The first report of *Enterobacter gergoviae* carrying *bla*<sub>NDM-1</sub> in Iran

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**ABSTRACT**

**Objective(s):** Prompt detection of extended-spectrum β-lactamases (ESBL) and carbapenemase-producing enterobacteriaceae is crucial for infection prevention and control strategies. The present study aimed to characterize the ESBL and carbapenemase genes among *Enterobacter* isolates from an Iranian inpatient population.

**Materials and Methods:** A total of 96 *Enterobacter* isolates obtained from inpatients between June 2016 and March 2017, were identified by the conventional microbiological methods and diagnostic kits. Antimicrobial susceptibility pattern was performed using the disk diffusion method. The ESBL and carbapenemase genes were screened using polymerase chain reaction (PCR).

**Results:** All clinical isolates of *Enterobacter* were classified as *E. gergoviae* (52, 54.2%), *E. aerogenes* (34, 35.4%), *E. cloacae* (7, 7.3%), *Cronobacter* (E), *sakazakii* (3, 3.1%). The highest and lowest antimicrobial resistance rates were observed against ampicillin (93.8%) and imipenem (21.9%). High prevalence of multi-drug resistance (MDR=96.9%) was substantial. Of the 96 *Enterobacter* isolates, 35 (36.5%) and 28 (29.2%) were phenotypically ESBL-positive and non-susceptible carbapenem, respectively. Overall, the frequency of evaluated genes was as follows: *bla*<sub>CTX-M</sub>=25 (26%), *bla*<sub>SHV</sub>=30 (31.3%), *bla*<sub>IMP</sub>=3 (3.1%), *bla*<sub>TEM</sub>=12 (12.5%), *bla*<sub>VIM</sub>=3 (3.1%), *bla*<sub>NDM</sub>=8 (8.3%), and *bla*<sub>KPC</sub>=0 (0%).

**Conclusion:** In this study, we report for the first time the presence of *E. gergoviae* harboring *bla*<sub>NDM-1</sub> from an Iranian population. Regarding the increase of MDR *Enterobacter* spp. in our region, strict hygiene rules will be needed to control the quick spread of ESBL and carbapenemase-producing *Enterobacter* isolates in healthcare facilities of developing countries.

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**Introduction**

Among enterobacteriaceae members, *Enterobacter* spp. is of particular concern, since it exhibits a higher level of resistance to antibiotics than other genera (1). The presence of β-lactamases, especially extended-spectrum β-lactamases (ESBLs) among Gram-negative bacteria is a major issue in clinical settings (2). The production of ESBLs is one of the most important mechanisms of resistance to extended-spectrum penicillins, third-generation of cephalosporins and monobactams, except for cephamycins and carbapenems (3-5). These enzymes have been reported in many enterobacteriaceae members, including *Enterobacter* spp (2, 3). The increasing prevalence of ESBL-producers is seen among both in and outpatients worldwide, ranging from 3–60% (2-4). The members of TEM, SHV, and CTX-M β-lactamases in *Klebsiella* and *Enterobacter* spp. are the most important ESBLs which have been growing all around the world (6).

Carbenpens are frequently used to treat infections due to cephalosporinase or ESBL-producing multidrug-resistant (MDR) Gram-negative rods such as *Enterobacter* species. However, the emergence of carbapenemases among these bacteria has restricted use of carbenpens in medical practice (7, 8). The main mechanism in emergence of carbapenem-resistant enterobacteriaceae (CRE), including *Enterobacter* spp., is the production of carbapenemases. Different carbapenemases have been described amongst these bacteria, including Ambler class A *bla*<sub>OGK</sub> metallo-β-lactamases (MBL) class B such as *bla*<sub>OGX</sub> *bla*<sub>OGX</sub> *bla*<sub>OGX</sub> etc. (9, 10).

Nosocomial infections caused by CRE are considered serious clinical challenges for physicians worldwide, and this issue is due to the capability of their rapid spread around the world. The mortality rate of infections caused by CRE is considerable, ranging from 30-44% (9-11). Moreover, infections caused by ESBL-producing bacteria, including *Enterobacter* spp., among inpatients are accompanied by increased mortality (12).

