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Antibiotic Sensitivity Patterns of *Escherichia coli* Isolated in Urine Samples of Patients Referred to Ayatollah Mousavi Hospital in Zanjan

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ABSTRACT

Background & Objective: *Escherichia coli* (*E. coli*) is considered to be the most common cause of urinary tract infections (UTIs) worldwide. Due to the recent rise in bacterial resistance to antibiotics and the appearance of multidrug-resistant *E. coli*, treatment options have been significantly limited, thus increasing the cost of treatment as well as morbidity and mortality rates, especially in developing countries. This study aimed to identify the antibiotic susceptibility patterns of *E. coli* for use in early empirical treatments and cultures of negative UTIs caused by previous antibiotic usage.

Materials & Methods: In the present study, 704 urine samples with a positive culture of *E. coli* were evaluated in terms of susceptibility to gentamycin, nitrofurantoin, ceftazidime, cefixime, meropenem, cefepime, azithromycin, ceftriaxone and ciprofloxacin using the Kirby-Bauer disc diffusion method. Data were collected based on age, sex, and hospitalization or ambulatory patient status. Data were analyzed using SPSS 22.0.

Results: *E. coli* showed the lowest resistance to nitrofurantoin (4.5%) and the highest resistance to cefixime (34.9%). There was a statistically significant relationship between antibiotic resistance and age, gender, and hospitalization status

Conclusion: Because of the high resistance rate of *E. coli* to cefixime, precautions should be taken before using cefixime to treat UTIs.

Keywords: Antibiotic sensitivity, *Escherichia coli*, Resistance, Urinary tract infections

Introduction

Escherichia coli (*E. coli*) is the most common organism causing urinary tract infections (UTIs) **(1)** and is the cause of about 90% of all UTIs in young women **(2)**. UTIs are very common infectious diseases, both as community- and hospital-acquired infections **(3,4)**. It is estimated that 10-20% of infections treated by primary care physicians and 30-40% of hospital-treated infections are due to UTIs **(4)**. These infections are more common in females and are found in about 40% of all females, with a 30% chance of relapse. UTIs are also more prevalent in the elderly than in younger people. The majority of UTI cases are mild **(5)**, but they can lead to end-stage renal scarring, hypertension, and renal dysfunction, especially when the kidney is affected **(6.)**. The purpose of treating UTIs is to provide a quick cure and prevent complications. To achieve this goal in patients suspected of having a UTI, empirical treatment should be initiated before preparing the urine culture results **(7,8)**. Due to the recent rise in bacterial resistance to antibiotics and the appearance of multidrug-resistant (MDR) *E. coli*, treatment options have been significantly limited; in developing countries, this situation may have a significant impact on treatment costs and increase the morbidity and mortality rates associated with UTIs **(4,9)**. UTIs caused by resistant pathogens have a longer recovery period and higher relapse rates, and they mostly depend on various courses of antibiotic therapy **(5)**. Thus, it is essential to be aware of the current antibiotic susceptibility patterns of *E. coli* (the main bacterial cause of UTIs) in order to determine the most appropriate choice for empiric treatment (4). Antibiotic susceptibility patterns differ by location and time, so periodically testing antibiotic resistance in different hospitals, cities, and nations is crucial (2,5,10). Although many factors affect antibiotic resistance, the inappropriate and widespread use of antibiotics plays an important role (11). This study aimed to identify the antibiotic susceptibility pattern of *E. coli* in order to determine effective early empirical treatments and cultures for negative UTIs caused by previous antibiotic usage. By increasing the quality of treatment and decreasing the duration of treatment, we can reduce complications and reduce the cost of treatment.

