Evaluating the antimicrobial resistance patterns and MDR frequency in *Escherichia coli* strains isolated from clinical specimens taken from patients in Iran: 2017–2019

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Research note

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Abstract

Objectives The purpose of this study was to evaluate the antimicrobial resistance and frequency of MDR strains in *E. coli* isolated from patients in two hospitals of Iran. In this descriptive-analytical study, 13322 clinical specimens were collected from two teaching hospitals. The *E. coli* isolates were identified using standard bacteriological methods. Then, the resistance patterns of the isolates were analyzed by disk agar diffusion method according to the CLSI.

Results Out of 13322 clinical samples, 964 (7.23%) isolates of *E. coli* were identified. In both hospitals, high resistance to ampicillin and cefalexin was presented in 621 (64.4%) and 402 (41.7%) isolates, respectively. The highest antibiotic resistance was observed in burn unit, burn ICU and burn restoration section, while all 8 strains which were isolated from the neonatal-ICU, were sensitive to all tested antibiotics except cefalexin, nitrofurantoin, nalidixic acid, and ampicillin. Also, the bacteria isolated from urine, wound, stool, and blood samples were resistant to all tested antibiotics. Increased resistance to different antibiotics in burn hospital has created increasing concern. Very high resistance to some antibiotics indicates that these drugs are misused in therapeutic centers and highlights that infection control measures should be arranged in the ICUs of our hospitals.

Introduction

Escherichia coli (*E. coli*) causes important infections such as sepsis, neonatal meningitis, wound infection, and urinary tract infections (UTIs) (1, 2). The UTI is the second most common bacterial infection after respiratory tract infection in outpatients and inpatients. The frequency of UTI is more common in women than in men, with approximately half of women experiencing it at least once in their lifetime (3, 4). UTI in infants, pregnant women, the elderly, diabetics, multiple sclerosis, and immunocompromised individuals is associated with an increased risk (5, 6). The prevalence of UTIs in the developing countries is estimated to be at least 250 million cases per year (6, 7). Beta-lactam antibiotics are often used to control infections caused by *E. coli*, however several different antibiotic resistance mechanisms have been identified in *E. coli* (8, 9). Failure to control and prevention of the antibiotic resistance will result in increased resistant isolates and augmented therapeutic failure in the treatment of infections caused by these isolates, resulting in prolonged course of the disease, increasing the likelihood of transmission of disease to others, increased length of hospital stay, need for more expensive medications, more complications for treatment and increased risk of death (1). The growing use of antibiotics and the subsequent increase in antibiotic resistance is one of the major concerns in health centers around the world. On the other hand, the antibiotic susceptibility pattern of *E. coli* clinical isolates varies in different regions of the world, different cities of a country, and even in different hospitals of a city or region. Awareness of the antibiotic resistance pattern of bacteria in each region is essential to effective treatment of the infections, control of the nosocomial infections and reduce the prevalence of resistant organisms. The purpose of this study was to evaluate the antimicrobial resistance and frequency of MDR strains in *E. coli* isolated from patients.

Methods

In this descriptive-analytical study, 13322 different clinical specimens (such as ulcer, urine, sputum, blood, stool, etc.) collected from patients admitted to Bu-Ali Sina hospital (a pediatric treatment center) and Zare hospital (A burn center) in Sari city from spring 2017 to winter 2019, were evaluated to identify *E. coli* isolates. Clinical samples were cultured on blood agar and eosin methylene blue agar (EMB) and incubated at 37° C for 24 hours. About urine culture, the patients with clinical symptoms such as fever or chills, dysuria, decreased urine volume, frequent or intense urge to urination, pain, pyuria, etc., along with a bacterial counting of $\geq 10^5$ cfu/ml in urine culture were included in this study (2). After isolation, Gram staining and conventional standard biochemical and microbiological tests were used to identification of the isolates (10). Also, the analytical profile index (API) (BioMérieux, Lyon, France) was used for final confirmation of the isolates. Next, the antibiotic resistance patterns of the isolates were evaluated by standard disk agar diffusion method (Kirby-Bauer) according to CLSI (Clinical and Laboratory Standard Institute) guidelines (11). Antibiotics used (MAST Diagnostic Co, UK) included imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), ceftazidime (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), gentamicin (10 µg), ampicillin (10 µg), cefalexin (30 µg), cefepime (30 µg), nalidixic acid (30 µg) and tobramycin (10 µg). Statistical analysis was performed using SPSS software version 16.

