



Prevalence and Determination of Pattern of Antibiotic Resistance of Enterobacteriaceae Causing Urinary Tract Infection in Clienteles Referred to the Laboratory of Valiasr Clinic in Mashhad in 2022

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ABSTRACT

Urinary tract infection is one of the most common bacterial infections affecting human health. Since Enterobacteriaceae bacteria are common causes of urinary tract infections, this study aimed to determine the frequency and pattern of Enterobacteriaceae antibiotic resistance in patients with urinary tract infections to prevent increased antibiotic resistance of these bacteria and prevent increased treatment costs. This cross-sectional analytical study was performed on 3 different age groups of Clienteles referred to Valiasr Clinic Laboratory in Mashhad. Detection of bacterial species was performed using conventional microbiological methods and biochemical tests, and according to the instructions of the Institute of Clinical and Laboratory Standard 2021, the pattern of antibiotic resistance of isolates was evaluated by the disk diffusion method. Out of 438 urine cultures with positive results in terms of Enterobacteriaceae, *Escherichia coli* with a prevalence of 76.7%, *Klebsiella* 14.4%, *Enterobacter* 4.3%, *Proteus* 3%, and other bacterial species with a prevalence of 1.6% were observed. Bacterial isolates showed the lowest antibiotic resistance against Piperacillin-Tazobactam (13%) and Amikacin (17.2%), and also had the highest antibiotic resistance against Cefazolin (81.5%) and Co-trimoxazole (62.5%). Due to the frequency of urinary tract infections and to prevent their serious complications, it is necessary to study the pattern of regional antibiotic resistance in medical centers for effective and timely treatment. Therefore, it is recommended that Amikacin and Piperacillin-Tazobactam antibiotics be considered for the experimental treatment of urinary tract infections caused by Enterobacteriaceae.

1. Introduction

Urinary tract infection (UTI) is caused by the presence of pathogenic microorganisms in the urinary tract and is one of the most common infectious diseases that affect human health (Millner et al., 2019). Also, failure to diagnose and treat it in time can have serious

complications. Cause urinary-renal disorders, hypertension, uremia, preterm delivery, and even miscarriage (Byron., 2019). The most common microorganisms that humans face in the development of urinary tract infections include members of the bacterial family

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Enterobacteriaceae. Most members of this bacterial family are facultative anaerobes. These bacteria ferment glucose, reduce nitrate to nitrite, and are oxidase negative. Except for *Shigella* and *Klebsiella*, which are immotile, these bacteria have a peritoneal flagellum that surrounds the bacterium (Hörl, 2011; Rock et al., 2014). Enterobacteriaceae include some of the normal flora of the gastrointestinal tract, such as the small and large intestines. Therefore, they are sometimes called enteric and cause asymptomatic colonization to symptomatic infection and inflammation of the urinary tract. In the acute type, it is classified into two categories: lower duct infection (urethritis and cystitis) and upper duct infection (pyelonephritis and prostatitis) (Rock et al., 2014; Donnenberg, 2014). *Escherichia coli* is one of the most common causes of urinary tract infections and accounts for about 85% of the samples obtained. After *Escherichia coli*, other genera such as *Klebsiella*, *Enterobacter*, and *Proteus* can also cause urinary tract infections (Gupta et al., 2017; Chart., 2012). The incidence of this type of infection in women is higher than in men due to the structure of the ureter and its proximity to the genitals. In fact, the shortness of the urethra and its proximity to the anus in women is one of the main reasons for the increase in urinary tract infections. However, the different anatomical systems of the male urinary tract, along with prostate secretions containing bactericidal substances and cationic proteins, can collectively play an important role in counteracting the invasion of urinary pathogenic bacteria (Johnson et al., 1989; Schollum et al., 2012). Elderly people are also more prone to urinary tract infections due to reduced immune function, the relative reduction in resistance to infections, and the use of invasive treatments such as urinary catheters (Rowe et al., 2014).