Materials and Methods

In this study, we examined all urinary samples (24,706 cases) from inpatients and ambulatory patients referred to the laboratory of Ayatollah Mousavi Hospital in Zanjan between August 21, 2015, and August 21, 2016. Urine sampling was performed as a mid-stream urine clean catch in a sterile container. In children under three years old, samples were collected in a urine bag. The samples were immediately transferred to the laboratory and cultured in an agar plate. The culture plates were incubated at 37°C for 48 hours. Positive cultures were identified through standard biochemical methods. The isolation of a single type with colony counts of more than 10^5 colony-forming unit/mL was regarded as positive urine cultures. Positive cultures other than *E. coli* were discarded. There were a total of 704 *E. coli* positive samples for which antibiotic susceptibility to gentamicin, nitrofurantoin, ceftazidime, cefixime, meropenem, cefotaxime, cefepime, azithromycin, ceftriaxone, and ciprofloxacin, was examined using the Kirby-Bauer disc diffusion method. One isolate per patient was included in the study. Data were collected based on age, sex, and hospitalization or outpatient status. Values were expressed as numbers and percentages. Comparisons for categorical variables were performed via a Chi-square test. A P-value<0.05 was regarded as statistically significant. The data were analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). This study was approved by the ethics committee of Zanjan University of Medical Sciences and has been registered with the code (zums.REC.1394.222).

Results

In this study, among 24,706 urine samples cultured in Ayatollah Mousavi Hospital Laboratory in Zanjan from August 21, 2015, to August 21, 2016, 704 *E. coli* positive urine samples were isolated. Of these isolates, 188 (26.7%) were derived from males, and 516 (73.3%) were derived from females. There were 216 (30.7%) inpatient samples and 488 (69.3%) from ambulatory settings. In terms of age distribution, 24 (3.4%) were under one month, 86 (12.2%) were 1-12 months, 68 (9.7%) were 1-5 years, 68 (9.7%) were 5-10 years, 30 (4.3%) were 10-18 years, 216 (30.7%) were 18-40 years, 132 (18.8%) were 40-65 years, and 80 (11.4%) were over 65 years old.

The sensitivities of isolated *E. coli* to common antibiotics are shown in Table 1, and their differences by gender are shown in Table 2. These findings showed that for all antibiotics, *E. coli* is more resistant in men than in women (*P*=0.00).

Based on the results of this study, *E. coli* samples obtained from hospitalized patients showed a higher rate of resistance than outpatients (*P*=0/00) (Table 3).

Antibiotic susceptibility findings of the *E. coli* isolated from urine samples of UTI patients by age are shown in Table 4.

Table 1. The sensitivity of the isolated *E. coli* from patients' urine samples

Antibiotics	Number of isolates (percent)		
	resistant	intermediate	sensitive
Meropenem	50(7.1)	10(1.4)	644(91.5)
Ceftazidime	102(14.5)	34(4.8)	568(80.7)
Cefepime	96(13.6)	40(5.7)	568(80.7)
Azithromycin	176(25)	20(2.8)	508(72.2)
Gentamicin	114(16.2)	18(2.6)	572(81.2)
Ceftriaxone	212(30.2)	20(2.8)	472(67)
Ciprofloxacine	180(25.5)	4(0.6)	520(73.9)
Cefixime	246(34.9)	18(2.6)	440(62.5)
Nitrofurantoin	32(4.5)	8(1.2)	664(94.3)

Table 2. The sensitivity of the isolated *E.coli* from patients' urine samples by gender

Antibiotics	Gender	Number of isolates (percent)			P-value
		resistant	intermediate	sensitive	
Meropenem	male	36(19.1)	4(2.1)	148(78.8)	0.001<
	female	14(2.7)	6(1.2)	496(96.1)	
Ceftazidime	male	62(33)	16(8.5)	110(58.5)	0.001
	female	40(7.8)	18(3.5)	458(88.8)	
Cefepime	male	62(33)	20(10.6)	106(56.4)	0.001
	female	34(6.6)	20(3.9)	462(89.5)	
Azithromycin	male	64(34)	4(2.1)	120(63.8)	0.08
	female	112(21.7)	16(3.1)	388(75.2)	
Gentamicin	male	60(31.9)	10(5.3)	118(62.8)	0.001<
	female	54(10.5)	8(3)	454(88)	
Ceftriaxone	male	98(52.1)	4(2.1)	86(45.8)	0.001<
	female	114(22.1)	16(3.1)	368(74.8)	
Ciprofloxacine	male	82(43.6)	-	106(56.4)	0.001<
	female	98(19)	4(0.8)	414(80.2)	
Cefixime	male	102(54.3)	4(2.1)	82(43.6)	0.001<
	female	144(27.9)	14(2.7)	358(69.4)	
Nitrofurantoin	male	26(13.8)	2(1.1)	160(85.1)	0.001<
	female	6(1.2)	6(1.2)	504(97.6)	

