

Broad-Spectrum Beta-Lactamases and Drug-Resistance Phenotypes of *Enterobacteriaceae* Isolated from Clinical Specimens in Gonbad-e Kavus, Golestan Province, Iran

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ABSTRACT

Introduction: This study aimed to determine the frequency of extended-spectrum beta-lactamases (ESBL) and different drug resistance phenotypes in *Enterobacteriaceae* isolated from clinical specimens in Gonbad-e Kavus, Golestan Province. **Methods:** 220 clinical samples of urine, blood, pus, sputum, CSF, body fluids, and ear and eye discharge were collected during six months from April to September 2021 at a referral hospital. The samples were cultured on blood and MacConkey agar and incubated overnight at 37 °C. Standard biochemical tests and the API20E enteric identification system were used to identify bacteria. The Kirby-Bauer disk diffusion method determined the antibiotic resistance pattern, and the phenotypic confirmatory test was used for detecting ESBL producers. **Results:** 108 *Enterobacteriaceae* isolates were identified from different clinical specimens out of the samples. The isolates were *Escherichia coli* (36.1%), *Klebsiella pneumonia* (25%), *Enterobacter cloacae* (18.5%), *Citrobacter freundii* (11.1%) and *Proteus mirabilis* (9.2%). The highest resistance and susceptibility among the isolates belonged to sulfamethoxazole-trimethoprim (68.5%) and meropenem (11.1%), respectively. The highest prevalence of multidrug-resistant (MDR) and ESBL were observed in *E. coli* and *Proteus mirabilis* isolates. **Conclusion:** In this study, the high frequency of MDR phenotypes in the isolates may suggest an increasing trend of antibiotic resistance in *Enterobacteriaceae*. This could greatly impact the management and treatment of infections caused by these drug-resistant bacteria. Therefore, infection-control measures and continuous monitoring is recommended for controlling the spread of ESBL-producing strains in different geographical areas.

INTRODUCTION

One of the most important causes of nosocomial and community-acquired infections is *Enterobacteriaceae*, a large family of facultative anaerobes Gram-negative bacilli with over 30 genera. Among the members of genera, some pathogens such as *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Escherichia coli*, *Shigella*, *Proteus*, *Serratia*, and other species are responsible for many infections, including urinary tract, respiratory tract, and bloodstream and wound infections [1, 2].

Beta-lactam antibiotics are among the most prescribed antibiotics to treat bacterial infections caused by *Enterobacteriaceae*. The widespread use, overuse and/or inappropriate use of beta-lactams has triggered the development of resistant *Enterobacteriaceae* [3]. One of the most common resistance mechanisms to beta-lactam

drugs among members of the *Enterobacteriaceae* family is the production of beta-lactamases, especially extended-spectrum beta-lactamases (ESBL). ESBL hydrolyze beta-lactam rings in a wide range of beta-lactams such as extended-spectrum penicillin, cephalosporins, and monobactams except for cephamycins and carbapenems [4- 6]. In the *Enterobacteriaceae* family, the enzymes primarily occur in *Klebsiella* spp. and *E. coli* and other genera such as *Enterobacter*, *Citrobacter*, *Proteus*, *Morganella*, *Providencia*, *Salmonella*, and *Serratia* [7].

In the last years, the increasing rates of antimicrobial resistance and the global burden of infectious diseases caused by these resistant strains have become a challenge and associated with a higher mortality rate, increased

length of hospital stay, health costs, and therapeutic failure [8].

Due to these concerns, continuous monitoring and surveillance of antimicrobial resistance and timely detection of ESBL-producing *Enterobacteriaceae* are crucial to establishing appropriate antimicrobial treatment and preventing their spread. Therefore, this study was performed to determine the incidence of ESBL and multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) phenotypes in *Enterobacteriaceae* isolated from clinical specimens at a referral hospital in Gonbad-e Kavus, Golestan Province, Iran.

MATERIAL AND METHODS

Sample collection and identification of isolates. This laboratory-based cross-sectional study was conducted from April to September 2021 on clinical specimens of hospitalized patients at a referral hospital in Gonbad-e Kavus, Golestan Province, Iran. According to the World Medical Association Declaration of Helsinki (ethical principles for medical research involving human subjects), written informed consent was obtained from all patients or their guardians, and their demographic characteristics were recorded anonymously. The samples were cultured on blood agar and MacConkey agar (Oxoid Ltd., UK). After incubation at 37 °C for 18–24 h, the isolates were identified using standard bacteriological methods and biochemical tests and confirmed with the API20E identification system (bioMérieux, Marcy l'Etoile, France) [6, 9].