Results

In this study, 389 (40.35%) and 575 (59.64%) isolates were collected from inpatients and outpatients, respectively. The mean age of the patients was 29.99 ± 33.57 , ranging from infant to 105 years old patients. Of all patients, 676 (70.1%) were female and 288 (29.9%) were male. Urine samples with 875 cases (90.8%), wound specimens with 50 cases (5.2%) and stool with 24 samples (2.5%) were the most prevalent clinical specimens included in this study. However, 870 (90.2%) clinical isolates were collected from Bu-Ali Sina hospital and others 94 (9.8%) were collected from Zare hospital (Table 1). *E. coli* collected from Zare hospital were isolated only from burn ward, intensive care unit, restoration and psychiatry wards. Totally, 64 (7.4%) bacterial strains were isolated from clinical specimens of the patients hospitalized in internal medicine ward. The most prevalent infections observed in Bu-Ali Hospital were UTIs in children under 10 years old, while most isolates of Zare Hospital were collected from wound samples and middle-aged patients. Out of 13322 clinical specimens, 964 isolates of *E. coli* were detected, while 638 isolates (66.18%) were from female urine samples. The most samples in Bu-Ali Sina hospital were collected from 0-10-year-old patients followed by 51-60-year-old persons. However, in Zare hospital, the samples collected from 51-60-year-old patients were higher than other age groups (Table 1). Also, urine was the most prevalent sample in both hospitals. Evaluation of the antibiotic resistance patterns of the isolated bacteria by disk agar diffusion method showed that in both hospitals there was high resistance to ampicillin in 621 (64.4%) isolates and cefalexin in 402 (41.7%) isolates, and the lowest resistance rate was observed against amikacin, while 39 (4%) isolates were resistant to this antibiotic (Table 2). In general, the percentage of antibiotic resistance was higher in Zare hospital than in Bu Ali Sina hospital. The most active antibiotics against the clinical isolates of *E. coli* in Bu-Ali Sina hospital were amikacin, tobramycin and imipenem, while this issue in Zare hospital was seen about amikacin, gentamicin and nalidixic acid. However, amikacin was the most effective antibiotic in both hospitals followed by gentamicin and tobramycin in all isolates of this study (Table 2). The highest resistance to all antibiotics was observed in the burn unit, and only 8 strains were isolated in the neonatal-ICU (NICU) which were sensitive to all tested antibiotics except cefalexin, nitrofurantoin, nalidixic acid and ampicillin (Table 3). According to table 3, ampicillin, cefalexin and nalidixic acid were the least

effective antibiotics in different units of the two studied hospitals. Resistance to both carbapenems was observed in *E. coli* isolated from all samples except sputum. Also, bacteria isolated from urine, wound, stool and blood samples were resistant to all tested antibiotics in this study. Moreover, one *E. coli* was isolated from semen culture which was sensitive to aminoglycosides (amikacin and gentamicin) (Additional file 1: Table S1). According to the results, 221 (22.9%) isolates were resistant to only one antibiotic, while 7 (0.72%) isolates were resistant to at least 9 antibiotics. In this study, 24 (2.4%), 78 (8.09%), 125 (12.9%), and 85 (8.8%) isolates were resistant to all antibiotics tested belonged the class of aminoglycosides, cephalosporins, quinolones, and carbapenems, respectively. Further information on these results is provided in (Additional file 2: Table S2). Based on statistical analysis, a significant relationship was observed between resistance to all antibiotics, except nalidixic acid, and variables such as hospital wards of bacterial isolation, clinical sample type, age and sex of the patients ($P < 0.05$). Also, 241 (25%) clinical isolates of *E. coli* were resistant to at least 3 antibiotics from 3 different classes and were considered as MDR isolates (Additional file 3: Table S3).

