

# Prevalence of antibiotic resistance in Enterobacteriaceae among the patients hospitalized in Al-Zahra hospital, Isfahan, Iran



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## Abstract

**Introduction:** *Enterobacteriaceae* are a high prevalent gram-negative species worldwide, which play a major role in various infections.

**Objectives:** The present study aims to assess the prevalence of antimicrobial resistance in *Enterobacteriaceae* at a tertiary hospital in Isfahan, Iran.

**Materials and Methods:** This cross-sectional laboratory-based study was performed during 2020 in Al-Zahra hospital, Isfahan, Iran. The isolates of all of the clinical samples submitted for the bacterial culture containing *Enterobacteriaceae* strains were checked for antibiotic resistance during March 2018 to March 2019.

**Results:** The results revealed that the specimens obtained from intensive care units (ICUs) exhibited more resistance than the others. Additionally, the highest abundant antibiotic resistance among inpatients was related to ampicillin/sulbactam, and carbapenem resistance was significantly high.

**Conclusion:** The high prevalence of antibiotic resistance indicated the inappropriate antibiotic prescription in this center. The results emphasized the importance of preventive strategies and restrictions in over-the-counter antibiotic consumption.

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## Introduction

Antimicrobial resistance is considered as a global disaster limiting the ability to treat bacterial infection (1). This phenomenon is a great danger to inpatients, especially those hospitalized in the intensive care unit (ICU), which can lead to resistant infections, and consequently a high mortality rate (2). Alexander Fleming in his Nobel prize speech in 1945 for penicillin discovery warned that inadequate and improper exposure to antimicrobial agents can cause resistance (3). World Health Organization (WHO) declared antimicrobial resistance as a global danger (2019), while several inter-organizational cooperation were launched to understand the extent of resistance worldwide (4).

Bacterial infections are the prevalent conditions which can involve different tissues and organs (5). There are various resistance mechanisms such as intrinsic or acquired pathways. In the intrinsic

## Key point

The results of the present study represented the high prevalence of antibiotic resistance in Enterobacteriaceae in the intensive care unit, which may be related to inappropriate antibiotic prescription.

pathway, all organisms of the species lack susceptibility or have defense mechanisms to resist the antibiotic class. Acquired resistance is an evolution in the bacterial genome which previously was susceptible to the antibiotics (6). The experts of the European Centre for Disease Prevention and Control (ECDC) and Centers for Disease Control and Prevention (CDC) held a meeting to define drug-resistant pathogenic bacteria in hospital settings based on the results of the *in vitro* antimicrobial tests of susceptibility. Multiple drug resistance (MDR), extensively drug-resistant (XDR), and pandrug-

resistant (PDR) is defined as resistance to three or more antimicrobial classes, or resistance to the most of the standard antimicrobial regimens, or also to the almost all commercially-available antimicrobials, respectively (7).

*Enterobacteriaceae* are a large group of gram-negative bacilli, which coexist as normal flora in the human intestine, 22 species of which were discovered so far (8, 9). In addition, they can lead to a broad spectrum of infections from uncomplicated cystitis, pyelonephritis, and hospital-acquired pneumonia to intra-abdominal infection such as cholangitis, appendicitis, and endocarditis (8, 9). For example, around 60% of hospital-acquired infections can be related to the species (10).

According to a recent WHO report on global antimicrobial surveillance (2021), surveillance is a cornerstone for evaluating the spread of antimicrobial resistance, and is an essential issue for examining and planning for global programs to prevent further disaster for future generations (4).

## Objectives

Collecting adequate information is the primary step for providing an accurate report for surveillance according to the increasing incidence of antibiotic resistant pathogens in our medical center. Therefore, the study evaluated the prevalence of antimicrobial resistance in *Enterobacteriaceae* at a tertiary hospital in Iran to help develop a broad perspective on antibiotic prescription.

## Materials and Methods

### Study design

This study was designed as an across-sectional laboratory-based research to provide data on the prevalence of antimicrobial resistance in the samples with *Enterobacteriaceae*.

