ORIGINAL ARTICLE

Prevalence and antibiotic resistance pattern of extended-spectrum beta-lactamase-producing *Escherichia coli* in clinical specimens

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Background: Extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae* seem to have an extended antibiotic resistance, but have different resistance patterns throughout different sites and regions. This study aimed to evaluate the antibiotic resistance pattern of ESBL-producing *Escherichia coli.* **Materials and Methods:** One hundred swab samples from patients hospitalized due to a clinical suspicion of any kind of infection (with manifestations such as fever, leukocytosis, and an active urinalysis result) were processed in Alzahra Microbiology Laboratory, Isfahan, Iran. Isolated *E. coli* were cultured on Mueller–Hinton agar and antibiotic susceptibility was tested by Kirby–Bauer disk diffusion method following the Clinical and Laboratory Standard Institute 2017 guidelines. **Results:** ESBL-producing samples had higher antibiotic resistance rates than ESBL-non-producing samples: ceftriaxone (58.8% vs. 27.3%), cefotaxime (73.5% vs. 30.3%), ceftizoxime (76.5% vs. 33.3%), cefixime (79.4% vs. 40.9%), and cefpodoxime (73.5% vs. 53%), except for carbenicillin (29.4% vs. 48.5%). Imipenem and meropenem were the least resisted antibiotics in ESBL-producing samples (5.9% and 11.8%). **Conclusion:** ESBL-producing *Enterobacteriaceae* have a high resistance rate to third-generation cephalosporins and high susceptibility to imipenem and meropenem.

Key words: Bacterial, beta-lactamases, drug resistance, *Escherichia coli*

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INTRODUCTION

One of the most important mechanisms of bacteria against antibiotics is the production of enzymes destroying β-lactam ring in the antibiotics structure. Extended-spectrum ß-lactamase (ESBL) is an important group of β-lactamases.[1] *Escherichia coli* is the most prevalent and hence the most important multidrug-resistant Gram-negative infection, especially in patients with urinary tract infection (UTI).[2,3] Throughout the recent century, ESBL-producing *Enterobacteriaceae* have been introduced in the literature.[4] ESBL-producing *E. coli* has been isolated in community and nosocomial settings as well.[5] This might be a result of extensive antibiotic usage and can cause antibiotic resistance in human pathogens. Infection with ESBL-producing *E. coli* has an ascending trend of growth in both community and hospital infections in Iran.[6-8]

Sufficient identification of ESBL-producing strains is essential to make an appropriate choice of antimicrobial regimen and evaluation strategy.[9] Because no comprehensive studies in the territory of ESBL-producing *E. coli* in Iran are available, we aimed to evaluate the prevalence and antibiotic resistance pattern of ESBL-producing *E. coli* in clinical specimens.

MATERIALS AND METHODS

Study design and target group

Throughout a cross-sectional study, we evaluated clinical specimens from hospitalized patients in Isfahan Alzahra Hospital, Center of Iran, from August to December 2015. Four milliliters of midstream

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urine was collected from each patient into a sterile tube. Samples were then transported to the hospital laboratory as soon as possible. Patients were instructed properly for the means of sampling.[10,11] Sources of the samples varied throughout the patients, in accordance with their symptoms, practitioners' clinical suspicion, and the standard diagnostic guidelines (with blood [24%], urine [44%], abscess [3%], CSF [4%], sputum [5%], rectal swab [3%], perianal swab [3%], and skin swab [13%], differing based on the patients' manifestations).

Laboratory assessment and extended-spectrum ß-lactamase detection

Two hours after the collection, 100 swab samples, isolated from urine specimen of patients hospitalized due to various reasons with a clinical suspicion of any kind of infection (fever, leucocytosis), were streaked directly on eosin methylene blue agar, MacConkey agar, and blood agar plates. Such plates were incubated at 37°C aerobically, and after overnight incubation, they were assessed for *E. coli* growth. *E. coli* existence was proved by their colony morphology, Gram staining characteristics, biochemical tests of glucose fermentation, Voges–Proskauer reaction (acetyl methyl carbinol production from dextrose) on the Triple Sugar Iron agar, gas producing, lactose metabolism, production of indole from tryptophan, sulfide-indole-motility, and methyl red Voges–Proskauer.