Antibiotic susceptibility test. Antibiotic susceptibility test of the isolates was performed on Mueller- Hinton agar (Himedia Company, India) plates by Kirby-Bauer disc diffusion method [9]. After incubation at 37 °C for 24 h, the results were interpreted by measuring the inhibition zone against each of the isolates. The antimicrobial agents including amikacin (30 µg), aztreonam (30 µg), ticarcillin-clavulanic (85 µg) acid, piperacillin-tazobactam (110 µg), ciprofloxacin (5 µg), gentamicin (10 µg), meropenem (10 µg), imipenem (10 µg), cefepime (30 µg), fosfomycin (200 µg), ceftazidime (30 µg), sulfamethoxazole-trimethoprim (1.25 µg), colistin (10 µg), cefotaxime (30 µg), chloramphenicol (30 µg), cefazolin (30 µg), amoxicillin-clavulanic acid (30 µg) and ampicillin-sulbactam (30 µg) were purchased from a commercial company (Padtanteb, Iran). The results were interpreted as resistant (R), intermediate (I), and susceptible (S) according to the criteria provided by CLSI guidelines. *Escherichia coli* ATCC 25922 (provided by Tehran University, Faculty of Veterinary Medicine) was used as a quality control strain to interpret the results). According to Magiorakos *et al.* (2012), an *Enterobacteriaceae* isolate was defined as MDR if it was non-susceptible to ≥ 1 antimicrobial agent in ≥ 3 antimicrobial categories. If the isolate was non-

susceptible to ≥ 1 antimicrobial agent in all the antimicrobial categories, except in ≤ 2 , it was considered XDR. An isolate that was non-susceptible to all antimicrobial agents in all antimicrobial categories was considered PDR [10].

Detection of ESBL-producing isolates. To determine ESBL-producing bacteria, the isolates showing inhibition zones of ≤ 22 mm for ceftazidime (CAZ) and ≤ 27 mm for cefotaxime (CTX) were considered as possible ESBL-producers and were selected to confirm ESBL production using double disk-diffusion test as recommended by CLSI guideline [9]. In this test, disks containing 30 µg of CAZ and CTX, with and without 10 µg of clavulanic acid, were placed at an appropriate distance on Mueller- Hinton agar plate inoculated with 0.5 McFarland standard bacterial suspension of the isolate. After overnight incubation at 37 °C, the inhibition zone diameter of each isolate was measured. If the inhibition zone diameter for a combination disk was > 5 mm than that produced around the ceftazidime or cefotaxime disc alone, the isolate was considered ESBL producer [11].

RESULTS

Sampling and Identification of Isolates. 220 samples were collected from a referral hospital in Gonbad-e Kavus from April to September 2021. Out of the samples, 108 *Enterobacteriaceae* isolates were identified from different clinical specimens: 30 from the urine, 19 from blood; 24 from pus, 17 from sputum, 4 from the ear and eye discharge, 8 from body fluids, and 6 from cerebrospinal fluid (CSF) (Table 1). Among the isolates, 68 (63%) were recovered from males and 40 (37%) from females. The most frequent isolates were *E. coli* (36.1%) and *K. pneumoniae* (25%). The isolates were obtained from patients aged one day to 87 years with a mean age of 31.1 years (standard deviation: 24.4). The highest incidence rate of *Enterobacteriaceae* (35.5%) was in the patients 30-40 years of age (39%). Most *Enterobacteriaceae* isolates were from urine (34.2%) and pus (20.3%) samples. The highest isolation rate was from the internal medicine ward (40%), followed by the surgery ward (18.5%).

Antibiotic Susceptibility Test. *E. coli* isolates showed a high degree of resistance to sulfamethoxazole-trimethoprim (69.2%) followed by chloramphenicol (61.5%). Among the *K. pneumoniae* isolates, the highest resistance was observed to gentamicin (81.4%) followed by sulfamethoxazole-trimethoprim and chloramphenicol (70.3%). In *E. cloacae* isolates, the highest resistance was to gentamycin (80%) followed by sulfamethoxazole-trimethoprim (75%), the same as *K. pneumoniae* isolates. *C. freundii* isolates showed a high degree of resistance to ampicillin-sulbactam (66.6%), followed by sulfamethoxazole-trimethoprim (58.3%). For *P. mirabilis* isolates, the highest resistance was to sulfamethoxazole-trimethoprim (60%), followed by ciprofloxacin (50%).