Reports about the prevalence of ESBL and carbapenemase-producing *Enterobacter* spp. from Iran are scarce. This study was undertaken to characterize infections caused by ESBL-positive and carbapenem-resistant *Enterobacter* spp. collected in Shiraz Namazi Hospital, Shiraz, Iran.

**Materials and Methods**

**Clinical isolates**

A total of 96 non-repetitive *Enterobacter* isolates were obtained from patients hospitalized at a university-affiliated medical center (Namazi) in Shiraz, Southwest of Iran from June 2016 to March 2017. Only one isolate was collected per patient. The isolates were recovered from different clinical samples, namely blood, wound, sputum, endotracheal tube aspirates, abdominal discharge, urine, and eye. *Enterobacter* spp. was initially identified by standard microbiological tests and confirmed using API 20E (bioMérieux, Marcy l’Etoile, France).
France) and Microgene™ GnA+B-1D system (Microgen Bioproducts Ltd, UK) diagnostic kits. Confirmed Enterobacter spp. isolates were stored in tryptic soy broth (TSB) (Merck Co., Germany) containing 20% glycerol at -70 °C until further study. This study was in accordance with the declaration of Helsinki and ethical approval was sought from the institutional Ethics Committee of Shiraz University of Medical Sciences (approval No. EC IR.SUMS.REC.C.1396.5526). However, because we only used leftovers from clinical specimens, the local ethics committee waived the need for informed consent.

Susceptibility testing

Antimicrobial susceptibility pattern was determined by the disk diffusion method on Muller-Hinton agar plates (Merck Co., Germany) following Clinical and Laboratory Standards Institute (CLSI) guidelines (13). Guidelines of the CLSI were used for ampicillin, ceftazidime, cefotaxime (30 µg), and ceftriaxone (30 µg) disks were applied alone and in combination with clavulanic acid (30/10 µg). An increase of ≥ 5 mm in the inhibition zone of the agent in combination with clavulanic acid ceftazidime (30 µg) and cefotaxime (30 µg) disks were applied alone and in combination with clavulanic acid (30/10 µg). An increase of ≥ 5 mm in the inhibition zone of the agent in combination with clavulanic acid was considered ESBL producer. E. coli ATCC 25922 was used as the quality control strain. MDR was defined as non-susceptibility to ≥1 agent in ≥3 different antibiotic classes (14).

ESBL phenotypic detection was performed using the combination disk method in accordance with CLSI recommendations (13). All ceftazidime (as a third-generation cephalosporin) resistant isolates were selected for evaluation of ESBL production. In this test, ceftazidime (30 µg) and cefotaxime (30 µg) disks were applied alone and in combination with clavulanic acid (30/10 µg). An increase of ≥ 5 mm in the inhibition zone of the agent in combination with clavulanic acid was considered ESBL producer. E. coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were used as negative and positive control strains, respectively.

Genotypic detection of ESBL and carbapenemase genes

Genomic DNA was extracted from overnight TSB culture using a Cinna-pure kit (CinnaGen Co., Iran) according to the manufacturer’s instructions. Molecular characterization of ESBLs (blaTEM, blaprov, and blaCTX-M) and carbapenemases (blaKPC, blavac, blahcm, and blaind) were screened in all isolates by PCR amplification using specific previously reported primers (15, 16). PCRs were performed using a thermal cycler 5530 (Eppendorf master, Germany) with 1 µl of each specific primer (1 µM), 3 µl DNA template, 2.5 µl PCR buffer (1X), 1 µl deoxyribonucleotide triphosphates solution (dNTPs, 200 µM), 1.5 µl MgCl2 (1.5 mM), and 0.25 µl Taq DNA polymerase (1 Unit) in a total volume of 25 µl. PCRs comprised 5 min at 94 °C initial denaturation, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing (the eventual annealing temperatures chosen were 45–60 °C for corresponding genes), extension of 1 min at 72 °C, and a final elongation step of 7 min at 72 °C. The positive PCR products were screened by electrophoresis on agarose 1.5% w/v gels and stained with safe stain load dye (CinnaGen Co., Iran) and visualized through UV transillumination.

DNA sequence analysis

To confirm the accuracy of amplified carbapenemase genes (one sample of each positive gene and three samples for blasmogene), the amplicons were submitted for sequencing (Bioneer Co., Munpyeongseo, Daedeok-gu, Daejeon, South Korea) and the sequences were compared using online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/). For ESBL genes, Klebsiella pneumoniae ATCC700603 was used as control strain.

