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Original Article

Prevalence of Multiple Drug Resistant Clinical Isolates of Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in Southeast Iran

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Abstract

Background: Multidrug resistance and production of extended spectrum β-lactamases (ESBLs) by enteric gram-negative rods in hospitals and community continue to be worsened. We aimed to characterize the multidrug resistance and determine the prevalence of ESBL production by clinical isolates of Enterobacteriaceae in southeast Iran.

Methods: Gram-negative bacteria isolated from clinical samples of hospital inpatients and outpatients from three hospitals in southeast Iran were tested for susceptibility to 10 commonly used antimicrobials. For 500 isolates which showed resistance to ≥3 antibiotics from different classes, minimum inhibitory concentration, and prevalence of ESBL production were determined by agar dilution and double disc synergy method respectively. The isolated bacterial species were compared in respect of antibacterial resistance, ESBL production, patients' gender, hospital ward, and type of specimen.

Results: The most frequent resistance was to trimethoprim/sulfamethoxazole, amoxicillin, and tetracycline. Imipenem with 99.8% and ceftizoxime with 83% susceptibility were the most active agents. A total of 53.8% of isolates expressed ESBL production. *Escherichia coli* and *Klebsiella pneumoniae* were most common in outpatients, and inpatients samples respectively. Higher rate of resistance to most antibacterial agents and ESBL production was found in samples of inpatients.

Conclusion: The present study showed high prevalence of ESBL-producing Enterobacteriaceae especially in the patients admitted to hospital. Infection control strategy with continuous resistance surveillance is essential to monitor in vitro susceptibility to antibacterial agents currently used in clinical practice. Determination of the type of involved ESBL enzymes is important for a better antimicrobial control and empirical therapy of critically ill patients in hospitals.
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Keywords ● Antimicrobial resistance ● Enterobacteriaceae ● outpatients ● inpatients

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Introduction

Gram–negative rods in the Enterobacteriaceae family are widely distributed in nature. These organisms are among the most important opportunistic human pathogens, causing various infectious diseases, especially urinary tract infections, septicemia, hospital and health care associated pneumonia, and various abdominal infections.[1] *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter* Spp, and S*erratia marcescens* are the most frequent isolated enteric bacteria in clinical specimens.[2]

Antibacterial resistance of Enterobacteriaceae, especially the emergence of multiple drug resistant (MDR) strains is an important clinical problem worldwide.[1,3] β-lactam antibiotics are the most common prescribed antibiotics.[4] The major mechanism of resistance to β-lactams, particularly in gram-negative bacteria, is the production of extended spectrum β-lactamases (ESBLs).[5] These enzymes are chromosomally or plasmid encoded, and are associated with mobile genetic elements such as transposons or integrons, carrying genes that encode resistance to other antimicrobial agents such as aminoglycosides, sulfonamides, trimethoprim/sulfamethoxazole, and quinolones.[1,3,6] ESBL producing isolates are particularly resistant to penicillins, cephalosporins, and monobactams, however, the bacteria retain susceptibility to cephamycin, fourth generation cephalosporins, and carbapenems.[5]

MDR strains of ESBL producing Enterobacteriaceae are of particular concern. This is because of their widespread acquisition of other resistant elements. Regional variation in resistance pattern is usual, and surveillance of antimicrobial resistance is recommended in each geographic region.[6,7] Many reports of the colonization with MDR or ESBL producing Enterobacteriaceae isolates have mainly focused on hospital inpatients especially in the intensive care units (ICU), or in the nursing homes' residents.[2,8,9] However there are reports on the community acquired ESBLs or the ESBLs originated from non-clinical sources such as pets and farm animals.[10,11]

The incidence of MDR and ESBL producing *E. coli and K. pneumoniae* in the hospital setting in Tehran (capital of Iran) has been reported to be high.[12-14] The present study was performed on clinical isolates of Enterobacteriaceae to determine in vitro susceptibility to antibacterial agents of the isolates from inpatients and outpatients in three major hospitals in southeast Iran and to determine the ESBL production by the MDR isolates.