Discussion

E. coli is the most common urinary tract pathogen (12). Moreover, most cases of *E. coli* infection in this study were related to the urinary system, accounting for more than half of the cases. Also, studies carried out in Iran and other neighboring and non-neighboring countries have confirmed this result (13, 14). In addition, women were the most infected individuals with *E. coli* (70.1%), which is consistent with other studies conducted in Tehran, Isfahan and Rasht (10, 15, 16). On the other hand, the bacterial isolates in the present study showed the lowest antibiotic resistance rates against amikacin in both hospitals. An Iranian study performed in 2019 showed that 2.2% of the clinical isolates of *E. coli* were resistant to this antibiotic (17). However, other studies conducted in Iran (18), Saudi Arabia (12) and Turkey (19), detected no amikacin resistant isolates of *E. coli*. These results indicate the good efficacy of amikacin as one of the effective antibiotics against infections caused by *E. coli*. Also, the results of this study showed low antibiotic resistance, except against ampicillin and cefalexin, in *E. coli* isolated from Bu-Ali Sina hospital compared to burn hospital. Due to the weakened immune system of burn patients, broad-spectrum antibiotics are used to treat bacterial infections in this group, which may increase the prevalence of antibiotic resistance (20). With the exception of carbapenems, the above results are consistent with other Iranian studies conducted in 2019 (21, 22). The antibiotic resistance rate of *E. coli* strains in Turkey was also much lower than in the present study (20). However, their research conducted on 21 urine samples, which may be the reason for lower resistance rate. Differences in antibiotic resistance rates in different regions of the world can be due to genetic differences in resistant strains, various antibiotic use policies in regional hospitals, levels of arbitrary use of antibiotics in the community and various study time (23). Among the antibiotics studied in this study, ampicillin was the least effective ones with the highest resistance rate, which was similar to the results obtained in other Iranian studies carried out in Ahvaz, Rasht and Kashan (15, 22, 24). These results indicate that unlike amikacin, ampicillin is not suitable for the treatment of *E. coli* infections in Iran. Moreover, resistance to carbapenems among isolates collected from Bu-Ali Sina hospital in this study was similar to other studies in Iran and neighboring countries (13, 19, 21, 22, 25), while the reported carbapenem resistance rates in non-neighboring countries such as Malaysia and Nigeria were 29.2% and 19.7%, respectively (26, 27). This discrepancies may be due to the differences in the type of sample, as Bu-Ali Sina hospital is a specialized pediatric hospital. However, resistance to carbapenems was observed extensively in the Zare as a burn hospital in the present study, which is justifiable given that the hospital is a specialized burn center. Beside, quinolones and fluoroquinolones such as nalidixic acid and ciprofloxacin are also used to treat UTIs. Similar studies, in Ahvaz (22) and Sanandaj (21), reported 64.2% and 78.8% ciprofloxacin resistance, respectively, which is consistent with the results of the burn hospital in the present study. In other studies (19, 27), the rate of quinolone resistance was higher than that of fluoroquinolones, which was consistent with the results of this study. This may be due to the greater use of drugs such as nalidixic acid to treat UTIs. Numerous factors, including supplementary use of antibiotics in agriculture and veterinary medicine, also contribute to the development of antibiotic resistance (28). Moreover, one of the serious problems worldwide is the multi-drug resistance and the development of MDR strains, which is a major concern in the treatment of infections caused by *E. coli* (8, 29). In this study, 80.9% of the urinary isolates of *E. coli* were MDR, which was very similar to the study performed in Ahvaz (22). In the research of Badamachi et al, (18), 54.6% of urinary isolates were MDR, while only 1 to 5-year-old children were studied. Since the prevalence of MDR *E. coli* strains, especially in burn patients, can increase morbidity and mortality rates (29), so their presence can be a major risk factor for the patients. Understanding the resistance rates of the clinical isolates of *E. coli* to common antibiotics in hospitals is essential to control the antibiotic resistance of this bacterium. According to the results of the present study and other studies, aminoglycosides are almost effective drugs against *E. coli* infections, which can be suggested as the selective antibiotics in the treatment of resistant strains in this area. On the other hand, caring for the elderly and the high risk patients for the infection with *E. coli*, such as burn and ICU patients, is of great importance, which can lead to reduced infection-related mortality and reduced rates of disease transmission.

Limitations

The present study had some limitations; Lack of information on the underlying disease status of the patients, Also, in order to achieve better results, detection of common antibiotic resistance genes is also necessary.

Abbreviations

MDR; Multidrug-Resistant, CLSI; Clinical and Laboratory Standards Institute, *E. coli*; Escherichia coli, ICU; intensive care unit, UTIs; urinary tract infections, EMB, eosin methylene, TSI; Triple sugar iron, API; Analytical Profile Index

Declarations

Ethics approval and consent to participate

All patients signed an informed consent to participation in the work. The study on human specimens was approved by Research Council and Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran with the following code of ethics (IR.MAZUMS.REC.1398.016).

Consent for publication

There is no limit to the publication.

Competing interests

The author reports no conflicts of interest in this work

Availability of data and materials

Data generated or analyzed during this study are included in this published article.

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Authors' contributions

Dr. H. Goli design of the study and supervision. Z. Norouzi Bazgir: collected the data, cultured the samples. Dr. M. Gholami: Interpreted the data and performed the statistical analysis, drafting of the manuscript in collaboration with Dr. H. Goli. All authors of the manuscript have read and agreed to its content.