Statistical analysis

The Chi-square (χ2) test was used to analyze significant differences between the studied resistance genes and the clinical outcome, using SPSS (ver. 21.0; IBM Co., Armonk, NY, USA) software. The results of demographic and clinical manifestations were presented as descriptive statistics in terms of relative frequency. A P-value<0.05 was considered as significant clinical relevance.

Results

Study population and clinical characteristics of Enterobacter isolates

The isolates were collected from 96 individuals admitted as inpatients, consisting of 62 (64.6%) men and 34 (35.4%) women with a median age of 42 years (range=9 days to 75 years). Distribution of isolation of Enterobacter spp. from different clinical samples was as follows: respiratory tract infection (RTI) (n=49, 51%), skin and soft tissue infection (SSTI) (n=18, 18.8%), urinary tract infection (UTI) (n=12, 12.5%), bloodstream infection (BSI) (n=7, 7.3%), abdominal infection (n=5, 5.2%), and eye infection (n=5, 5.2%). Moreover, the recovered Enterobacter isolates from Intensive Care Unit (ICU), Internal, Surgery, and Transplantation wards were 55 (57.3%), 36 (37.5%), 4 (4.2%), and 1 (1%), respectively. All 96 clinical isolates of Enterobacter were classified as E. gergoviae (n=52, 54.2%), E. aerogenes (n=34, 35.4%), E. cloacae (n=7, 7.3%), and Cronobacter (E) sakazakii (n=3, 3.1%).

Antimicrobial resistance among Enterobacter isolates

The results of susceptibility testing are depicted in Table 1. All 96 clinical isolates revealed resistance to all antimicrobials with different proportions. The highest resistance (non-susceptible isolates) rate was seen to β-lactams, including ampicillin, amoxicillin-clavulanate, cefoxitin, and ceftazidime. Conversely, the lowest resistance rate was against imipenem (29.2%), followed by amikacin (30.2%). Among different Enterobacter spp., E. gergoviae represented the highest (90%) resistance to antimicrobial agents. The majority of isolates (n=93, 96.9%) exhibited a multi-drug resistant (MDR) phenotype. Except for E. gergoviae isolates whose MDR rate was 94.2%, all the remaining isolates from other species were MDR.

Totally, among 96 Enterobacter isolates, 35 (36.4%) were positive for the ESBL phenotype. The prevalence of ESBL in E. gergoviae, E. aerogenes, E. cloacae, and C. sakazakii was 28.8% (15/52), 50% (17/34), 28.6% (2/7), and 33.3% (1/3), respectively. Among the antimicrobial agents evaluated, imipenem was the
most active antibiotic (80%) against the ESBL-positive isolates, and ciprofloxacin had a notable in vitro activity (68.6%). There was no significant correlation between ESBL production and higher antibiotic resistance, except for ceftazidime (Table 1). All ESBL producers were MDR; however, compared to non-ESBL producers (95.1%) the differences were not statistically significant ($P = 0.18$).

**Characterization of ESBL and carbapenemase genes**

Of the 35 isolates identified as ESBL-producers, 16 (45.7%) isolates harbored the TEM type enzyme, and 15 (42.8%) and 8 (22.8%) carried CTX-M and SHV type enzymes, respectively. A statistically significant difference was determined between ESBL-positive isolates and the presence of TEM, CTX-M, and SHV genes with values 0.021, 0.004, and 0.02, respectively; $bla_{TEM}$ and $bla_{CTX-M}$ was found to be the frequent combination (n=9, 9.4%), followed by $bla_{TEM}$+$bla_{SHV}$+$bla_{CTX-M}$ (n=3, 3.1%) (Table 3). Among ESBL-producers, $bla_{IMP}$ and $bla_{NDM}$ genes were sought in 2 (5.7%) and 5 (14.3%) of the isolates, respectively. Furthermore, there was no significant correlation between any of the mentioned genes among ESBL-producing isolates.