Materials and Methods

Bacterial Strains

Enteric bacteria were isolated from the clinical samples including blood, urine, stool, and body fluids of patients at three major hospitals located in three different regions of Kerman city, southeast Iran, from November 2006 to August 2007. Laboratory heads were asked to provide information about the date of isolation, site of infection, patients' gender, and the patients' location within hospitals. Only one sample from each patient was included. The isolates were identified by their cultural characteristics and reactions to standard biochemical tests,[15] and were stored in trypticase soy broth with 40% glycerol at -70 ˚C.

Determination of MDR Phenotype

The following antibacterial agents were used in the study: amoxicillin, cephalexin, ceftazidime, ceftizoxime, imipenem, nalidixic acid, ciprofloxacin, tetracycline, gentamicin, and trimethoprim/sulfamethoxazole. Bacterial isolates were tested for susceptibility to the antibacterial agents at the cut off concentrations for susceptible isolates,[16] by using CLSI standard agar dilution method.[17] The minimum inhibitory concentrations (MICs) were defined as the lowest antimicrobial concentration able to fully inhibit bacterial growth. Of 948 samples tested for susceptibility, a total of 500 isolates from different classes showed resistance to ≥3 antibacterial agents. These isolates were regarded as MDR, and included in the study. The isolated bacteria were obtained from 265 hospital outpatients (73 male and 191 female patients, and one neonate) and 235 hospital inpatients (89 male and 113 female patients, and 33 neonates). The samples were obtained from various specimens of patients, which included 431 urine, 41 blood, 15 wound, and 13 other samples from miscellaneous body sites. The MIC of each antibacterial agent was determined by standard agar dilution method.[17]

ESBL Detection

All isolates were screened for ESBL production in Muller-Hinton agar using cefotaxime (2 mg/L).[18] For the isolates resistant to cefotaxime or third generation cephalosporins, ESBL production was tested by the MAST (MAST Chemical Co, England) combined disc method for ESBL detection. The

bacterial suspension was prepared for agar dilution method matching the 0.5 MacFarland standard. Three sets of discs were used in this study,[19] included: ceftazidime (30 µg), ceftazidime (30 µg) plus clavulanic acid (10 µg), cefotaxime (30 µg), cefotaxime (30 µg) plus clavulanic acid (10 µg), and cefpodoxime (30 µg), cefpodoxime (30 µg) plus clavulanic acid (10 µg). Muller Hinton agar was inoculated with the bacterial suspension and ESBL detection discs were placed on the surface of agar. Diameter of inhibition zone was measured after 18-24 hours of incubation at 37 ˚C. In accordance with the MAST instruction for ESBL detection, the following formula was used to determine the presence of ESBL in the test organisms:

$$\text{Diameter of inhibition zone (mm)} = \frac{\text{Ceftazidime}}{\text{Ceftazidime plus clavulanic acid}}$$

≥ 1.5 positive, < 1.5 negative

The same equation was repeated and the results were considered positive if the equation for any disc combination was ≥1.5. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains for antibacterial susceptibility tests and *K. pneumoniae* ATCC 700603 was used as a positive control for ESBL determination.

Statistical Analysis

Data was analyzed using SPSS software version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The Fisher exact test was used for categorical data. P≤0.05 was considered significant (two-tailed test).

Results

E. coli (67.8%) was the most frequently isolated bacteria from all specimens except wound infections. The prevalence of this bacteria in neonatal specimens was low (38.2%, P<0.0001). *K. pneumoniae* (9.2%) was the second most prevalent isolated bacteria and was the most common in the neonatal samples (41.2%, P<0.0001). *E. coli* (78.5%) was the most frequent isolated bacteria from the outpatients' samples (P≤0.0001), however, *K. pneumoniae* and *C. freundii* were predominant in the inpatients' samples (P≤0.002; table 1). Pediatric ward was the most common place from which the MDR bacteria were isolated (23.4%), followed by neonatal intensive care unit (12.5%) and internal medicine ward (11.5%).

Imipenem had an excellent antimicrobial activity against all MDR isolates and 99.8% of the isolates were susceptible to imipenem. The susceptibility test results for other antibacterial agents and the prevalence of bacterial isolates are presented in table 1. Ceftizoxime was the second most active agent. 12% of the outpatients' and 22.5% of the inpatients' isolates were resistance to ceftizoxime. The MIC_{50} of

Table 1: Antibacterial resistance pattern of multidrug resistant members of Enterobacteriaceae isolated from different clinical samples of outpatients and inpatients, southeast Iran.