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Tables

Table 1 Distribution of *E. coli* clinical isolates in different types of clinical samples and various age groups

year-old	Hospital	No. (%) of different samples collected in two hospitals studied							
		Wound	Urine	Sputum	Semen	Blood	Stool	Trachea	Total
0-10	B	5 (1.4)	340 (91.9)	1 (0.3)	1 (0.3)	4 (1.1)	19 (5.1)	-	370 (100)
	Z	3 (60)	2 (40)	-	-	-	-	-	5 (100)
11-20	B	-	47 (94)	1 (2)	-	-	2 (4)	-	50 (100)
	Z	-	5 (71.4)	1 (14.3)	-	1 (14.3)	-	-	7 (100)
21-30	B	-	61 (100)	-	-	-	-	-	61 (100)
	Z	1 (16.7)	5 (83.3)	-	-	-	-	-	6 (100)
31-40	B	-	48 (100)	-	-	-	-	-	48 (100)
	Z	2 (20)	7 (70)	-	-	-	1 (10)	-	10 (100)
41-50	B	-	40 (100)	-	-	-	-	-	40 (100)
	Z	7 (50)	5 (35.7)	-	-	1 (7.14)	1 (7.14)	-	14 (100)
51-60	B	-	89 (100)	-	-	-	-	-	89 (100)
	Z	13 (48.1)	12 (44.4)	-	-	1 (3.7)	-	1 (3.7)	27 (100)
61-70	B	1 (1.2)	81 (98.8)	-	-	-	-	-	82 (100)
	Z	14 (73.7)	3 (15.8)	1 (5.3)	-	-	1 (5.3)	-	19 (100)
71-80	B	-	58 (96.7)	2 (3.3)	-	-	-	-	60 (100)
	Z	4 (100)	-	-	-	-	-	-	4 (100)
81-90	B	-	58 (100)	-	-	-	-	-	58 (100)
	Z	-	2 (100)	-	-	-	-	-	2 (100)
91-100	B	-	11 (100)	-	-	-	-	-	11 (100)
	Z	-	-	-	-	-	-	-	-
101-110	B	-	1 (100)	-	-	-	-	-	1 (100)
	Z	-	-	-	-	-	-	-	-
Total		50 (5.2)	875 (90.8)	6 (0.6)	1 (0.1)	7 (0.7)	24 (2.5)	1 (0.1)	964 (100)

Notes: B, Bu-Ali Sina hospital; Z, Zare hospital.

Table 2 Antibiotic resistance pattern of *E. coli* clinical isolates in two studied hospitals

Antibiotics	No. (%) of isolates with different antibiotic resistance pattern in								
	Bu-Ali Sina hospital			Zare hospital			Total		
	(n=870)			(n=94)			(n=964)		
	R	I	S	R	I	S	R	I	S
Ciprofloxacin	126 (14.5)	12 (1.4)	732 (84.1)	68 (72.3)	-	26 (27.7)	194 (20.1)	12 (1.2)	758 (78.6)
Nalidixic acid	232 (26.7)	22 (2.5)	616 (70.8)	35 (37.2)	-	59 (62.8)	267 (27.7)	22 (2.3)	675 (70)
Cefalexin	342 (39.3)	7 (0.8)	521 (59.9)	60 (63.8)	2 (2.1)	32 (34)	402 (41.7)	9 (0.9)	553 (57.4)
Cefepime	104 (12)	9 (1)	757 (87)	54 (57.4)	2 (2.1)	38 (40.4)	158 (16.4)	11 (1.1)	795 (82.5)
Ceftazidime	131 (15.1)	10 (1.1)	729 (83.8)	44 (46.8)	4 (4.3)	46 (48.9)	175 (18.2)	14 (1.5)	775 (80.4)
Ampicillin	544 (62.5)	17 (2)	309 (35.5)	77 (81.9)	-	17 (18.1)	621 (64.4)	17 (1.8)	326 (33.8)
Nitrofurantoin	88 (10.1)	18 (2.1)	764 (87.8)	48 (51.07)	-	46 (48.93)	136 (14.1)	18 (1.9)	810 (84)
Gentamicin	44 (5.1)	19 (2.2)	807 (92.8)	31 (33)	2 (2.1)	61 (64.9)	75 (7.8)	21 (2.2)	868 (90)
Amikacin	15 (1.7)	4 (0.5)	851 (97.8)	24 (25.5)	2 (2.1)	68 (72.3)	39 (4)	6 (0.6)	919 (95.3)
Tobramycin	41 (4.7)	6 (0.7)	823 (94.6)	44 (44.8)	2 (2.1)	48 (51.1)	85 (8.8)	8 (0.8)	871 (90.4)
Imipenem	41 (4.7)	6 (0.7)	823 (94.6)	45 (47.9)	2 (2.1)	47 (50)	86 (8.9)	8 (0.8)	870 (90.2)
Meropenem	52 (6)	1 (0.1)	817 (93.90)	47 (50)	2 (2.1)	45 (47.9)	99 (10.3)	3 (0.3)	862 (89.4)