Of the 96 Enterobacter spp., 28 (29.2%) were phenotypically non-susceptible carbapenem isolates (Table 1); however, 3 (3.1%) and 8 (8.3%) of them harbored $bla_{IMP}$ and $bla_{NDM}$ genes. No PCR products were detected for any of the $bla_{VIM}$ and $bla_{KPC}$ genes investigated (Table 2). Meanwhile, sequencing results confirmed that all of the tested $bla_{NDM}$ positive isolates were NDM-1 variant.

**Discussion**

An increase in the emergence of MDR Enterobacter spp. producing ESBLs and carbapenemases has limited therapeutic options. Therefore, to reduce the mortality of nosocomial infections caused by these species, their early identification is necessary (17, 18). In the

### Table 1. Distribution of antibiotic resistant Enterobacter isolates according to ESBL production

| Antibiotic          | Total (N=96) | ESBL-Positive (N=35) | P-value $^a$ |
|---------------------|--------------|----------------------|--------------|
|                     | R            | I                    | S            | R            | I          | S            |              |
| Ampicillin          | 90 (93.8)    | 5 (5.2)              | 1 (1)        | 31 (88.6)    | 4 (11.4)   | 0            | 0.45         |
| Amoxicillin-clavulanate | 84 (87.5) | 6 (6.3)              | 6 (6.3)      | 30 (85.7)    | 2 (5.7)    | 3 (8.6)      | 0.48         |
| Cefoxitin           | 80 (80.1)    | 7 (7.3)              | 9 (9.4)      | 30 (85.7)    | 1 (2.9)    | 4 (11.4)     | 0.60         |
| Ceftazidime         | 70 (72.9)    | 2 (2.1)              | 24 (25)      | 30 (85.7)    | 2 (5.7)    | 3 (8.6)      | 0.005        |
| Imipenem            | 21 (21.9)    | 7 (7.3)              | 68 (70.8)    | 5 (14.3)     | 2 (5.7)    | 28 (80)      | 0.13         |
| Gentamicin          | 39 (40.6)    | 1 (1)                | 56 (58.3)    | 14 (40)      | 0          | 21 (60)      | 0.80         |
| Amikacin            | 22 (22.9)    | 7 (7.3)              | 67 (69.8)    | 6 (17.1)     | 6 (17.1)   | 23 (65.7)    | 0.51         |
| Trimethoprim         | 45 (46.9)    | 6 (6.3)              | 45 (46.9)    | 19 (54.3)    | 4 (11.4)   | 12 (34.3)    | 0.061        |
| sulfamethoxazole     |              |                      |              |              |            |              |              |
| Nitrofurantoin       | 68 (70.8)    | 14 (14.6)            | 14 (14.6)    | 22 (62.9)    | 5 (14.3)   | 8 (22.9)     | 0.082        |
| Ciprofloxacin        | 31 (32.3)    | 4 (4.2)              | 61 (63.5)    | 8 (22.9)     | 3 (8.6)    | 24 (68.6)    | 0.44         |

$^a$ Compared with susceptibility rates of ESBL-negative isolates
R: resistant; I: intermediate-resistant; S: susceptible

### Table 2. Distribution of ESBL and carbapenemase genes among Enterobacter spp.

| Species            | ESBL genes No. (%) | Carbenapenemase genes No. (%) |
|--------------------|-------------------|-----------------------------|
|                    | CTX-M | TEM | SHV | IMP | NDM | VIM | KPC |
| E. gergoviae (N=52)| 14 (26.9) | 19 (36.5) | 9 (17.3) | 0 | 6 (11.5) | 0 | 0 |
| E. aerogenes (N=34)| 8 (23.5) | 8 (23.5) | 2 (5.9) | 3 (8.8) | 1 (2.9) | 0 | 0 |
| E. cloacae (N=7)   | 2 (28.6) | 2 (28.6) | 1 (14.3) | 0 | 1 (14.3) | 0 | 0 |
| C. sakazakii (N=3) | 1 (33.3) | 1 (33.3) | 0 | 0 | 0 | 0 | 0 |
| Total (N=96)       | 25 (26) | 30 (31.3) | 12 (12.5) | 3 (3.1) | 8 (8.3) | 0 | 0 |
Table 3. Resistance genes pattern identified among Enterobacter isolates