### Sample collection

The study was carried out on the clinical specimens (e.g., urine, blood, respiratory tract, wound, and others) of 2761 inpatients and 1682 outpatients (1734 male and 2709 female) submitted for bacterial cultures in the microbiology laboratory of Al-Zahra hospital. Inpatients were defined as the patients admitted to the hospital, while outpatients were considered as those who did not contact hospitals recently or were not hospitalized in 2 weeks. Urine samples were collected from midstream urine, suprapubic, or catheter depending on the patient situation. Further, the secretions aspirated from wounds through employing a sterile method were used as wound specimens. Regarding respiratory tract samples, different techniques such as bronchial lavage, sputum, or swab were applied according to the estimated location of the infection in the tract. Finally, blood samples were cultured in an automated blood culture system (BD BACTEC™), or traditional blood culture media, followed by incubation at 35°C for 18-24 hours (1).

### Bacterial identification

In the present study, standard microbiological methods were implemented to recognize the bacteria recovered from specimens. The samples were cultured and incubated for 24 hours at 35°C on blood, Eosin-methylene blue, and MacConkey agar media. Then, the isolates were identified based on the standard bacteriological, gram staining, and biochemical techniques like indole production, catalase, oxidative, urea test, triple iron sugar utilization, oxidative-fermentative test with glucose, methyl red-Voges-Proskauer, and ortho-nitrophenyl-β-galactoside test (10).

### Antibiotic susceptibility test

Kirby-Bauer disc diffusion method on Mueller-Hinton agar was used to determine the antibiotic susceptibility pattern of the isolates to the antibiotic discs determined by Clinical and Laboratory Standards Institute (CLSI, 2018), as well as the available pharmacopoeia of the hospital. In general, the antibiotics utilized against the isolates included beta-lactam and non-beta-lactam agents such as amikacin (30 µg), ampicillin-sulbactam (10/10 µg), cefazolin (30 µg), cefepime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefixime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamycin (10 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), meropenem (10 µg), piperacillin/tazobactam (100/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and tetracycline (30 µg). Along with antibiotics, all of the *Escherichia coli* and *Klebsiella pneumoniae* species taken from the samples were tested by using a phenotypic approach according to the CLSI for extended-spectrum beta-lactamase (ESBL) production. The CLSI guideline was applied to interpret the results and the discs utilized for antimicrobial susceptibility tests were prepared by BD BBL Sensi-Disc (Becton Dickinson, Sparks, MD). Regarding each species, the type of resistance was reported based on the antibiotic class resistance, as well as the definition of MDR, XDR, and PDR provided by ECDC and CDC in 2009 (7).

### Data collection

The data were obtained from the microbiology laboratory of Al-Zahra hospital and imported in WHONET 5.6 software as a standard format. Then, the duplicate data were eliminated by considering the CLSI-2018 guideline providing the standards and guidelines for medical laboratories.

### Statistical analysis

Chi-square test was employed to analyze the differences in the frequency distribution of antibiotics, as well as the type of resistance among the groups of organisms. The frequency distribution of age, gender, department, specimen type, and source was extracted by using Microsoft Office Excel 2010. *P* values < 0.05 rejected the primary hypothesis and SPSS version 26 was utilized for

statistical analysis.

## Results

A total of 4443 *Enterobacteriaceae* were isolated from clinical samples during March 2018 to March 2019, the distribution of which were *E. coli* (2081), *K. pneumoniae* (1990), *Klebsiella aerogenes* (186), *Proteus mirabilis* (138), *Citrobacter freundii* (22), *Klebsiella oxytoca* (21), *Proteus vulgaris* (2), *Citrobacter koseri* (1), *Shigella flexneri* (1), and *Shigella sonnei* (1). Table 1 summarizes the distribution of age and genders in each species. Additionally, 1682 specimens were obtained from outpatients, whereas the rest (2761) were taken from inpatients.