Bacterial species & number of isolates Outpatient (Inpatient)	% resistant to antibacterial agents Outpatient (Inpatient)								
	LEX	LEX	CAZ	ZOX	NAL	CIP	TET	GEN	SXT
All isolates 265 (235)	89 (94)	55.5 (82)	49 (73.5)	12 (22.5)^{S}	66.4 (68.9)	40.4 (55.5)^{S}	84.5 (76.5)	36.2 (57.8)^{S}	93.6 (94)
Escherichia coli 200 (139)	89 (94.2)	54.2 (75.5)	47.5 (67.6)	9.9 (17.3)	69.5 (75.5)	41 (50.3)	85.5 (80.6)	34.8 (45.3)	93.5 (92.8)
Klebsiella pneumoniae 11 (35)	81.8 (97.1)	81.8 (97.1)	81.8 (91.4)	36.3 (34.3)	63.6 (42.8)	36.3 (25.7)	63.6 (60)	36.3* (82.8)^{S}	81.8 (91.4)
Citrobacter Diversus 15 (21)	93.3 (100)	46.4* (80.9)^{S}	53.3 (76.2)	6.6 (19)	66.6 (80.9)	60 (71.4)	80 (90.5)	13.3* (57.1)^{S}	100 (100)
*Citrobacter freundii*4 (18)	100 (100)	100 (100)	100 (100)	27.3 (33.3)	68.2 (66)	22.7 (16.6)	100 (72.2)	7 (83.3)	100 (100)
Proteus mirabilis 13 (6)	84.6 (33.3)^{S}	46.1 (50)	30.8 (50)	14.4 (33.3)	46.1 (50)	14.4 (33.3)	92.3 (83.3)	46.1 (83.3)^{S}	100 (100)
Enterobacter Aerogenes 9 (9)	88.9 (100)	55.5 (100)^{S}	55.5 (100)^{S}	22.2 (22.2)	66.6 (55.5)	44.4 (33.3)	100 (77.8)	77.8 (100)	88.9 (88.9)
Other isolates* 13 (7)	92.3 (85.7)	58.8* (100)	38.5 (71.4)	23.1 (42.8)	38.5 (71.4)	25 (42.8)	69.2 (42.8)	30.7 (71.4)	92.3 (100)

AMX: Amoxicillin, LEX: Cephalexin, CAZ: Ceftazidime, ZOX: Ceftizoxime, NAL: Nalidixic acid, CIP: Ciprofloxacin, TET: Tetracycline, GEN: Gentamicin, SXT: Trimethoprim-sulfamethoxazole.
S= Significant difference in the resistance of isolates from inpatients compared with that of the outpatients.
*= All bacteria with the isolation frequency of less than 3% were regarded as other isolates, which included: *Klebsiella oxytoca* (n=7), *Proteus Vulgaris* (n=4), *Enterobacter Cloacae* (n=3), *Serratia Marcescens* (n=4); and *Shigella Sonnei* (n=2).

ceftizoxime for all of the isolated bacterial species was not in the resistance category (≥32 µg/ml).[16] For other antibacterial agents except ciprofloxacin the MIC that inhibits the growth of 50% (MIC_{50}) or 90% (MIC_{90}) were in the resistance category (table 2). Resistance to antimicrobials was generally more common in the isolated bacteria from inpatients, and the difference between cephalexin, ceftazidime, ceftizoxime, ciprofloxacin, and gentamicin was significant (P<0.001).

Resistance to the three antibacterial agents was detected in 15.2% of the isolates, and the most common phenotype was simultaneous resistance to trimethoprim-sulfamethoxazole, amoxicillin, and tetracycline (table 3). Resistance to the three antimicrobial agents was more common in the isolates from outpatients (22.6%) compared with inpatients (6.8%, P=0.00001). The difference in the resistance to four, five, and six antimicrobial agents was not significant in the outpatients and inpatients samples, while resistance to ≥8 antimicrobials was significantly higher in the inpatients, compared with the outpatients samples (28.9% and 12.4%, respectively; P=0.001). The resistance to nine antimicrobial agents in the clinical isolates was observed in *E. coli* (n=14), *K. pneumoniae* (n=9), *C. diversus* (n=2), *C. freundii* (n=1), and *E. aerogenes* (n=2).