| Gene pattern | Frequency | Percent |
|--------------|----------|---------|
| No gene      | 50       | 52.1    |
| TEM          | 10       | 10.4    |
| SHV          | 4        | 4.2     |
| CTX-M        | 7        | 7.3     |
| NDM          | 1        | 1.0     |
| TEM/SHV      | 2        | 2.1     |
| TEM/CTX-M    | 9        | 9.4     |
| TEM/IMP      | 2        | 2.1     |
| TEM/NDM      | 1        | 1.0     |
| SHV/NDM      | 1        | 1.0     |
| CTX/NDM      | 2        | 2.1     |
| TEM/SHV/CTX-M| 3        | 3.1     |
| TEM/CTX-M/IMP| 1        | 1.0     |
| TEM/CTX-M/NDM| 1       | 1.0     |
| SHV/CTX-M/NDM| 1       | 1.0     |
| TEM/SHV/CTX-M/NDM| 1 | 1.0 |
| Total        | 96       | 100.0   |

present study, we characterized the antimicrobial resistance pattern and the presence of seven ESBL and carbapenemase genes among 96 clinical isolates of Enterobacter recovered from an Iranian population. In the literature, E. cloacae and E. aerogenes have been suggested as the most common species of Enterobacter (1, 8). In our survey, by contrast, E. gergoviae was found the most frequently isolated species (54.2%), followed by E. aerogenes, E. cloacae, and C. sakazakii with frequencies 35.4%, 7.3%, and 3.1%, respectively. To our knowledge, there has been no further report of this species as an emerging nosocomial pathogen until this work in Iran. But in studies in Germany, Spain, and Hong Kong, E. gergoviae was isolated from clinical samples with frequencies of 26.1%, 6.6%, and 2.9%, respectively (19-21). In another survey from a nosocomial outbreak of bacteremia, 11 E. gergoviae were isolated from 11 babies in neonatal ICU (NICU) (22).

Enterobacter spp. are responsible for a wide variety of nosocomial infections, particularly wound infections, bacteremia, and pneumonia (1, 23). In the current study, most isolates (51%) were recovered from RTIs. Consistent with our work, Qin and co-workers (11) and Hoffmann et al. (17) isolated 91% and 37.8% of strains from respiratory tract samples, respectively. In contrast, in several studies from Brazil (7), China (24), a global surveillance program (25), and Korea (8), blood and abdominal samples were the most common sites of Enterobacter isolation. In our study, 57.3% of isolates were obtained from the ICU ward. Likewise, two authors from Germany (17) and Spain (26) showed most strains were isolated from ICU.

Members of ESBL-producing and CRE, including Enterobacter spp., have been emerging and increasing around the world and become a matter of great concern (11, 23, 27). By analysis of susceptibility testing, it is found the majority of our isolates were remarkably resistant to most of the antimicrobials tested, with 96.9% of strains showing MDR phenotype, making them a public health concern in our area. This finding does not coincide with two previously reported works from Iran with prevalence of 17.5% and 47.5% (28, 29). Carbapenem resistance was defined as resistance to one or more carbapenems according to CLSI guidelines (7). In the current study, 29.2% of isolates were non-susceptible to imipenem (carbapenem-resistant). In several studies from different areas, these rates were reported 8.7%, 25.7%, 35.1%, 18.3%, and 5.1% (8, 23, 28, 30, 31). Although CRE isolates are usually extensively drug-resistant, some isolates may be still susceptible to amikacin and ciprofloxacin. Hu and co-workers reported the rate of susceptibility of their isolates to amikacin and ciprofloxacin were 10.4 and 13%, respectively (32). Instead, 69.8% and 63.5% of our isolates were fortunately susceptible to amikacin and ciprofloxacin, correspondingly, indicating an alternative choice to treatment of infections caused by Enterobacter resistant isolates, especially ESBL-producers.

Thirty-five (36.4%) of our isolates were ESBL-producers using the phenotypic tests. The result was less than those observed by two other studies from Iran with prevalence of 52.6% and 44.2%, respectively (28, 29). The use of antimicrobials, including cefoxitin and ceftazidime in Iran, could partly explain this slightly high rate of ESBL among Enterobacter isolates. In agreement with our findings, in two investigations performed in Korea (33) and Germany (17), 35.4% and 40% of E. cloacae were ESBL-positive, respectively; however, Villa and colleagues (31) and Yu et al. (34) detected only 5.1% and 15% of isolates as ESBL-producers, correspondingly. On the other hand, in a report from China, ESBL-producing Enterobacter isolates comprised 65.7% (23). These discrepancies might be due to the differences in the epidemiology of isolates or sample sizes of studies.