Among the outpatients, *E. coli* was the dominant species (1089 isolates, 64%) which was typically found in urine samples (66%). The other species included *K. pneumoniae* (31%), *K. aerogenes* (0.6%), *P. mirabilis* (2.7%), *C. freundii* (0.4%), *K. oxytoca* (0.5%), and *P. vulgaris* (0.05%). Further, urine and blood were the most prevalent specimens, and the most abundant pathogens obtained were *E. coli* (1629, 66%) from urine and *K. pneumoniae* (54%) from blood.

In the inpatients, *K. pneumoniae* (1471, 53%) was detected as the highest dominant species, which was often isolated from urine samples (43%), while it was obtained in 89% of respiratory tract specimens. The other species were distributed as *E. coli* (36%), *K. aerogenes* (6%), *P. mirabilis* (3%), *C. freundii* (0.5%), and *K. oxytoca* (0.4%), whereas the distribution of the rest was almost 0%. In terms of sample, urine (1459) and blood (446) were the most common, respectively. *E. coli* (51%) was more prevalent in the urine, while *K. pneumoniae* was the most frequent in

blood (43%), respiratory tract (89%), wound (65%), and other samples (64%).

Furthermore, the frequency of organisms among the inpatients was categorized by department. A majority of isolates were collected from the emergency room (966), the most dominant pathogen of which was *E. coli* (48%). Regarding the other departments, the frequency of *K. pneumoniae*, as the highest abundant organism, was equal to 86, 48, 50, and 70% in ICU, internal medicine, surgery, and neonatal intensive care unit (NICU), respectively.

Based on the final results, MDR was observed among 7% of outpatient and 20% of inpatient specimens, whereas 12% of inpatients and 2% of outpatients exhibited possible XDR. PDR survey was impossible according to the pharmacopeia restriction. Among the organisms, *K. pneumoniae* represented the maximum rate of MDR (35%) and possible XDR (22%), while the highest MDR (51%) and possible XDR (32%) were detected in respiratory tract samples. With respect to the department, MDR (54%) and possible XDR (39%) were the most prevalent in the ICU. More details are provided in Tables 2 and 3.

The results of antimicrobial resistance tests demonstrated that resistance rate was maximized regarding cefazolin among outpatients (80.6%) and ampicillin/sulbactam in inpatients (97%). In terms of the type of organisms, the most common antibiotic resistance was related to *E. coli* (cefazolin, 82.8%), *K. pneumoniae* (levofloxacin, 81.3%), *K. aerogenes* (cefazolin, 90%), *P. mirabilis* (nitrofurantoin, 85.4%), and others (cefazolin, 87.5%). Finally, the distribution of ESBL was 96.8% in *E. coli*, 81% in *K. pneumoniae*, 98.1% among inpatient specimens, and

**Table 1.** Frequency of different genus of *Enterobacteriaceae* in clinical samples according to gender and age groups

Organisms	Total	Gender		Age group	
		Male, No. (%) <sup>*</sup>	Female, No. (%)	>20, No. (%)	<20, No. (%)
<i>K. pneumoniae</i>	1990	912 (46%)	1078 (54%)	1780 (89%)	210 (10%)
<i>E. coli</i>	2081	633 (30%)	1448 (73%)	1861 (89%)	220 (10%)
<i>K. aerogenes</i>	186	97 (52%)	89 (48%)	159 (85%)	27 (14%)
<i>P. mirabilis</i>	138	69 (50%)	69 (50%)	103 (75%)	35 (25%)
<i>C. freundii</i>	22	15 (68%)	7 (32%)	18 (82%)	4 (18%)
<i>K. oxytoca</i>	21	6 (28%)	15 (71%)	15 (71%)	6 (28%)
<i>P. vulgaris</i>	2	1 (50%)	1 (50%)	1 (50%)	1 (50%)
<i>C. koseri (diversus)</i>	1	1 (100%)	0	1 (100%)	0
<i>S. flexneri</i>	1	0	1 (100%)	1 (100%)	0
<i>S. sonnei</i>	1	0	1 (100%)	0	1 (100%)

\* Number of isolated organism/total number of isolates (percentage).