Table 3. The sensitivity of the isolated *E. coli* from patients' urine samples by Hospitalization status

Antibiotics	Hospitalization status	Number of isolates (percent)			P-value
		resistant	intermediate	sensitive	
Meropenem	inpatient	24(11.1)	4(1.9)	188(87)	<0.001
	outpatient	26(5.3)	6(1.2)	546(93.4)	
Ceftazidime	inpatient	54(25)	16(7.4)	146(67.6)	<0.001
	outpatient	48(9.8)	18(3.7)	422(86.5)	
Cefepime	inpatient	62(28.7)	12(5.6)	142(65.7)	<0.001
	outpatient	34(7)	28(5.7)	426(87.3)	
Azithromycin	inpatient	80(37)	2(0.9)	134(62)	<0.001
	outpatient	96(1907)	18(3.7)	372(76.2)	
Gentamicin	inpatient	58(26.9)	8(3.7)	150(69.4)	<0.001
	outpatient	56(11.5)	10(2)	422(86.5)	
Ceftriaxone	inpatient	106(49.1)	4(1.8)	106(49.1)	<0.001
	outpatient	106(21.7)	16(3.3)	366(75)	
Ciprofloxacine	inpatient	90(41.7)	-	126(58.3)	<0.001
	outpatient	90(18.5)	4(0.8)	394(80.7)	
Cefixime	inpatient	116(53.7)	2(0.9)	98(45.4)	<0.001
	outpatient	130(26.6)	16(3.3)	342(70.1)	
Nitrofurantoin	inpatient	28(13)	2(0.9)	186(86.1)	<0.001
	outpatient	4(0.8)	6(1.2)	478(98)	

Table 4. **Frequency and percent of antibiotic susceptibility of E. coli samples by age**

Antibiotics	Age	Number of isolates (percent)			P-value
		resistant	intermediate	sensitive	
Meropenem	<1month	2(8.3)	-	22(91.7)	0.03
	1-12 months	14(16.3)	-	72(83.7)	
	5-1years	2(2.9)	-	66(97.1)	
	10-5 years	4(5.9)	2(2.90	62(91.2)	
	18-10 years	2(6.7)	-	28(93.3)	
	40-18 years	10(4.6)	6(2.8)	200(92.6)	
	65-40 years	8(6.1)	-	70(93.9)	
	≤65 years	8(10)	2(2.5)	70(87.5)	
Ceftazidime	<1month	4(16.7)	-	20(83.3)	<0.001
	1-12 months	20(23.3)	2(2.3)	64(74.4)	
	5-1years	4(5.9)	6(8.8)	58(85.3)	
	10-5 years	8(11.8)	2(2.9)	58(85.3)	
	18-10 years	6(20)	-	24(80)	
	40-18 years	20(9.3)	6(2.7)	190(88)	
	65-40 years	14(10.6)	10(7.6)	108(81.8)	
	≤65 years	26(32.5)	8(10)	46(57.5)	
Cefepime	<1month	4(6.7)	-	20(83.3)	<0.001
	1-12 months	22(25.6)	2(2.3)	62(72.1)	
	5-1years	4(5.9)	8(11.8)	56(82.4)	
	10-5 years	12(17.6)	-	56(82.4)	
	18-10 years	2(6.7)	2(6.7)	26(86.7)	
	40-18 years	18(8.3)	4(1.9)	194(89.8)	
	65-40 years	12(9.1)	16(12.1)	104(78.8)	
	≤65 years	22(27.5)	8(10)	50(62.5)	
Azithromycin	<1month	4(16.7)	-	20(83.3)	0.016
	1-12 months	32(37.2)	4(4.7)	50(58.1)	
	5-1years	14(20.6)	2(2.9)	50(73.5)	
	10-5 years	16(32.5)	2(2.9)	50(73.5)	
	18-10 years	8(26.7)	2(6.7)	20(66.7)	
	40-18 years	48(22.2)	6(2.8)	162(75)	
	65-40 years	40(30.3)	2(2.5)	90(68.2)	
	≤65 years	14(17.5)	2(2.5)	64(80)	
Gentamicin	<1month	4(6.7)	-	20(83.3)	0/032
	1-12 months	18(20.9)	2(2.3)	66(76.8)	
	5-1years	14(20.6)	-	54(79.4)	
	10-5 years	8(11.8)	-	60(88.2)	
	18-10 years	8(26.7)	-	22(73.3)	