It has been suggested that CTX-M and SHV-type beta-lactamases have been the predominant ESBLs in Enterobacter spp. (35). Conversely, beta-lactamases belonging to the TEM (31.3%) family were the ESBLs encountered most frequently in our isolates, followed by CTX-M (26%) and SHV (12.5%) types. Likewise, Ghanavati and colleagues reported blaTEM and blaSHV as the
most and less prevalent ESBL genes in their Enterobacter species (28). In a study from Brazil, blaTEM and blaCTX-M were the frequently identified ESBL genes with no blaSHV among E. aerogenes and E. cloacae isolates studied (7). In several studies from Algeria, Spain, and Korea, high rates of the CTX-M type with frequencies of 76%, 52.3%, 53.3%, and 60.8% have been reported, respectively (12, 26, 33, 36). Conversely, in an investigation from England (37), in none of Enterobacter spp. isolated from blood and urine samples, the blaCTX-M gene was detected. This result is not in agreement with our findings. The rate blaIMP was observed in our study was similar to another study with frequency of 10% (12), but much lower than those identified (52%) in Korea (36). On the contrary, in a work from Spain (26), no blaSHV and blaTEM were identified among Enterobacter obtained isolates.

Among evaluated carbapenemase genes, only blaNDM (n=8, 8.3%) and blaKPC (n=3, 3.1%) were detected. In other words, blaNDM was the most prevalent MBL as a mechanism of resistance to carbapenems in our Enterobacter spp. This study is the second reported presence of blaNDM among clinical isolates of Enterobacter in Iran. While in the first report 2.5% of isolates were carried blaNDM, the species and origin of isolates were not mentioned (30). In our work, 6 (75%) NDM-positive isolates were related to E. gergoviae, which is the first report of this species in Iran, and two other cases belonged to E. aerogenes and E. cloacae. This result is not consistent with those published in other countries such as China (23), Spain (38), Korea (8), and Mexico (9) with frequencies of 2.8%, 0%, 0%, and 100%, respectively, where blaNDM had been identified in E. aerogenes and/or E. cloacae.

blaKPC has been reported as the predominant carbapenemase gene associated with CRE intrahospital infections (9). The importance of KPC enzymes is due to high-level resistance to all beta-lactams and distinct levels of resistance to the carbapenem antibiotics (39). A study in Brazil showed 88.6% of E. aerogenes and 100% of E. cloacae isolates harbored blaKPC, and 8 other carbapenemase genes evaluated were not detected in any isolate (7). In an investigation in the United States, 11 (25%) isolates of the 44 carbapenem-nonsusceptible Enterobacter isolates were found to be KPC-producer (40). In our study, by contrast, no Enterobacter isolates harboring blaKPC were diagnosed. This result is consistent with results from other researchers who reported the rate of 0% for the blaKPC gene (8). However, in the studies from China and Spain, the frequencies of 19.3% and 6.8% were determined, respectively (11, 31).

It has been mentioned that carbapenemase production is mostly related to the presence of VIM and IMP types (23). Indeed, VIM-1-producing Enterobacter isolates, especially E. cloacae, have been frequently reported in some European countries and particularly in Spain and become major nosocomial pathogens in southern Europe and Asia (31, 38). In our investigation, however, no isolate carrying blaIMP was found and only 3.1% of isolates (3 E. aerogenes isolates) harbored the blaIMP gene. In accordance with the literature, 52% and 100% (7 isolates) of E. cloacae isolates in two studies from Spain were found to be blaIMP producers (31, 38). On the other hand, in research from the Far East the rates of blaIMP (0.5%) and blaKPC (0.25%) were reported rare (8), similar to our findings. Furthermore, in a recent work from Iran, no carbapenemase gene was detected among clinical isolates of Enterobacter spp. (28). Taken together, these discrepancies in results are probably due to the distribution of geographically different regions and genetic heterogeneity of strains.

A limitation of the current study is the relatively small sample size. Another limitation of the work is that we could not evaluate the presence of other ESBL and carbapenemase genes from different classes of beta-lactamases to better assess beta-lactam resistance in our isolates.