Of 11 isolated bacteria in the transplant

Table 2: Comparative antimicrobial susceptibility of major species of multidrug resistant Enterobacteriaceae isolated from different clinical samples, southeast of Iran

Isolate (Number)	MIC (µg/ml) to selected antimicrobial agents								
	AMX 50%/90% (Range)	LEX 50%/90% (Range)	CAZ 50%/90% (Range)	ZOX 50%(90%) (Range)	NAL 50%/90% (Range)	CIP 50%/90% (Range)	TET 50%/90% (Range)	GEN 50%/90% (Range)	SXT 50%/90% (Range)
Escherichia coli (339)	≥1024≥1024 (≤32-≥1024)	128/≥1024 (≤32-≥1024)	64/≥1024 (≤32-≥1024)	≤32/64 (≤32-128)	128/≥1024 (≤32-≥1024)	≥4/≥1024 (≤4-≥1024)	128/512 (16-≥1024)	≤8/256 (8-≥1024)	≥1024/≥1024 (≤8-≥1024)
Klebsiella pneumoniae (46)	≥1024/≥1024 (≤32-≥1024)	≥1024/≥1024 (≤32-≥1024)	128/≥1024 (≤32-≥1024)	≤32/512 (≤32-512)	64/≥1024 (≤32-≥1024)	≥4/512 (≤4-512)	256/512 (16-≥1024)	64/≥1024 (8-≥1024)	≥1024/≥1024 (≤8-≥1024)
Citrobacter. diversus (36)	≥1024/≥1024 (≤32-≥1024)	≥1024/≥1024 (≤32-≥1024)	128/≥1024 (≤32-≥1024)	≤32/64 (≤32-128)	≥1024/≥1024 (≤32-≥1024)	16/≥1024 (≤4-≥1024)	128/512 (16-≥1024)	128/512 (8-≥1024)	≥1024/≥1024 (16-≥1024)
Citrobacter. freundii (22)	≥1024/≥1024 (128-≥1024)	≥1024/≥1024 (64-≥1024	≥1024/≥1024 (128-≥1024)	≤32/64 (≤32-512)	128/≥1024 (≤32-≥1024)	≥4/512 (≤4-≥1024)	128/512 (16-≥1024)	128/512 (8-≥1024)	128/512 (16-≥1024)
Proteus. mirabilis (19)	128/≥1024 (128-≥1024)	128/≥1024 (128-≥1024)	32 /128 (≤32-≥1024)	≤32/≤32 (≤32-512)	≥1024/≥1024 (≤32-≥1024)	≥4/8 (≤4-16)	64/512 (16-512)	16/256 (8-≥1024)	≤32/≥1024 (≤32-≥1024)
Enterobacter. aerogenes (189)	≥1024/≥1024 (≤32-≥1024)	512 /≥1024 (≤32 -≥1024)	512/≥1024 (≤32-≥1024)	32/128 (≤32-≥1024)	128/512 (≤32-≥1024)	≥4/256 (≤4-≥1024)	128/512 (16-≥1024)	128/512 (8-≥1024)	512/512 (≤8-≥1024)

AMX: Amoxicillin, LEX: Cephalexin, CAZ: Ceftazidime, ZOX: Ceftizoxime, NAL: Nalidixic acid, CIP: Ciprofloxacin, TET: Tetracycline, GEN: Gentamicin, SXT: Trimethoprim-sulfamethoxazole.

Table 3: Antimicrobial resistance phenotype and ESBL production for 500 members of Enterobacteriaceae family isolated from clinical samples, southeast Iran.

