Isolation and determination of antibiotic resistance patterns of urinary tract infections pathogens in patients who referred to Pol-e Dokhtar medical centers during 2016-2018

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Abstract

Background: Urinary tract infection (UTI) is one of the most common human bacterial infections. The frequent use of antibiotics is associated with increased resistance of many bacterial agents to the treatment.

Materials and Methods: A total of 9456 urine specimens were collected from patients referring to Pol-e Dokhtar health centers. All urine samples were inoculated on blood agar plates (BAP) and eosin methylene blue (EMB) agar. Those specimens positive for selected pathogen were finally identified. The antimicrobial susceptibility and resistance patterns of the UTI pathogens to common antibiotics were determined using disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of 9456 investigated urine samples, 1036 samples (10.95%) were positive, of which 823 samples (79.4%) belonged to female subjects and 213 samples (20.6%) belonged to males. The most common identified urine tract pathogens were *Escherichia coli* (69.4%), *Staphylococcus epidermidis* (15.15%) and *Staphylococcus aureus* (7.05%). Also, co-trimoxazole (sulfamethoxazole/trimethoprim) displayed the highest antibiotic resistance (58.1%) and nitrofurantoin (90.1%) and gentamicin (88%) were shown to be the most frequent cause of sensitivity.

Conclusion: The findings of our study demonstrate that *E. coli* is the most common UTI pathogen. Given the high antibiotic resistance of co-trimoxazole, it is recommended to avoid indiscriminate consumption of this antibiotic and perform antibiogram tests for the treatment of UTIs.

Keywords: Urinary tract infection, Antibiotic resistance patterns, Escherichia coli

1. Introduction

Urinary tract infections (UTIs) as a urinary tract inflammatory response against invasion of pathogenic microorganisms (1) are one of the most common bacterial infections and the second most commonly encountered microbial infection following respiratory tract infections in human beings (2-7). Many UTIs are caused by underlying diseases in the urinary system. Host susceptibility, structural urinary tract abnormalities, and pathogenicity of microorganisms are the most important early causes of UTI occurrence and recurrence, and lack of proper diagnosis and treatment of UTIs may lead to chronic and severe renal complications such as renal parenchymal scar, high blood pressure, chronic renal failure, uremia, and some pregnancy complications including preterm labor and abortion in pregnant women (5, 8-10). The annual global incidence of UTIs is estimated as at least 250 million in developing countries (5, 6, 11). Nowadays, Escherichia coli is the most common cause

of UTIs, accounting for 80-90% both male and female cases of UTI (12), in all age groups. It should be noted that due to anatomical differences, younger females are more likely to have an *E. coli* infection (5, 6). *Staphylococcus saprophyticus* is responsible for 5-10% of UTIs. Other bacterial agents involved in UTIs include Klebsiella, Proteus, *Pseudomonas aeruginosa* and Enterobacter (5, 13). The indiscriminate consumption of antibiotics in the treatment of UTIs has increased the resistance to the existing and common antibiotics, resulting in changes in susceptibility pattern of bacteria to different antibiotics over time in various geographical areas (6, 8, 11, 14). As a result, antimicrobial treatment for UTIs should be developed based on information regarding antimicrobial susceptibility and resistance patterns (13). Thus, the present paper was aimed to isolate and determine pattern of antibiotic resistance in UTI pathogens among patients referring to healthcare centers of Pol-e Dokhtar region.

2. Materials and Methods

A total of 9456 urine specimens were collected from patients referring to healthcare centers of Pol-e Dokhtar. All urine specimens were inoculated on blood agar plates (BAP) and eosin methylene blue (EMB) agar using a standard inoculating loop. After 24 hours' incubation of all dilutions at 37°C, samples positive for selected pathogen (samples with colonies equal or higher than 105 CFU/ml were identified using common microbiological methods. For this purpose, we directly prepared lams from specimens inoculated on BAP and EMB agars, and the morphology of the pathogens were determined to identify negative-gram bacteria using Triple Sugar Iron (TSI) agar, Urea agar, Methyl Red (MR) and Voges–Proskauer (VP) test, Sulfide Indole Motility (SIM), and Simmons citrate agar. Also, positive-gram bacteria were identified by using catalase test and for those catalase-positive bacteria the bacterial sensitivity to bacitracin, mannitol fermentation, DNase, coagulase, novobiocin, urease and o-nitrophenyl-Beta-D-galactopyranoside (ONPG) were evaluated to determine the species of the grown bacteria. The Kirby-Bauer disk diffusion procedure was used with Mueller-Hinton agar plate with pH of 2.7-7.7 as a standard antibiogram test to determine antibiotic susceptibility (13). Following bacteria growth on Mueller-Hinton agar, the discs of nalidixic acid, ciprofloxacin, cefixime, gentamicin, co-trimoxazole, cephaloxin, ceftriaxone, nitrofurantoin, amikacin, ampicillin, tetracycline, stiffness zoxime and cephalothin were inoculated on the target media. Then, all dilutions were incubated for 24 hours at 37°C. After incubation, all plates were investigated and diameter of inhibition zone of adjacent antibiotic discs was measured using a standard ruler according to CLSI-2016 guidelines. Data analysis was performed using SPSS, version 20.0.