Table 3 Antibiotic resistance pattern of *E. coli* clinical isolates in terms of hospital wards

Hospital Wards	No. (%) of isolates with different antibiotic resistance patterns against									
	Amikacin	Gentamicin	Imipenem	Meropenem	Tobramycin	Ceftazidime	Cefepime	Cefalexin	Ciprofloxacin	Nalidixic acid
Pediatric Surgery (n=56)	1 (1.8)	1 (1.8)	-	1 (1.8)	-	10 (17.9)	5 (8.9)	18 (32.1)	8 (14.3)	11 (19.6)
Infectious (n=40)	-	3 (7.5)	1 (2.5)	1 (2.5)	1 (2.5)	9 (22.5)	4 (10)	18 (45)	8 (20)	16 (40)
Oncology (n=5)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)
Neurology (n=38)	3 (7.9)	5 (13.2)	5 (13.2)	7 (18.4)	5 (13.2)	8 (21.1)	12 (31.6)	26 (68.4)	10 (26.3)	18 (47.4)
Eye (n=3)	-	-	-	-	-	1 (33.3)	-	1 (33.3)	1 (33.3)	1 (33.3)
Neonates (n=21)	-	1 (4.8)	-	-	-	3 (14.3)	-	6 (28.6)	3 (14.3)	3 (14.3)
Intensive Care Unit (ICU) (n=47)	3 (6.4)	7 (14.9)	7 (14.9)	8 (17)	7 (14.9)	19 (40.4)	18 (38.3)	34 (72.3)	17 (36.2)	27 (57.4)
Pediatrics (n=14)	-	1 (7.1)	-	-	-	-	1 (7.1)	2 (14.3)	1 (7.1)	3 (21.4)
Post ICU (PICU) (n=13)	1 (7.1)	4 (30.8)	1 (7.7)	1 (7.7)	1 (7.7)	1 (7.7)	3 (23.1)	7 (53.8)	4 (30.8)	3 (23.1)
Neonatal ICU (NICU) (n=8)	-	-	-	-	-	-	-	1 (12.5)	1 (12.5)	-
Internal medicine (n=65)	1 (1.5)	6 (9.2)	8 (12.3)	8 (12.3)	8 (12.3)	8 (12.3)	16 (24.6)	30 (46.2)	5 (7.1)	20 (30.8)
Men and women (n=5)	-	-	-	-	-	2 (40)	2 (40)	3 (60)	-	1 (20)
Outpatient (n=575)	7 (1.2)	17 (3)	25 (4.3)	32 (5.6)	25 (4.3)	78 (13.6)	49 (8.5)	203 (35.3)	71 (12.3)	135 (23.5)
Burn ICU (BICU) (n=14)	5 (35.7)	5 (35.7)	5 (35.7)	5 (35.7)	5 (35.7)	7 (50)	6 (42.9)	6 (42.9)	10 (71.4)	3 (21.4)
Burn (n=11)	7 (63.3)	8 (72.7)	8 (72.7)	9 (81.8)	7 (63.6)	6 (54.5)	10 (90.9)	11 (100)	10 (90.9)	5 (45.5)
Restoration (n=39)	9 (23.1)	16 (41)	23 (59)	24 (61.5)	23 (59)	21 (53.8)	30 (76.9)	33 (84.6)	35 (89.7)	19 (48.7)
Psychiatry (n=9)	1 (11.1)	-	2 (22.2)	2 (22.2)	2 (22.2)	1 (11.1)	1 (11.1)	2 (22.2)	3 (33.3)	1 (11.1)
Total (n=964)	39 (4)	75 (7.8)	86 (8.9)	99 (10.3)	85 (8.8)	175 (18.2)	158 (16.4)	402 (41.7)	194 (20.1)	267 (27.7)

Supplementary Files

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- [TableS2.docx](#)
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