Antibiotics	Age	Number of isolates (percent)			P-value
		resistant	intermediate	sensitive	
	40-18 years	26(12)	4(1.9)	186(86.1)	
	65-40 years	20(15.2)	6(4.5)	106(80.3)	
	≤65 years	16(20)	6(7.5)	58(72.5)	
Ceftriaxone	<1month	4(16.7)	-	20(83.3)	<0.001
	1-12 months	34(39.5)	4(4.7)	48(55.8)	
	5-1years	14(20.6)	2(2.9)	52(76.5)	
	10-5 years	10(14.7)	4(5.9)	54(79.4)	
	18-10 years	(26.7)	2(6.7)	20(66.7)	
	40-18 years	48(22.2)	6(2.8)	162(75)	
	65-40 years	52(39.4)	2(1.5)	78(59.1)	
	≤65 years	42(52.5)	-	38(47.5)	
Ciprofloxacine	<1month	4(16.7)	-	20(83.3)	<0.001
	1-12 months	32(37.2)	-	54(62.8)	
	5-1years	12(17.6)	2(2.9)	54(79.5)	
	10-5 years	8(11.8)	-	60(88.2)	
	18-10 years	6(20)	-	24(80)	
	40-18 years	36(16.7)	2(0.9)	178(82.4)	
	65-40 years	46(34.8)	-	86(65.2)	
	≤65 years	36(45)	-	44(55)	
Cefixime	<1month	4(16.7)	-	20(83.3)	<0.001
	1-12 months	48(55.8)	-	38(44.2)	
	5-1years	18(26.5)	2(2.9)	48(70.6)	
	10-5 years	16(23.5)	4(5.9)	48(70.6)	
	18-10 years	10(33.3)	-	20(66.7)	
	40-18 years	58(26.9)	2(2.9)	156(70.2)	
	65-40 years	50(37.9)	10(7.6)	72(54.5)	
	≤65 years	42(52.5)	-	38(47.5)	
Nitrofurantoin	<1month	2(8.3)	-	22(91.7)	0.636
	1-12 months	4(4.7)	2(2.3)	80(93)	
	5-1years	2(2.9)	-	66(97.1)	
	10-5 years	2(2.9)	2(2.9)	64(94.1)	
	18-10 years	2(6.7)	-	28(93.3)	
	40-18 years	8(3.7)	4(1.9)	204(94.4)	
	65-40 years	6(4.5)	-	126(95.5)	
	≤65 years	6(7.5)	-	74(92.5)	

Discussion

Our study showed that the antibiotic resistance of *E. coli* isolates varied from 4.5% to nearly 35% with different antibiotics (12-15).

Kullkarni *et al.* examined 1,000 urine samples of patients suspected of having a UTI. Of these, 395 had positive *E. coli* cultures, 170 (43%) of which showed MDR. The resistance rates against ampicillin and ceftriaxone were 82.53% and 66.58%, respectively. However, the sensitivity to imipenem was 96.71%, and sensitivity to nitrofurantoin was 92.41% (16).

In a five-year study on the first UTI with positive *E. coli* culture, Fasugba found that the highest antibiotic resistance rates were found for ampicillin (41.9%) and co-trimoxazole (20.7%). The lowest antibiotic resistance rates were reported for meropenem (0.0%) and nitrofurantoin (2.9%) (17).

