2)	3.865		0.276			5.452	
	***P*-value**	0.049		0.704			0.020	
Gender	Male	2 (18.2)	9 (81.8)	6 (54.5)	5 (45.5)	11 (100)	–	11 (100)
	Female	14 (66.7)	7 (33.3)	13 (61.9)	8 (38.1)	21 (100)	2 (9.5)	19 (90.5)
	Chi-square (X^2)	6.788		0.162			1.117	
	***P*-value**	0.012		0.721			0.534	
Age groups	< 20	4 (66.7)	2 (33.3)	3 (50.0)	3 (50.0)	6 (100)	1 (16.7)	5 (83.3)
	21–40	2 (28.6)	5 (71.4)	6 (85.7)	1 (14.3)	7 (100)	–	7 (100)
	41–60	6 (60.0)	4 (40.0)	5 (50.0)	5 (50.0)	10 (100)	1 (10.0)	9 (90.0)
	> 60	4 (44.4)	5 (55.6)	5 (55.6)	4 (44.4)	9 (100)	–	9 (100)
	Chi-square (X^2)	2.463		2.657			2.418	
	***P*-value**	0.505		0.483			0.550	

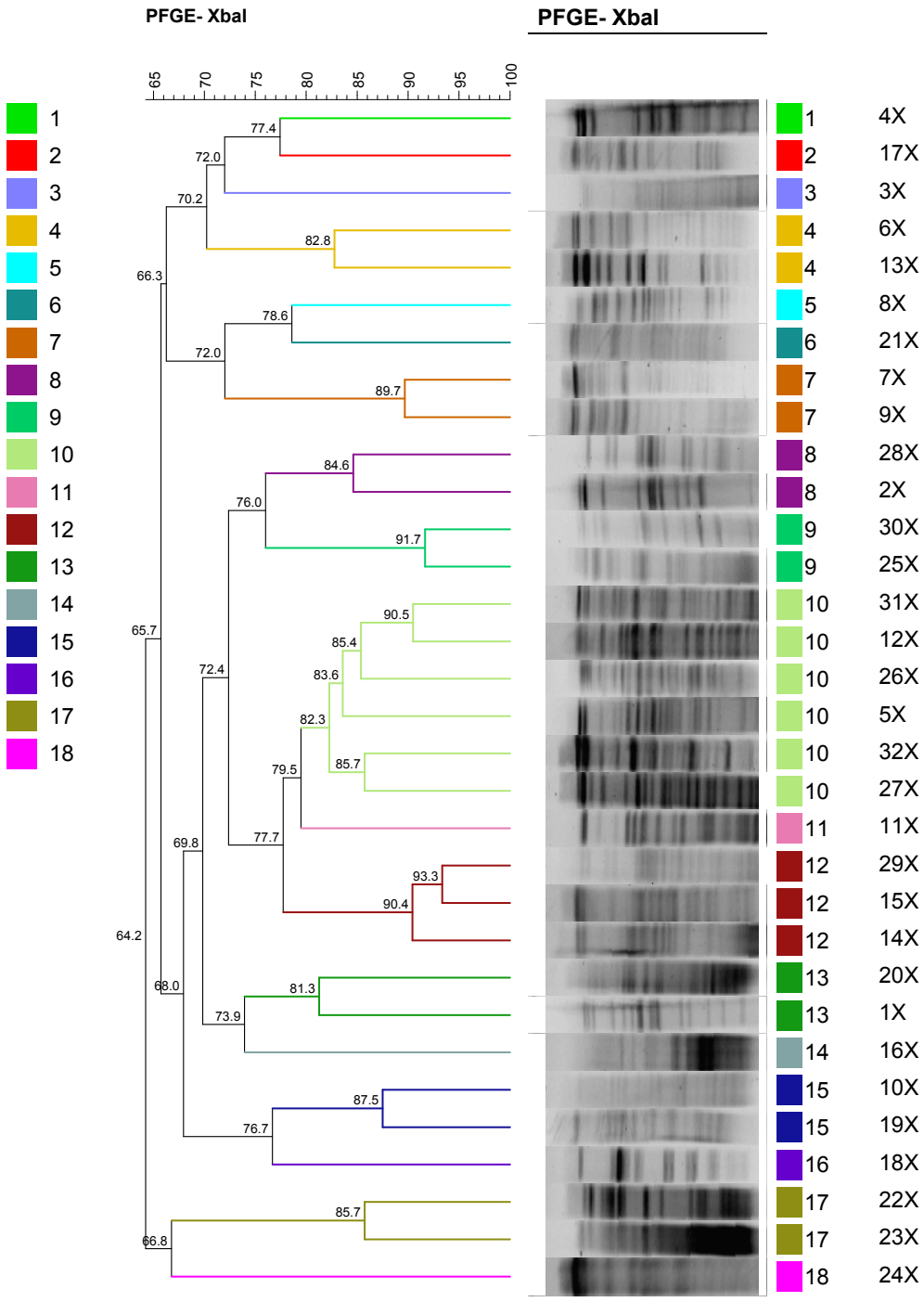

Fig. (1). PFGE typing of the tested *K. oxytoca* isolates. Electropherogram of PCR products from 52 tested samples, digested with the *Xba1* enzyme. Isolates were divided into 18 distinct clusters. The largest cluster (cluster no. 10) contained 7 isolates collected from the outpatient ward. The fragments were electrophoresed by the CHEF MAPPER apparatus (Bio-Rad, USA) for 22 h at 14°C. Gels were stained with ethidium bromide and visualized by the UVItec Gel Doc system (UVItec, UK). The electrophoretic patterns obtained were compared using BioNumerics software, version 7.1

One of the main reasons for the heterogeneity among *K. oxytoca* strains in our study could be the diversity in sample collection, wards, and the inclusion of inpatients and outpatients. In addition, Shahid Rahimi and Shohada Ashayer hospitals are considered provincial referral hospitals, which leads to the admission of diverse patients from across the province, resulting in genetic diversity of strains.

The high prevalence of ESBL-producing *K. oxytoca* has significant clinical implications, including limited therapeutic options, increased reliance on carbapenems, prolonged hospitalization, higher healthcare costs, and increased mortality. Therefore, strict infection control measures, rational antibiotic use, and implementation of antimicrobial stewardship programs are essential. As the first study to investigate *K. oxytoca* isolates in Lorestan Province, this research provides valuable baseline data on antimicrobial resistance patterns and the distribution of ESBL genes. Further studies focusing on additional resistance mechanisms, including metallo-β-lactamases, are recommended to improve treatment strategies and limit the spread of antimicrobial resistance.