Isolated *E. coli* were cultured on Mueller–Hinton agar (MHA), and antibiotic susceptibility was tested by Kirby–Bauer disk diffusion method after the Clinical and Laboratory Standard Institute (CLSI) guidelines, 2017.[12] Below is the list of drug concentrations used for disc diffusion testing: ceftazidime (30 µg; inhibition zone (IZ) size equal or smaller than 22 mm); amikacin (30 µg), ampicillin (10 µg), piperacillin (100 µg), cefixime (5 µg), cefotaxime (30 µg; IZ ≤27 mm), amoxicillin/clavulanic acid (30 µg), ceftriaxone (30 µg; IZ ≤ 25 mm), ciprofloxacin (5 µg), cotrimoxazole (23.75 µg sulfamethoxazole/1.25 µg trimethoprim), ceftizoxime (30 µg), imipenem (10 µg), meropenem (30 µg), nalidixic acid (30 µg), gentamicin (10 µg), carbenicillin (100 µg), and cefpodoxime (30 µg; IZ ≤ 17 mm).

Isolates showing IZs less than the values stated above were interpreted as screening positive for ESBL production. Only *E. coli* were screened for ESBL production.

For ESBL confirmation, 2–3 colonies of the organisms were suspended in 0.5 ml of sterile broth and the turbidity matched to 0.5 McFarland. Using a sterile cotton swab, the broth culture was uniformly swabbed on MHA. All the *E. coli* isolates resistant to at least ceftazidime, ceftriaxone, and/or cefotaxime were tested for confirmation using cefotaxime–clavulanic acid (30 µg + 10 µg), cefotaxime (30 µg), ceftazidime–clavulanic acid (30 µg + 10 µg), and ceftazidime (30 µg) combination disks. The tests were interpreted according to the most recent CLSI guidelines (2017), and a difference of 5 mm between IZ of a single disk and in combination with clavulanic acid (inhibitor) was confirmed to be produced by an ESBL-positive isolate.

Data analysis

Statistical analysis of data was performed using SPSS 22.0 software. To compare qualitative variables between groups, Chi-square test was performed. The normal distribution of all studied parameters was checked with Kolmogorov–Smirnov test. Student's *t*-test was used for variables which were distributed in a normal way, besides Mann–Whitney and Wilcoxon tests were performed for variables that have not normal distribution. Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

The results of the study showed that ESBL-producing *E. coli* was found in 34% of all samples (ergo 34 ESBL screening-positive samples). ESBL-producing samples had higher antibiotic resistance rate to third-generation cephalosporins than ESBL-non-producing samples such as ceftriaxone (58.8% vs. 27.3%, $P < 0.001$), cefotaxime (73.5% vs. 30.3%, $P < 0.001$), ceftizoxime (76.5% vs. 33.3%, $P < 0.001$), cefixime (79.4% vs. 40.9%, $P < 0.001$), and cefpodoxime (73.5% vs. 53%, $P = 0.045$). On the other hand, carbenicillin in ESBL-producing samples had lower antibiotic resistance rate than ESBL-non-producing samples (29.4% vs. 48.5%, $P = 0.031$), which is a rather strange finding. Furthermore, we found that imipenem and meropenem had the lowest antibiotic resistance rate in ESBL-producing samples (5.9% and 11.8%) [Tables 1 and 2].

Table 1: Demographic characteristics of patients and studied variables on account of extended-spectrum ß-lactamase production

Variables	ESBL		*P*
	Positive (%)	Negative (%)	
Age (years)	46.35±12.97	45.93±11.8	0.873
Sex			
Male (53)	15 (44.1)	38 (57.6)	0.201
Female (47)	19 (55.9)	28 (42.4)	
Clinic sample			
Blood (24)	7 (20.6)	17 (25.8)	0.176
Urine (44)	14 (41.2)	31 (47)	
Abscess (3)	0	3 (4.5)	
CSF (4)	2 (5.9)	2 (3)	
Sputum (5)	1 (2.9)	4 (6.1)	
Rectal swab (3)	2 (5.9)	1 (1.5)	
Perianal swab (3)	0	3 (4.5)	
Skin swab (13)	8 (23.5)	5 (7.6)	

ESBL=Extended-spectrum ß-lactamase

Table 2: Antibiotic susceptibility patterns on account of extended-spectrum ß-lactamase production