Table 1. Distribution of *Enterobacteriaceae* isolates against specimen types

Specimens	Distribution of <i>Enterobacteriaceae</i> isolates, No. (%)				
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>C. freundii</i>	<i>Proteus mirabilis</i>
Urine (37)	14 (12.9)	7 (6.4)	10 (9.2)	3 (2.7)	3 (2.7)
Blood (19)	8 (7.4)	4 (3.7)	3 (2.7)	2 (1.8)	2 (1.8)
Pus (22)	7 (6.4)	5 (4.6)	6 (5.5)	2 (1.8)	2 (1.8)
Sputum (10)	3 (2.7)	4 (3.7)	0 (0)	3 (2.7)	0 (0)
CSF (8)	1 (0.9)	5 (4.6)	0 (0)	0 (0)	2 (1.8)
Body fluids (8)	4 (3.7)	2 (1.8)	1 (0.9)	0 (0)	1 (0.9)
Ear & Eye discharge (4)	2 (1.8)	0 (0)	0 (0)	2 (1.8)	0 (0)
Total (No. = 108)	39 (36.1)	27 (25)	20 (18.5)	12 (11.1)	10 (9.2)

The highest susceptibility was to meropenem (11.1%) among the isolates, followed by imipenem (12%). About 56.5%, 52.5%, 57%, 50%, and 70% of the *E. coli*, *K. pneumoniae*, *E. cloacae*, *Citrobacter freundii*, and *P.*

mirabilis isolates exhibited MDR phenotypes, respectively. No XDR and PDR phenotypes were observed among the isolates.

Table 2. Frequency of antimicrobial resistance among the isolated organisms

Antimicrobial agents	Resistant species, No. (%)				
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>C. freundii</i>	<i>P. mirabilis</i>
Imipenem (10µg)	6 (15.3)	2 (7.4)	1 (5)	3 (25)	1 (10)
Meropenem (10 µg)	2 (5.12)	8 (29.6)	0 (0)	2 (16.6)	0 (0)
Aztreonam (30 µg)	10 (25.6)	4 (14.8)	6 (30)	0 (0)	2 (20)
Ciprofloxacin (5µg)	25 (64.1)	12 (44.4)	10 (50)	6 (50)	5 (50)
Gentamicin (10µg)	13 (33.3)	22 (81.4)	16 (80)	5 (41.6)	2 (20)
Cefotaxime (30µg)	22 (56.4)	18 (66.6)	10 (50)	6 (50)	2 (20)
Ceftazidime (30µg)	21 (53.8)	16 (59.2)	13 (65)	5 (41.6)	3 (30)
Cefazolin (30 µg)	13 (33.3)	12 (44.4)	7 (35)	6 (50)	2 (20)
Cefepime (30 µg)	8 (20.5)	9 (33.3)	4 (20)	4 (33.3)	0 (0)
Ticarcillin-clavulanic acid (85 µg)	10 (25.6)	11 (40.7)	13 (65)	5 (41.6)	2 (20)
Sulfamethoxazole-trimethoprim (1.25 µg)	27 (69.2)	19 (70.3)	15 (75)	7 (58.3)	6 (60)
Chloramphenicol (30 µg)	24 (61.5)	19 (70.3)	7 (35)	6 (50)	0 (0)
Amikacin (30µg)	5 (12.8)	5 (18.5)	4 (20)	0 (0)	3 (30)
Fosfomycin (200 µg)	8 (20.5)	3 (11.1)	4 (20)	1 (8.3)	4 (40)
Ampicillin-sulbactam (30 µg)	19 (48.7)	10 (37)	13 (65)	8 (66.6)	2 (20)
Amoxicillin-clavulanic acid (30 µg)	15 (38.4)	11 (40.7)	10 (50)	5 (41.6)	3 (30)
Piperacillin-tazobactam (110 µg)	13 (33.3)	9 (33.3)	11 (55)	6 (50)	2 (20)
Colistin (10 µg)	2 (5.12)	2 (7.4)	1 (5)	0 (0)	0 (0)

Detection of ESBLs: The isolates with inhibition zones ≤ 22 mm for ceftazidime (CAZ) and ≤ 27 mm for cefotaxime (CTX) were examined for ESBLs by the combined disk assay. Among the screened isolates, the most frequent ESBLs producers were *E. coli* (46.1%)

followed by *K. pneumoniae* (37 %). ESBL producers were frequently recovered from the surgical ward (40.5%), followed by ICUs (27%). The most prevalent MDR bacterial isolates were *P. mirabilis* isolates (70%). The frequency of ESBLs and MDR among the *Enterobacteriaceae* isolates is shown in Table 3.