Number of agents to which isolates were resistant	Total number	Number (%) isolates resistance to:										Number (%) ESBL harboring isolates
		SXT	AMX	TET	LEX	NAL	CAZ	CIP	GEN	ZOX	IPM	
3	76 (15.2)	66 (86.8)	61 (80.3)	60 (78.9)	4 (5.3)	14 (18.4)	5 (6.6)	9 (11.8)	9 (11.8)	0 (0)	0 (0)	5 (6.6)
4	63 (12.6)	53 (84.1)	54 (85.7)	39 (61.9)	34 (54)	32 (50.8)	21 (33.3)	6 (9.5)	13 (33.3)	3 (4.8)	0 (0)	20 (31.7)
5	101 (20.2)	91 (90.1)	93 (92.1)	67 (66.3)	57 (56.4)	59 (58.4)	51 (50.5)	37 (36.6)	46 (45.5)	3 (3)	0 (0)	47 (46.5)
6	90 (18)	89 (98.9)	83 (92.2)	72 (80)	7.5 (83.3)	66 (73.3)	66 (73.3)	30 (33.3)	44 (48.9)	15 (16.7)	0 (0)	56 (62.2)
7	69 (13.8)	69 (100)	66 (95.7)	67 (97.1)	69 (100)	66 (95.7)	64 (92.8)	39 (56.5)	30 (43.5)	12 (17.4)	0 (0)	51 (73.9)
8	73 (14.6)	73 (100)	72 (98.6)	71 (97.3)	73 (100)	73 (100)	73 (100)	62 (84.9)	64 (87.7)	24 (32.9)	0 (0)	63 (86.3)
9	28 (5.6)	28 (100)	28 (100)	28 (100)	28 (100)	28 (100)	27 (96.4)	28 (100)	28 (100)	28 (100)	1 (3.6)	26 (92.8)
Total	500 (100)	469 (93.8)	457 (91.4)	404 (80.8)	340 (68)	338 (67.6)	307 (61.4)	211 (42.2)	234 (46.8)	85 (17)	1 (0.2)	268 (100)

The most common phenotype of MDR in each resistant category is presented by gray shadow.
AMX: Amoxicillin, LEX: Cephalexin, CAZ: Ceftazidime, ZOX: Ceftizoxime, NAL: Nalidixic acid, CIP: Ciprofloxacin, TET: Tetracycline, GEN: Gentamicin, SXT: Trimethoprim-sulfamethoxazole.

ward, nine isolates (81.8%) were resistant to ≥8 antibacterial agents, and 86.6% of the 15 isolates, in the infectious diseases ward were resistant to ≥7 antibacterial agents (results are not shown). Resistance to 2 mg/L cefotaxime as a screening test for ESBL production was detected in 369 (73.8%) isolates. For 254 isolates (50.8%) the ESBL phenotype was positive by combined disc method. However for 14 isolates the diameter of inhibition zone for all of the cephalosporin/cephalosporin inhibitor combinations discs was equal to zero. Considering these isolates as ESBL producer, 268 isolates (53.6%) were ESBL positive. ESBL production by different bacterial species in a descending order was *K. pneumoniae* (82.6%), *C. freundii* (81.1%), *E. coli* (63%), *E. aerogenes* (61.1%), *C. diversus* (58.3%), and *P. mirabilis* (50%). The difference in ESBL production in the outpatients' samples in comparison with the inpatients' samples was significant in case of *E. coli, C. diversus,* and *E. aerogenes (*P≤0.001). The number of isolates harboring ESBL phenotype in those organisms that were resistant to three antimicrobial agents was low (5.32%) and increased in the isolates that were resistant to more antimicrobial agents. Therefore, in the isolates that were resistant to nine antimicrobials, ESBL production was 92.8%, (P=0.00001; table 3). The bacterial isolates from urinary tract infections had the lowest percent of ESBL production (48.5%), while bacterial isolates from blood cultures (87.8%), different body fluids (71.4%), and wound infections (86.6%) had the highest levels of ESBL production (P=0.00002). In hospitals, the higher prevalence of ESBL positive isolates were from oncology/transplantation wards (100%), neonate intensive care units (87.9%), infectious disease wards (86.6%,), ICU (73%), and pediatrics wards (71%).

Discussion

Escherichia coli, which is the most common cause of urinary tract infections was the most frequent bacterial isolate in the present study, followed by *K. pneumoniae.* This finding is similar to the observation of Sader and Andrade and their co-workers.[20,21] However, *P. mirabilis* that is reported to be the second most common Enterobacteriaceae isolate in European countries and the USA,[2,22] was not common in southeast Iran. More frequent isolation of *E. coli* in the outpatients' samples, in comparison with *K. pneumoniae* and *C. freundii* that were more prevalent in the hospital inpatients in our study concur with the report of McGown and colleagues in which the high prevalence of antimicrobial resistant organisms in the hospitals or hospital inpatient specimens was not caused solely by antimicrobial use but rather the presence of some other factors that permitted better survival of the bacteria in or around the medical devices and equipments in the hospitals.[23]