**Table 2.** Type of antimicrobial resistance according to organism

	Organism					P value	Total (N=4443)
	<i>E. coli</i> No. (%) <sup>*</sup> (n =2081)	<i>K. pneumoniae</i> No. (%) (n = 1990)	<i>K. aerogenes</i> No. (%) (n = 186)	<i>P. mirabilis</i> No. (%) (n =138)	Other <i>Enterobacteriaceae</i> No. (%) (n =48)		
MDR	85 (4%)	705 (35%)	28 (15%)	28 (20%)	6(12%)	0.00	852 (19%)
XDR	4 (0%)	449 (22%)	3 (2%)	3 (2%)	0 (0%)	0.00	459 (10%)
PDR	0	0	0	0	0	-	0

\*Number of isolated organism (Percentage).

MDR, Multiple Drug Resistance; XDR, extensive drug resistance; PDR, pandrug-resistant.

91.1% among outpatient ones. Tables 4 and 5 outline the data of total antimicrobial susceptibility.

## Discussion

Nowadays, antimicrobial resistance is considered as a vital problem, which influences medication outcomes and mortality. Antibiotic overuse and over-the-counter availability can be addressed as some of the leading causes of bacterial resistance to the antibiotics. Numerous studies have focused on determining the resistance rate in their regions for effective surveillance on antibiotic use. However, more research is needed due to the rapid evolution of bacterial genes over time. The present study assessed the antimicrobial resistance frequency among the

*Enterobacteriaceae* taken from the clinical samples of the intended medical center.

The results suggested *E. coli* and *K. pneumoniae* as the highest prevalent organisms among outpatient and inpatient isolates, respectively. Regarding the blood specimen, *K. pneumoniae* was the most common pathogen among the isolates of out- and inpatients. In addition, *E. coli* was dominant in the urine samples of inpatients, while the highest abundant organism among their respiratory tract and wound specimens was *K. pneumoniae*. *Klebsiella pneumoniae* was found as the most common pathogen in the isolates from ICU, internal medicine, surgery, and NICU departments, whereas *E. coli* had the maximum frequency in the emergency room. The MDR and

**Table 3.** Type of antimicrobial resistance according to specimen type

	Specimen type					P value	Total (N=4443)
	Blood No. (%)* (n =469)	Urine No. (%) (n = 3088)	Respiratory tract No. (%) (n = 379)	Wound No. (%) (n =139)	Other specimen No. (%) (n =368)		
MDR	64 (14%)	378 (12%)	195 (51%)	48 (34%)	6 (1%)	0.00	637 (14%)
XDR	35 (7%)	175 (6%)	121 (32%)	30 (21%)	1(0%)	0.00	362 (8%)
PDR	0	0	0	0	0	-	0

\*Number of isolated organism (Percentage).

MDR, Multiple Drug Resistance; XDR, extensive drug resistance; PDR, pandrug-resistant.

**Table 4.** Antimicrobial resistance of *Enterobacteriaceae* according to specimen source

Antibiotic	Source		P value	Total
	Outpatient No. (%)	Inpatient No. (%)		
Amikacin	35 (34.3%)	708 (28.3%)	0.137	743
Amoxicillin/Clavulanic acid	509 (42.6%)	-	-	509
Ampicillin/Sulbactam	-	96 (97%)	-	96
Cefazolin	990 (80.6%)	53 (94.6%)	0.205	1043
Cefepime	62 (62%)	1504 (60.3%)	0.325	1566
Ceftazidime	65 (65%)	1656 (65.4%)	0.832	1721
Cefotaxime	394 (42.3%)	5 (45.5%)	0.710	399
Ceftriaxone	154 (39.2%)	68 (90.7%)	0.00	222
Cefixime	502 (41%)	-	-	502
Ciprofloxacin	392 (36.8%)	1176 (58.8)	0.00	1568
Colistin	-	0 (0%)	-	0
Chloramphenicol	-	3 (12.5%)	-	3
ESBL	102 (98.1%)	236 (91.1%)	0.018	338
Gentamycin	168 (13.8%)	75 (69.4%)	0.000	243
Levofloxacin	4 (36.4%)	326 (79.3%)	0.000	330
Nitrofurantoin	181 (14.8%)	-	-	181
Meropenem	40 (38.8%)	883 (35.2%)	0.318	923
Piperacillin/Tazobactam	43 (46.2%)	1052 (42%)	0.346	1095
Trimethoprim-sulfamethoxazol	597 (48.3%)	1540 (63.3%)	0.000	2137
Tetracycline	-	16 (64%)	-	16

\* Number of isolated organism (percentage).