Antibiotics	ESBL						*P*
	Positive (%)			Negative (%)			
	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	
Antibiotic susceptibility							
Ampicillin	1 (2.9)	8 (23.5)	25 (73.5)	2 (3)	21 (31.8)	43 (65.2)	0.683
Amikacin	1 (2.9)	16 (47.1)	17 (50)	1 (1.5)	24 (36.4)	41 (62.1)	0.487
Amoxicillin/clavulanic acid	7 (20.6)	13 (38.2)	14 (41.2)	10 (15.2)	28 (42.4)	28 (42.4)	0.781
Ceftriaxone	4 (11.8)	10 (29.4)	20 (58.8)	40 (60.6)	8 (12.8)	18 (27.3)	<0.001
Cefotaxime	0	9 (26.5)	25 (73.5)	24 (36.4)	22 (33.3)	20 (30.3)	<0.001
Ceftizoxime	0	8 (23.5)	26 (76.5)	34 (51.5)	10 (15.2)	22 (33.3)	<0.001
Cefixime	0	7 (20.6)	27 (79.4)	18 (27.3)	21 (31.8)	27 (40.9)	<0.001
Carbenicillin	9 (26.5)	15 (44.1)	10 (29.4)	21 (38.1)	13 (19.7)	32 (48.5)	0.031
Ciprofloxacin	2 (5.9)	10 (29.4)	22 (64.7)	7 (10.6)	29 (43.9)	30 (45.5)	0.185
Cefpodoxime	0	9 (26.5)	25 (73.5)	8 (12.1)	23 (34.8)	35 (53)	0.045
Trimethoprim	5 (14.7)	13 (38.2)	16 (47.1)	9 (13.6)	17 (25.8)	40 (60.6)	0.383
Imipenem	31 (91.2)	1 (2.9)	2 (5.9)	64 (97)	1 (1.5)	1 (1.5)	0.42
Meropenem	29 (85.3)	1 (2.9)	4 (11.8)	57 (86.4)	4 (6.1)	5 (7.6)	0.645
Sulfamethoxazole	9 (26.5)	13 (38.2)	12 (35.3)	21 (31.8)	17 (25.8)	28 (42.4)	0.435
Piperacillin	5 (14.7)	9 (26.5)	20 (58.8)	11 (16.7)	17 (25.8)	38 (57.6)	0.968
Nalidixic acid	15 (44.1)	6 (17.6)	13 (38.2)	26 (39.4)	14 (21.2)	26 (39.4)	0.873
Gentamicin	14 (41.2)	7 (20.6)	13 (38.2)	19 (28.8)	21 (31.8)	26 (39.4)	0.357

ESBL=Extended-spectrum ß-lactamase

DISCUSSION

The present piece of research focused solely on the prevalence and antibiotic resistance pattern of ESBL-producing *E. coli* due to shortage of the project budget.

We found that the prevalence of ESBL-producing bacteria in clinical samples of the hospital was 34 %. This is a completely high amount for such a prevalent microorganism which would be realy catastrophic in the treatment approaches. This value has been reported in lower amounts in some of the other studies,[13-17] whereas other studies reported higher prevalence as compared to our results.[18,19] As reported in a cross-sectional study by Mihankhah *et al*., *E. coli* is among the most prevalent Gram-negative specimens obtained from clinical samples of UTIs in Iran with 37.8% of the whole.[20]

ESBLs are enzymes destroying β-lactam ring in the antibiotic structure, such as monobactams (e.g., aztreonam), third-generation cephalosporins (e.g., ceftriaxone, ceftazidime, and cefotaxime), and carbapenems (e.g., imipenem, meropenem, and ertapenem), but not the cephamycins (e.g., cefoxitin and cefotetan).[21] Such enzymes are sensitive to β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam).[22] Bacterial resistance has increased during the recent decades.[23,24] As our statistical data witness, although third-generation cephalosporins are strong and widely used antibiotics, there is a high rate of resistance and they are not a good choice. The most prominent sensitivity it is to imipenem and meropenem and they are better choices. We recommend performing antibiogram in hospital-admitted UTI patients and select the best choice of antibiotics.

CONCLUSION

Our results showed high prevalence of ESBL in hospital samples in Isfahan, Iran. Because Alzahra Hospital is a major and characteristic hospital laboratory dealing specifically with exceptional patients, the conduction of this study in that specific laboratory setting in Isfahan should interest readers from clinical and epidemiological perspective. Our data confirmed that ESBL had high resistance rate to third generation of cephalosporins and high susceptibility to imipenem and meropenem. These findings suggest further studies in this field.

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Conflicts for interest

There are no conflicts for interest.

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