3. Results

A total of 9456 urine samples were collected and investigated between 2016 and 2018. Results showed that 1036 cases were positive for target pathogen, of whom 823 subjects (79.4%) were females and 213 subjects (20.6%) were males. The average age of the participants was 35 years, ranged from one month to 85 years. The most frequent UTI pathogen was *E. coli* (69.4%), and the least frequent pathogen was Citrobacter in urine samples of the patients. The frequency of the isolated pathogens is shown in figure 1.
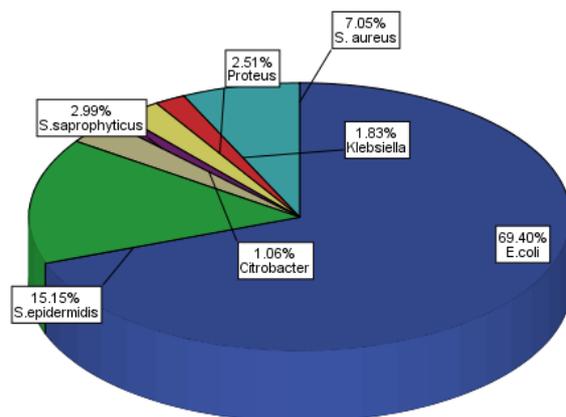

Figure 1. Frequency of bacteria causing UTIs.

According to the results of our study, co-trimoxazole showed the highest resistance rate to UTIs in both genders, and nitrofurantoin (FM) and gentamicin (GM) caused the highest antimicrobial susceptibility of UTI pathogens. The distribution of antibiotic susceptibility of isolated pathogens from the urine samples of UTI patients are listed in Table 1. Also, 21.1% of all UTI patients had kidney stones, and 10.3% had high urinary protein excretion.

Table 1. Antibiogram of bacteria in urine samples of the study subjects

(S,I,R) Antibiotic	Sensitive	Intermediate	Resistan
Nalidixic acid	٪65/6	٪6/2	٪28/2
Co-trimoxazol	٪34/4	٪6/8	٪58
Gentamicin	٪88	٪10/7	٪1/3
Nitrofurantoin	٪90/1	٪5/5	٪4/4
Ceftrixon	٪42	٪22/5	٪35/5
Cefixime	٪42	٪15	٪43
Ciprofloxacin	٪70/1	٪7/2	٪22/7
Cephalexin	٪38/8	٪17/9	٪43/3
Ampicillin	٪33	٪21/7	٪45/3
Ceftizoxime	٪35/7	٪23/3	٪41
Tetracycline	٪35/7	٪28/8	٪37/5
Amikacin	٪47/5	٪30	٪22/5
Cefalotin	٪45/5	٪32	٪22/5

4. Discussion

Urinary tract infections (UTIs) are one of the most common infectious diseases throughout the world. These bacterial infections are a serious health threat to the community, with millions of people becoming infected or hospitalized worldwide each year (4, 10, 15). Escherichia coli is one of the major pathogens of the urinary, responsible for 80-90% of UTIs, and its resistance to common antibiotics has shown a constantly increasing trend (9, 11, 16). The results of this study show that *E. coli* (69.4%) and Staphylococcus epidermididis (15.15%) are the most common causes of UTI, which is in accordance with results of previous studies. For example, Ehsani and colleagues investigated 848 urine samples and found that *E. coli* and Staphylococcus were the most frequent pathogens in all samples. They also stated that UTI was more prevalent among female patients than males, which was in accordance with the results of our study (9). Also, Mohammadi et al collected 4133 samples and stated that *E. coli* and Staphylococcus were the most common pathogens in all samples and women were more likely to develop UTIs, which was consistent with the present study (11). Raiszadeh and colleagues investigated 114 samples and found that *E. coli* and Staphylococcus were most commonly observed pathogens in urine samples of the patients, and UTIs were more prevalent in female patients, which was in accordance with our study (13). Klingeberg and colleagues examined 1245 urine samples, and stated that *E. coli* was the most common pathogens (74.5%) in all samples, which was supported by results of our study (17). Asafo and colleagues also proposed *E. coli* as the most prevalent urine tract pathogen (33.3%), which was in compliance with our study (18). The findings of the present study suggest that co-trimoxazole displayed the highest resistance rate to UTIs in both genders, and nitrofurantoin and gentamicin caused the highest antimicrobial susceptibility of UTI pathogens. As such, Mohammadi and colleagues proposed that *E. coli* as the most common UTI pathogen had the highest and lowest antimicrobial resistance to ampicillin (43.8%) and nitrofurantoin (3.6), respectively (5). Erdem and colleagues introduced *E. coli* (66.6) and Klebsiella pneumonia (16.6%) as the most frequent UTI pathogens and Fosfomycin was most active against *E. coli*, followed by nitrofurantoin (7.4%) (19). In addition, Molana and colleagues stated that nitrofurantoin and ampicillin induced the highest antimicrobial susceptibility and resistance of UTI pathogens, respectively (20). Due to the growing use of antibiotics and the subsequent increase in antibiotic resistance, control of the emergence and spread of resistance is inevitably essential, and effective interventions aimed at reducing improper use of

antibiotics should be established (6). Antibiotic resistance pattern of different pathogens varies in different regions, with an increasing trend in antimicrobial resistance to new antibiotics. All antibiotic resistance patterns have been annually examined every few years to develop more efficient experimental interventions for treatment of UTIs. According to the results of this study, nitrofurantoin is the best antibiotic for the treatment of UTIs caused by specific UTI pathogens. Therefore, nitrofurantoin is recommended by a number of studies for treatment of antibiotic-resistant UTIs.

Acknowledgements

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