ESBL, extended spectrum beta-lactamase.

Table 5. Total antimicrobial resistance of *Enterobacteriaceae*<sup>b</sup> According to Fisher's exact test.

Antibiotic	Organisms					P value	Total
	<i>E. coli</i> No. (%) <sup>a</sup>	<i>K. pneumoniae</i> No. (%)	<i>K. aerogenes</i> No. (%)	<i>P. mirabilis</i> No. (%)	Other No. (%)		
Amikacin	50 (5.5%)	641 (45.7%)	10 (5.9%)	39 (41.5%)	3 (10.7%)	0.000	743
Amoxicillin/Clavulanic acid	344 (41.7%)	142 (46.1%)	-	10 (24.4%)	9 (60%)	0.005	505
Cefazolin	714 (82.8%)	278 (78.3%)	9 (90%)	28 (66.7%)	14 (87.5%)	0.503 <sup>b</sup>	1043
Cefepime	485 (53.7%)	1011 (72.3%)	24 (14.2%)	41 (43.2%)	15 (17.9%)	0.000	1566
Ceftazidime	515 (56.6%)	1075 (75.9%)	77 (42.8%)	37 (39.4%)	14 (46.7%)	0.000	1718
Cefotaxime	288 (44.7%)	100 (39.8%)	-	7 (17.1%)	-	0.005	395
Ceftriaxone	27 (42.6%)	101 (58.7%)	-	4 (33.3%)	218.2%)	0.000	222
Cefixime	380 (44.9%)	113 (35.6%)	-	5 (11.9%)	5 (31.2%)	0.000	503
Ciprofloxacin	798 (51.2%)	697 (57.6%)	24 (14%)	34 (39.1%)	14 (36.8%)	0.000	1567
Colistin	-	0 (0%)	-	-	-	-	0
Chloramphenicol	-	0 (0%)	-	-	-	-	0
ESBL	261 (96.8%)	68 (81%)	-	-	-	0.000	329
Gentamycin	111 (13.1%)	127 (31.6%)	2 (12.5%)	2 (4.5%)	1 (5.9%)	0.000	132
Levofloxacin	20 (57.1%)	296 (81.3%)	-	11 (64.7%)	-	0.004 <sup>b</sup>	327
Nitrofurantoin	43 (5.1%)	100 (32.2%)	-	35 (85.4%)	2 (12.5%)	0.000	178
Meropenem	15 (1.7%)	857 (60.9%)	10 (5.9%)	34 (35.8%)	7 (24.1%)	0.000	923
Piperacillin/Tazobactam	125 (13.9%)	922 (65.8%)	15 (8.8%)	26 (28%)	5 (17.2%)	0.000	1093
Trimethoprim- sulfamethoxazol	1075 (64%)	899 (54.7%)	73 (42.7%)	74 (56.5%)	17 (37.8%)	0.000	2138
Tetracycline	-	6 (46.2%)	-	-	-	-	6

ESBL, extended spectrum beta-lactamase.

<sup>a</sup> Number of isolated organism (percentage).

possible XDR resistance were more prevalent among inpatient samples than the outpatient ones. In terms of specimen type, MDR and possible XDR were dominant in respiratory tract samples. Further, the ICU isolates had the greatest resistance with respect to antibiotic type. The most abundant resistance was related to cefazolin among outpatients and ampicillin/sulbactam among inpatients, and colistin was the only agent susceptible in all isolates. The results indicated the high prevalence of resistance among *Enterobacteriaceae* and warned us about the preservation of susceptible antibiotics.