Conclusion
ESBL-positive and carbapenem-resistant Enterobacter spp., particularly E. gergoviae have become a concern in our area. With respect to the findings, amikacin may still be suitable for treatment of infections caused by MDR Enterobacter isolates. Additionally, educational programs for healthcare workers about diminishing risk of transmission of Enterobacter isolates as serious nosocomial pathogens should be implemented in our hospitals.

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Conflicts of Interest
The authors declare that there are no conflicts of interest.

References
1. Perez A, Poza M, Fernández A, Fernández Mdel C, Mallo S, Merino M, et al. Involvement of the AcrAB-TolC efflux pump in the resistance, fitness, and virulence of Enterobacter cloacae. Antimicrob Agents Chemother 2012;56:2084-2090.
2. Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Agassounou-Djikpo Tchibazo M, Bankole HS, et al. Antibiotic resistance and genotype of beta-lactamase producing Escherichia coli in nosocomial infections in Cotonou, Benin. Ann Clin Microbiol Antimicrob 2015;14:5.
3. Khanfar HS, Bindayna KM, Senok AC, Rotta GA. Extended spectrum beta-lactamases (ESBL) in Escherichia coli and Klebsiella pneumoniae: trends in the hospital and community settings. J Infect Dev Ctries 2009;3:295-299.
4. Kohlenberg A, Schwab F, Rüden H. Wide dissemination of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli and Klebsiella spp. in acute care and rehabilitation hospitals. Epidemiol Infect 2012;140:529-534.
5. Haghighatpanah M, Mozaffari Nejad AS, Mojtahedi A, Amirmozafari N, Zeighami H. Detection of extended-spectrum beta-lactamase (ESBL) and plasmid-borne blaTEM and blaSHV genes among clinical strains of Escherichia coli isolated from patients in the north of Iran. J Glob Antimicrob Resist 2016;7:110-113.
6. Yoo JS, Byeon J, Yang J, Yoo JI, Chung GT, Lee YS. High prevalence of extended-spectrum beta-lactamases and plasmid-mediated AmpC beta-lactamases in Enterobacteriaceae isolated from long-term care facilities in Korea. Diagn Microbiol Infect Dis 2010;67:261-265.
7. Rosa JF, Rizek C, Marchi AP, Guimaraes T, Miranda L, Carrilho C, et al. Clanotoxicity, outer-membrane proteins profile and efflux pump in KPC-producing Enterobacter spp. in Brazil. BMC Microbiol 2017;17:69.
8. Lee JY, Hong YK, Lee H, Ko KS. High prevalence of non-clonal imipenem-nonsusceptible Enterobacter spp. isolates in Korea and their association with porin down-regulation. Diagn Microbiol Infect Dis 2017;87:53-59.
9. Bocanegra-Ibarias P, Garza-González E, Morfín-Otero R, Barrios H, Villarral-Treviño L, Rodríguez-Noriega K, et al. Molecular and microbiological report of a hospital outbreak of NDM-1-carrying Enterobacteriaceae in Mexico. PLoS One 2017;12:e0179651.
10. Tácao M, Correia A, Henriques IS. Low Prevalence of carbapenem-resistant bacteria in river water: resistance is mostly related to intrinsic mechanisms. Microb Drug Resist 2015;21:497-506.
11. Qin X, Yang Y, Hu F, Zhu D. Hospital clonal dissemination of Enterobacter aerogenes producing carbapenemase KPC-2 in a Chinese teaching hospital. J Med Microbiol 2014;63:222-228.
12. Nedjai S, Barguiga A, Djamila N, Jamali L, Zerouali K, Dekhil M, et al. Prevalence and characterization of extended spectrum beta-lactamase-producing Enterobacter cloacae strains in Algeria. Infect Dev Ctries 2013;7:804-811.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 26th Informational Supplement. CLSI document M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. 2016.
14. Majjorokas AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-281.
15. Dallenne C, Da Costa A, Decrè D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010;65:490-495.
16. Poirel L, Walsh TR, Cuvelier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119-123.