5. LIMITATIONS

The following are some of the limitations of this study:

A) The number of isolates studied was relatively limited, which may affect the generalizability of the results.

B) Samples were collected only from selected hospitals in one city; therefore, the resistance pattern in other regions or treatment centers may be different.

C) Due to financial and equipment limitations, it was not possible to examine a wider range of resistance genes or complementary molecular typing methods.

CONCLUSION

Given the increasing use of antibiotics in hospitals and the community, and the growing spread of ESBL-producing isolates, it is necessary to identify ESBL-producing isolates and determine their antibiotic resistance patterns before prescribing antibiotics. This will allow patients with serious infections to be prescribed beta-lactam antibiotics with the greatest possible efficiency.

AUTHORS' CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: G.H.G.: Conceived the study and supervised the project; P.S.H and G.H.G.: Curated the data and wrote the manuscript; H.À.: Performed the data analysis; T.Z.: Contributed to the content of the present manuscript and participated in revising the paper.

LIST OF ABBREVIATIONS

K. oxytoca	=	Klebsiella oxytoca
ESBLs	=	Extended-spectrum beta-lactamases
PFGE	=	Pulsed-field gel electrophoresis
CLSI	=	Clinical and Laboratory Standards Institute

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures related to clinical samples were approved by the Ethics Committee of Lorestan University of Medical Sciences, Iran (IR.LUMS.REC.1400.166).

HUMAN AND ANIMAL RIGHTS

All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Not applicable.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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