Centers for Disease Control and Prevention (CDC) in 2019 announced carbapenem-resistant *Enterobacteriaceae* (CRE) as a public health threat due to resistance to almost all available agents. Pokharel et al, in a cross-sectional study, mentioned that 27% of the studied *Enterobacteriaceae* were resistant to carbapenem (11). Colistin, known as polymyxin E, is the last-line agent used in gram-negative resistance. Büchler et al reported colistin resistance because of spreading carbapenem-resistance across the world (12). In the present study, CRE was examined by using meropenem although the frequency of resistance to meropenem was significant, especially in *K. pneumoniae* isolates. Furthermore, colistin resistance was negative, providing an opportunity to protect the susceptibility of

last-line agent.

The public health was concerned about ESBL-producing *Enterobacteriaceae*, in which the different types of genes are involved, for over two decades (13, 14). Based on the results of a review study, the worldwide spread of ESBL-producing *Enterobacteriaceae*, particularly *E. coli* and *K. pneumoniae*, is a challenge for a practical choice (15). The results are consistent with those of the present study revealed the significance of ESBL-producing among *E. coli* and *K. pneumoniae*. Uc-Cachón et al introduced ICU admission as a significant risk factor for highly prevalent antibiotic resistance, especially gram-negative bacilli as the most abundant pathogens in this department. Additionally, a significant difference was found in the resistance patterns of antibiotics in various ICUs, and the maximum frequent resistance was related to ampicillin, followed by cefuroxime and piperacillin (16). Ibrahim et al, in a retrospective study, reported that a majority of gram-negative obtained from ICUs was MDR and resistance rate was maximized regarding cefuroxime (17). The results of a retrospective study represented the gram-negative pathogens as the most abundant among the ICU isolates, blood samples as the most common sources, and nitrofurantoin as the least frequent resistance (18). The results are in line with those of the present study reflected the highest prevalent

resistance in the specimens taken from ICUs. Based on the specimen source, the highest abundant antibiotic resistance among inpatients was observed in ampicillin/sulbactam, cefazolin, and ceftriaxone, respectively. The differences in resistance frequency can be attributed to the various prevalent choices of antibiotics in empirical and prophylactic therapies or availability of an agent.

### Conclusion

The results revealed *E. coli* and *K. pneumoniae* as the most prevalent pathogens extracted from the isolates of outpatients and inpatients, especially ICU ones, respectively. The maximum frequency of MDR and possible XDR was obtained in *K. pneumoniae* isolates, which is in agreement with the results of the previous studies. The cefazolin and ampicillin/sulbactam were respectively detected as the most resistant agents among outpatients and inpatients, respectively. Thus, the frequency of resistance was high in the present study. Extensive national surveillance programs are essential to control and prevent resistance progression in remaining susceptible antibiotics.

### Limitations of the study

Based on the results, international studies should be carried out to determine the precise rate in Iran. The incompleteness of antimicrobial resistance tests in the intended hospital was a significant limitation in this study, which limited the resistance pattern to identify PDR among the isolates.

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### Author's contribution

AB, SM, and AN were the principal investigators of the study, and FE, SN, and SA were included in preparing the concept and design. SR revisited the manuscript and critically evaluated the intellectual contents. All authors participated to prepare the final draft of the manuscript, revised the manuscript, and critically examined the intellectual contents. They approved the manuscript content and confirmed the accuracy or integrity of any part of the work.

### Conflicts of interest

The authors declare no conflict of interest.

### Ethical issues

This study was extracted from M.D., thesis by Fatemeh Esfahanian in Isfahan University of Medical Sciences, which followed the tenets of the Declaration of Helsinki. This study and its protocols were approved by the Ethics Committee and Institutional Ethical Committee of Isfahan University of Medical Sciences, respectively (IR.MUI.MED.REC.1398.549). Accordingly, written informed consent was taken from all participants before any intervention. The authors have observed ethical issues such as plagiarism, data fabrication, and double publication.

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