Based on the pattern of antimicrobial resistance of infectious pathogens, experimental treatment with broad-spectrum antibiotics is performed (Zalewska-Piątek et al., 2019). Incidence of increased antibiotic resistance along with resistance acquisition mechanisms including resistance gene transfer through horizontal gene transfer (plasmid, bacteriophage), DNA recombination, and chromosomal mutation. Finally, due to the different patterns of antibiotic resistance in different geographical areas or clinical centers,

knowledge of common pathogens and their pattern of antibiotic resistance is very useful and necessary to adopt a practical and targeted treatment strategy (Rozwandowicz et al., 2018; Waller et al., 2018).

This study aimed to determine the frequency of Enterobacteriaceae bacterial family members causing urinary tract infections by gender and different age groups and also their pattern of antibiotic resistance in Valiasr Clinic in Mashhad.

2. Materials and Methods

2.1. Sample Collection:

In this cross-sectional analytical study conducted in a quarterly period in 2022, 2922 samples of urine culture of 3 different age groups of 15-25, 25-45, and 45-65 years in males and females were referred to Valiasr Clinic Laboratory in Mashhad were examined.

2.2 Sample processing method:

Samples were cultured by loop on Blood Agar (HI-Media) India, Eosin Methylene Blue (EMB), or McConkey Agar (Merck) Germany, and positive bacterial growth in Enterobacteriaceae species if bacterial colony grows. Samples with a colony count greater than 10^5 CFU/ml per unit were considered as the target group (Wu., 2006).

Morphological examination and bacterial identification were performed using hot staining and standard biochemical and microbiological laboratory tests. In the case of gram-negative bacteria, tests for the ability to ferment sugars in the culture medium of Triple Sugar Iron Agar (TSI), oxidase, Simmons Citrate Agar (SC), the ability to Motility-produce Indole and Hydrogen Sulfide (SIM), Methyl Red and Voges-Proskauer (MR-VP), Urease and Lysine used (NCCLS)(National Committee for clinical and laboratory standards., 2003; 1999).

2.3 Antibigram Test:

The pattern of antibiotic resistance was determined using the guidelines of the Clinical & Laboratory Standards Institute 2021 (CLSI 2021) by disk diffusion method and using the half McFarland standard and cultivated on Müller Hinton Agar (Merck) Germany. By CLSI

2021 instructions, antibiotic discs prepared by (Padten Teb Company) IRAN, were used as follows; For Enterobacteriaceae, the antibiotic discs Cefotaxime (30 ug), Cefepime (30 ug), Amikacin (30 ug), Cefazolin (30 ug), Gentamicin (10 µg), Nitrofurantoin (300 ug), Co-trimoxazole (25 ug), and Piperacillin-Tazobactam (100/10 ug) were used.

2.4. Statistical analysis:

The findings and data were statistically analyzed by SPSS software version 24. Also, the relationship between qualitative variables was determined using the Chi-square test with a statistically significant level of P -value < 0.05 .

3. Results

Out of 2922 urine samples collected, the result of the culture of 438 cases (about 15%) was positive for Enterobacteriaceae bacteria (Table 1).

Escherichia coli with a frequency of 76.7% had the highest value, followed by *Klebsiella* 14.4%, *Enterobacter* 4.3%, *Proteus* 3% and, other species with a frequency of 1.6% were observed, respectively (Table 1. Abundance distribution of Enterobacteriaceae family bacteria isolated from the urine of patients with urinary tract infection).

Frequency distribution of Enterobacteriaceae bacteria causing urinary tract infections by sex of the statistical population, there was no statistically significant difference (P -value > 0.05).

Out of 438 samples, represented were female and 68 (15.5%) represented were male subjects and, in both sexes, *Escherichia coli* had the highest frequency and prevalence in the cases observed (Table 2. Abundance (Frequency) of Enterobacteriaceae family bacteria by urinary tract infection based on the gender of the statistical population).

Distribution of prevalence and frequency of Enterobacteriaceae bacteria causing urinary tract infections in different age groups of the statistical population showed, that there was no statistically significant difference (P -value > 0.05).

Also, in different age groups of the statistical population, *Escherichia coli* had the highest frequency, and the highest prevalence was observed in the age group of 25-45 years with an abundance of 220 people, and the lowest prevalence was observed in the age group of 15-25 years with an abundance of 48 people (Table 3. Abundance (Frequency) of Enterobacteriaceae bacteria in urinary tract infections in different age groups of the statistical population).