Carbapenems are still the most effective agents against Enterobacteriaceae with susceptibility of over 98% in many countries including Iran,[12,13,20] that is consistent with our results. In the present study, resistance to ceftizoxime, the second most effective antimicrobial agent was more frequent in *C. freundii* and *E. aerogenes*. Resistance to trimethoprim-sulfamethoxazole, amoxicillin, and tetracycline, which was the most common phenotype in the MDR isolates in our study was similar to the results obtained for urinary tract infection isolates in southeast Iran in 2001.[24] Those bacterial isolates that were resistant to higher number of antibacterial agents were isolated from hospital wards such as transplant and oncology wards where the patients were critically ill with long hospital stay. Higher rate of resistance to antibacterial agents, especially in the bacterial isolates from blood stream infections and ICU, in comparison with community acquired infections has been reported.[1,2,23]

ESBLs producing bacterial isolates are the major causes of resistance to β-lactam antibiotics and have been reported to be involved in drug resistance, especially against fluoroquinolones.[3,8,19] In the Asia-Pacific surveillance program, prevalence of a negative ESBL confirmation test result after a positive ESBL screening test result were 8.9% for *E. coli* and 20.3% for *K. pneumoniae.*[25] In the present study, the prevalence was 1.47% for *E. coli* and 8.7% for *K. pneumoniae*. Because multi drug resistance is more important than the type of the enzymes involved,[26] according to the recommendation by Bell and others, these isolates were regarded as ESBLs in the present study.[25] The lowest percentage of ESBL positive isolates were seen in the *P. mirabilis* isolated only from urine samples. This is consistent with other findings that wild types *P. mirabilis*, were susceptible to all penicillins and cephalosporins, and chromosomally encoded β-lactamase production have not been reported for these bacteria.[27]

Prevalence and distribution of ESBL varies considerably with geographical location, time, and bacterial species.[2] It may also depend on the type and origin of specimen including blood stream infections and ICU patients.[23] The highest percentage of ESBL positive isolates has been reported for *K. pneumoniae*, especially the blood stream isolates, and ranged from 7.2% in European countries to 35.6% in Singapore, 30.7% in main land China, 28.1% in South Africa, 33.8% in Turkey, and lower frequencies in Philippine, Australia, and Japan.[7,9] In Iran, the rate of isolation of ESBL for all species within the Enterobacteriaceae family is high. The rates range from 76.6% for *K. pneumoniae* isolated from the ICU patients,[28] to 33% in other studies from non-ICU patients.[14,20]

The prevalence of ESBL positive *E. coli* in Iran is 60.6% to 67.2% for various clinical samples.[28] In the present study, we only tested the MDR isolates for ESBL production, and therefore the higher prevalence of ESBL positive isolates was not unpredictable. ESBLs were detected infrequently in the enteric bacteria other than E. *coli*, *K. pneumoniae,* and *K. oxytoca* isolates from 53 hospitals in USA.[29] However, there is a report from Barcelona,[18] that is similar to the present study. The interesting point of the present study was a correlation between multiple antibacterial resistances and the prevalence of ESBL positive phenotype. Resistance to antibacterial agents and ESBL production was higher in the isolates from hospital wards where the patients were more ill and had a longer hospital stay, with higher antimicrobial consumption.

Conclusion

The high prevalence of ESBL producing isolates and presence of isolates resistance to 9 out of 10 antibacterial agents in the present study, especially among species other than *K. pneumoniae* and *E. coli* is distressing. Observation of a single imipenem-resistant isolate in one hospital can alarm the emergence of more resistant isolates. Therefore, there is a need for using this and other carbapenems with caution. The present data clearly showed a higher rate of resistance to all antibacterial agents in the ESBL positive isolates, especially among the isolates from hospitals. Resistant organisms can be transferred from patients to patients and between strains of the same or a different bacterial species in hospitals. Ongoing surveillance will be particularly important to monitor changes in susceptibility to all classes of clinically important antimicrobials. Detection of ESBL phenotype in clinical laboratories is essential for appropriate management of patients. Efforts to maintain current therapeutic options for ESBL positive bacteria by limiting the use of extended spectrum antibacterial agents and 3rd generation cephalosporins are essential. Further studies are needed to establish the molecular type of ESBLs, and clonal occurrence of multi-drug resistance gene in the Enterobacteriaceae family in southeast Iran.

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