Table 3. Frequency of ESBLs and MDR among the *Enterobacteriaceae* isolates

No (%)	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>C. freundii</i>	<i>P. mirabilis</i>
ESBLs	18 (46.1)	10 (37)	4 (20)	3 (25)	2 (20)
MDR	22 (56.4)	15 (55.5)	11 (55)	6 (50)	7 (70)

DISCUSSION

Drug-resistant and ESBL-producing *Enterobacteriaceae* have become a global health challenge. The majority of the *Enterobacteriaceae* isolates are resistant to β -lactam antibiotics and have

caused the major therapeutic failure with a higher mortality rate [12]. Hence, to provide updated epidemiological data, which will be helpful in the improvement of patient care and advice on antibiotic prescription in clinical practice, we aimed to determine

the incidence of ESBL and MDR, XDR, and PDR phenotypes in *Enterobacteriaceae* isolates.

In the present study, the most frequent isolates were *E. coli* and *K. pneumonia* from urine samples. Among the isolates, the highest and lowest susceptibility was shown to meropenem (11.1%) and sulfamethoxazole-trimethoprim (68.5%). Of the 108 isolates, 61 (56.4%) were MDR strains, and *E. coli* was the highest ESBL producer, followed by *K. pneumonia*. In agreement with the current work, in some studies in Iran and Ethiopia, *E. coli* and *K. pneumonia* were the most frequent *Enterobacteriaceae* in clinical samples with the highest resistance against sulfamethoxazole-trimethoprim (77%); moreover, the incidence of ESBL production among these organisms was in line with our findings [2, 13, and 14]. In Tabriz, Iran, the frequency of ESBL production among the *E. coli* and *Enterobacter* isolates was similar to the current study [15].

Similar to the present study, in southeast Iran, *E. coli* and *K. pneumonia* were the most common bacteria in the clinical samples, and the most frequent resistance and susceptibility were to sulfamethoxazole-trimethoprim and carbapenems, respectively [16]. In contrast to the present study, in Gorgan, Iran, the highest ESBL producers were among the *P. mirabilis*, *E. cloacae*, and *C. freundii* recovered from clinical specimens [17]. In Iran, two studies from Kalaleh reported imipenem as the effective antibiotic against *E. coli* and *K. pneumonia* from urine samples. The frequency of ESBL was also in line with our results, but in Bandar-e Torkaman, Iran, lower rates for ESBL production were reported [18-20]. In Saudi Arabia, the *K. pneumoniae* and *E. coli* isolates from patients' clinical specimens were common ESBL producers, and the prevalence of ESBL production among *K. pneumonia* and *E. cloacae* isolates was similar to our findings, but it was lower than the present study for *E. coli* strains [21]. In Ahvaz, Iran, in contrast with the current study, the second most frequently *Enterobacteriaceae* isolate was *Enterobacter* (27.1%), followed by *Klebsiella* (1.2%), and the incidence of ESBL production among the isolates were higher than our results [22].

Most studies, including ours, revealed high resistance rates to sulfamethoxazole-trimethoprim for *Enterobacteriaceae* isolates, but the prevalence of ESBL producers and MDR phenotypes varied in different studies. These differences may be due to geographic location, availability of healthcare facilities, overuse, and misuse of antibiotics. Therefore, knowledge of these organisms and continuous monitoring in different regions are essential for controlling the spread of drug-resistant bacteria and helping physicians choose appropriate treatment strategies. In addition, avoiding self-medication, appropriate treatment, and rational use of antibiotics is a necessity for their control.

The limitation of the present study was that the samples were only from a single-center study for six months at a referral hospital in Gonbad-e Kavus. To reveal the

increasing prevalence of antibiotic resistance in *Enterobacteriaceae*, performing a multicenter study for at least one year is recommended.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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