Dehbani Pour *et al.* examined *E. coli* in Esfahan; it showed high resistance to ceftriaxone (13). In another study by Ferdowsi *et al.* in Babol in 2013, *E. coli* resistance to cefixime and ceftriaxone was reported to be ≥ 35.1%, which is consistent with the results of the current study (18). Overall, these data indicate that resistance to nitrofurantoin is low among *E. coli* isolates. There are several factors involved in preventing *E. coli* resistance to nitrofurantoin. Of course, nitrofurantoin is not widely used because of its unpleasant taste and inefficiency in pyelonephritis due to its low tissue penetration.

Sanchez GV *et al.* conducted a population-based study from 2000 to 2010 in the United States to investigate the in vitro antimicrobial resistance of urine *E. coli* isolates. They found that resistance to nitrofurantoin increased from 0.8% to1.6% over this time. However, the resistance level for ciprofloxacin increased from 3% to 17.1% (19). In 2012, Kahlmeter G. *et al.* showed a low rate of resistance to nitrofurantoin, while the resistance during 2007-2008 was ≤ 2% (15). In the present study, *E. coli* exhibited the greatest resistance to cefixime, supporting the findings of Mirsoleymani *et al.* in Bandar Abbas, Iran, from 2009-2012 (12). Our study showed that for all antibiotics, *E. coli* resistance in men was greater than in women. This is similar to the results presented by Rossana Rosa, Alos, and Gupta (14,20,21). In general, all cases of UTI are considered to be complicated in men, especially when they occur in infants and children or in association with neurological disorders such as bladder outlet obstruction (prostatic hyperplasia) or urinary tract manipulation. However, uncomplicated UTIs also occur in a small number of men between the ages of 15-50 years. (22) We also studied the sensitivity of *E. coli* to antibiotics in terms of hospitalization and outpatient. Our results revealed that urine isolated *E. coli* from hospitalized patients showed greater resistance to all antibiotics when compared to ambulatory patients. This difference was statistically significant (*P*=0.00). Dehbanipour also reported higher overall antibiotic resistance and multidrug resistance in hospitalized patients than in ambulatory patients (13). Hospital pathogens typically show greater resistance than community-acquired pathogens (13). Of course, for a variety of reasons, hospitalized patients have poorer immune systems than ambulatory patients, and the underlying factor that leads to hospitalization may also be added to the resistance problem. Regarding the pattern of antibiotic resistance based on age group, the findings of this study showed that antibiotic resistance is higher in the newborn infants and patients over 65 years old than in other age groups. This may be due to comorbidities in elderly people and the weakened immune systems of these individuals. On the other hand, hospitalization rates in infants and the elderly are higher than other age groups, which, as mentioned, can increase the rate of antibiotic resistance (23). Our results showed that—except in the age group below one month and above 65 years, for which UTIs were more prevalent in males—the majority of cases were females. This is similar to the results presented by Dehbanipour (13) but different from those provided by Mirsoleymani, who found that UTIs were more common in males than females (12). This discrepancy may be due to differences in the populations that comprised the study groups. In our study, all age groups were included, whereas only pediatric populations were evaluated by Mirsoleymani (12).

Conclusion

In this study, as predicted, the highest prevalence of *E. coli* infection was observed in the age group of 18-40 years, especially in women. Due to sexual activity and the anatomy of the genital tract, women in this age group are susceptible to UTIs. Considering the high resistance of *E.* coli to cefixime and ceftriaxone, this study suggests that these drugs are not appropriate antibiotics for UTI therapy, possibly due to the overuse of these antibiotics in recent years.

Therefore, it is suggested that cefixime should be used more cautiously for empiric therapy in cystitis. For treating severe cases of pyelonephritis, dual-agent therapy (ceftazidime or cefepime with an aminoglycoside), or carbapenem alone, is recommended. This study also emphasized the importance of the appropriate use of antibiotics, especially cefixime and ceftriaxone, in avoiding further increases in antibiotic resistance.

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Conflict of Interest

Authors declared no conflict of interest.

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