The results of distributing the frequency of antibiotic resistance of Enterobacteriaceae bacteria in urinary tract infections were as follows; *Escherichia coli* (96%), *Klebsiella* (98%), *Enterobacter* (91%) to Cefazolin, and *Proteus* (100%) to Nitrofurantoin, showed the highest level of antibiotic resistance.

Also, *Escherichia coli* (3%) to Amikacin, *Klebsiella* (28%), *Enterobacter* (19%) to Piperacillin-Tazobactam, and *Proteus* (0%) to Piperacillin-Tazobactam, Cefepime, and Amikacin, Showed the lowest level of antibiotic resistance.

Overall, clinical isolates of Enterobacteriaceae causing urinary tract infections had the lowest antibiotic resistance to Piperacillin-Tazobactam (13%), Amikacin (17.2%), Gentamicin (35%), and Cefepime (38.7%). And showed the highest antibiotic resistance to Cefazolin (81.5%), Co-trimoxazole (62.5%), and Cefotaxime (47.2%). As expected, *Proteus* bacteria, according to their inherent resistance, showed 100% resistance to Nitrofurantoin (Table 4.).

Table 1. Differential tests for common types of Enterobacteriaceae that cause urinary tract infections

Type of Bacteria	H ₂ S	Indole	Urease	Lysine	Abundance
<i>Escherichia coli</i>	-	+	-	+	336
<i>Klebsiella</i>	-	-	+	+	63
<i>Enterobacter</i>	-	-	-	+	19
<i>Proteus</i>	+	+	+	-	13

Table 1. Abundance distribution of Enterobacteriaceae family bacteria isolated from the urine of patients with urinary tract infection

Type of Bacteria	Abundance	Frequency
<i>Escherichia coli</i>	336	76.7
<i>Klebsiella</i>	63	14.4
<i>Enterobacter</i>	19	4.3
<i>Proteus</i>	13	3
Other	7	1.6

Table 2. Abundance (Frequency) of Enterobacteriaceae family bacteria by urinary tract infection based on the gender of the statistical population

Gender	<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Enterobacter</i>	<i>Proteus</i>	Other
Female	284 (76.8)	54 (14.6)	16 (4.3)	10 (2.7)	6 (1.6)
Male	52 (76.5)	9 (13.2)	3 (4.4)	3 (4.4)	1 (1.5)

Table 3. Abundance (Frequency) of Enterobacteriaceae bacteria in urinary tract infections in different age groups of the statistical population

Age	<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Enterobacter</i>	<i>Proteus</i>	Other
15 - 25 Y	36 (75)	6 (12.5)	3 (6.2)	2 (4.2)	1 (2.1)
25 - 45 Y	165 (75)	33 (15)	11 (5)	7 (3.2)	4 (1.8)
45 - 65 Y	135 (79.4)	24 (14.1)	5 (2.9)	4 (2.4)	2 (1.2)

Table 4. Distribution of frequency of antibiotic resistance of Enterobacteriaceae bacteria causing urinary tract infection

Type of Bacteria	Piperacillin-tazobactam	Nitrofurantoin	Cefotaxime	Cefepime	Cefazolin	Gentamicin	Amikacin	Co-trimoxazole
<i>Escherichia coli</i>	5	8	58	47	96	25	3	73
<i>Klebsiella</i>	28	35	68	51	98	51	34	69
<i>Enterobacter</i>	19	79	59	57	91	40	32	27
<i>Proteus</i>	0	100	4	0	41	24	0	81

4. Discussion

The highest rate of infection in developed countries is related to urinary tract infections, but the difference in the prevalence of these infections in different countries and even in different parts of a country can be related to differences in lifestyle, economic conditions, and health status of the people in that community (Mohiuddin., 2019; Cardwell et al., 2016).