17. Hoffmann H, Stürenburg E, Heesemann J, Roggenkamp A, Ho PL, Baquero F. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of Enterobacter cloacae and Klebsiella pneumoniae at an Indian tertiary care hospital: plasmid-mediated colistin resistance. Int J Infect Dis 2008;12:256-264.
18. Stock I, Wiedemann B. Natural antibiotic susceptibility of Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter gergoviae and Enterobacter sakazakii strains. Clin Microbiol Infect 2002;8:546-578.
19. Cantón R, Oliver A, Coque TM, Varela Mdel C, Pérez-Díaz JF, Baquero F. Epidemiology of extended-spectrum beta-lactamase-producing Enterobacter isolates in a Spanish hospital during a 12-year period. J Clin Microbiol 2002;40:1237-1243.
20. Ho PL, Stedman N, Chow KH, Duan RS, Mak GC, Lai EL, et al. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of Enterobacter spp. in Hong Kong. 2000-2002. J Antimicrob Chemother 2005;55:326-332.
21. Ganeswire R, Thong KL, Puthucheary SD. Nosocomial outbreak of Enterobacter gergoviae bacteremia in a neonatal intensive care unit. J Hosp Infect 2003;53:292-296.
22. Escherichia coli. Multiclonal spread of VIM-1-producing Enterobacter cloacae isolates from a teaching hospital in Shanghai, China. J Med Microbiol 2012;61:132-136.
23. Park YJ, Park SY, Oh EJ, Park JJ, Lee KY, Woi GI, et al. Occurrence of extended-spectrum beta-lactamases among chromosomal AmpC-producing Enterobacter cloacae, Citrobacter freundii, and Serratia marcescens in Korea and investigation of screening criteria. Diagn Microbiol Infect Dis 2005;51:265-269.
24. Xu F, Chen S, Xue X, Guo Y, Liu Y, Zhu D, et al. Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai, China. J Med Microbiol 2012;61:132-136.
25. Park YJ, Park SY, Oh EJ, Park JJ, Lee KY, Woi GI, et al. Evaluation of phoenix automated microbiology system for detecting extended-spectrum beta-lactamase-producing Enterobacter cloacae isolates associated with In624 and In488 integrons located in an IncHI2 plasmid. Int J Antimicrob Agents 2012;43:451-455.
26. Hu F, Chen S, Xu X, Guo Y, Liu Y, Zhu D, et al. Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a district teaching hospital in Taiwan. Clin Microbiol Infect 2006;12:597-598.
27. Nilsen E, Haldorsen BC, Sundsfjord A, Simonsen GS, Ingebrigsten A, Naseer U, et al. Large IncHI2-plasmids encode extended-spectrum beta-lactamases. J Antimicrob Chemother 2013;19:E516-E518.
28. Park YJ, Yu K, Lee S, Park JJ, Oh EJ. Evaluation of phoenix automated microbiology system for detecting extended-spectrum beta-lactamase-producing Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, and Serratia marcescens. Ann Clin Lab Sci 2007;37:75-78.
29. Swayne R, Emaneini M, Kalantar-Neyestanaki D, Maraji AS, Dalvand M, Beigverdi R, et al. Clanotoxic relation and antimicrobial resistance pattern of extended-spectrum beta-lactamase- and AmpC beta-lactamase-producing Enterobacter cloacae isolates collected from different clinical samples in Tehran, Iran. Iran J Basic Med Sci, Vol. 23, No. 9, Sep 2020
harbouring \( \text{bla}_{\text{VIM-1}}, \text{bla}_{\text{CTX-M-9}}, \text{aac(6')-lb} \) and \( qnrA \) genes in the spread of multiresistant \( \text{Enterobacter cloacae} \) and \( \text{Klebsiella pneumoniae} \) strains in different units at Hospital Vall d’Hebron, Barcelona, Spain. Int J Antimicrob Agents 2012;39:514-517.

39. Tuon FF, Scharf C, Rocha JL, Cieslinsk J, Becker GN, Arend LN. KPC-producing \( \text{Enterobacter aerogenes} \) infection. Braz J Infect Dis 2015;19:324-327.

40. Ahn C, Syed A, Hu E O’Hara JA, Rivera JI, Doi Y. Microbiological features of KPC-producing \( \text{Enterobacter} \) isolates identified in a U.S. hospital system. Diagn Microbiol Infect Dis 2014;80:154-158.