In this study, 84.5% of patients with this infection were female and 15.5% were male, due to the difference in prevalence between the two sexes and the higher incidence of these

infections in women than men can be due to anatomical differences in the urinary tract such as short length of the urethra, the proximity of the urethra to the anus and vagina, as well as the hot and humid environment of the perineum. Obviously, age-related problems such as; Long-term use of urinary catheters, immobility, incomplete urine discharge, chronic diseases, decreased fluid intake, stones and tumors, urethral stricture, chronic prostatitis, and enlarged prostate in men, who are likely to develop the disease with age, increases it is a predisposing factor and increases urinary tract infections in the elderly (Dielubanza et al., 2011; Coyle et al., 2009). In the study of Baghani Aval

et al., which was performed in 2016 in Sabzevar Vasei Hospital, 59.7% of the 256 samples were female and 40.3% were male (Keikha et al., 2017). In the study of Keikha et al., which was conducted in 2016 at Nabi Akram Hospital in Zahedan, out of a total of 87 samples, 70.2% of the isolates belonged to women and 29.8% from men (Baghani-Aval et al., 2018). Also, in the study by Raeeszadeh et al. in Sanandaj in 2014, it was shown that 96% of patients were women and 4% were men (Raeeszadeh et al., 2016), which was consistent with this study.

Escherichia coli is the predominant pathogen of urinary tract infections as a member of the normal human intestinal flora with the ability to colonize the urinary tract, which facilitates its proliferation by inflammatory reactions that increase bacterial survival (Lüthje et al., 2014; Subashchandrabose., 2015). According to the findings of the present study, the most common pathogens causing urinary tract infections were *Escherichia coli* with a frequency of 76.7% and *Klebsiella* with a frequency of 14.4%, respectively. Similar to this study, in the study by Biset et al., which was conducted in 2017, in northwestern Ethiopia, *Escherichia coli* with a prevalence of 49.2% was the main cause of uropathogenic pathogenicity (Biset et al., 2020). In the study conducted by Mansori et al. In 2015-2016 in Ghaem Hospital of Mashhad, the highest prevalence was reported in *Escherichia coli* isolates (55%) (Mansori et al., 2019). Also, in the study by Guermazi-Toumi et al. conducted in 2015-2016 in southern Tunisia, the highest frequency of bacterial isolates causing urinary tract infections was related to *Escherichia coli* (67.1%) and *Klebsiella pneumoniae* (12.8%) (Guermazi-Toumi et al., 2018).

Since overuse of antibiotics leads to an increase in antibiotic resistance, and given that *Escherichia coli* is the most common cause of urinary tract infections, it is important to know how its antibiotic resistance in any geographical area or clinical center is necessary to begin the treatment process before the outcome of the urine culture is determined. The results of the pattern of antibiotic resistance of *Escherichia coli* in the present study showed that this bacterium showed the highest level of antibiotic resistance to Cefazolin and the lowest level of antibiotic resistance to Amikacin. Also, in total, clinical isolates of Enterobacteriaceae causing

urinary tract infections in the recent study included the lowest levels of antibiotic resistance to Piperacillin-Tazobactam, Amikacin, Gentamicin, and Cefepime. And showed the highest level of antibiotic resistance against Cefazolin, Co-trimoxazole, and Cefotaxime.

In the same way, in the study conducted by Ramezani in 2015 at Resalat Hospital in Tehran, *Escherichia coli* was identified as the main cause of urinary tract infections in women and was highly sensitive to amikacin (Ramezani., 2016). Also, in the study conducted by Rahimi et al. In 2015 to investigate the pattern of antibiotic resistance of *Escherichia coli* strains isolated from patients with urinary tract infections in Isfahan, all *Escherichia coli* strains were sensitive to amikacin (Rahimi et al., 2016). Finally, the study by Al-Naqshbandi et al. in Erbil showed that gram-negative isolates showed significant resistance to Cefepime, which was not consistent with this study (Al-Naqshbandi et al., 2019), and could be due to a different pattern of antibiotic resistance in each geographical area compared to another area.

Conclusion

Overall, the findings of this study showed that the pattern of antibiotic resistance of Enterobacteriaceae causes urinary tract infections due to the development of antibiotic resistance and the use of antibiotics without clinical prescription changes over time, so Controlling its emergence is important. Therefore, it is recommended that clinical administration of Cefazolin and Co-trimoxazole antibiotics be performed to a lesser extent due to the occurrence of a significant amount of antibiotic resistance to them in the initial treatment of urinary tract infections. Finally, more comprehensive clinical statistical research is needed to complete the findings and results of this clinical trial.

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Conflict of interest

There is no conflict of interest between the authors.

Refereces

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