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Antibiotic Resistance in Urinary Isolates of *Escherichia coli*

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ABSTRACT

Objectives: The aim of this study was to examine the presence of antimicrobial resistance / susceptibility strains of *Escherichia coli* in inpatients and outpatients. **Materials and methods:** It is a retrospective study carried out at the Department of Microbiology, Parasitology and Virology Faculty of Medicine, University of Sarajevo. In cooperation with the Microbiological laboratory of the Cantonal Hospital Zenica and the Microbiological laboratory of the General Hospital Tesanj, 3863 urine samples were processed in the period from March 1st to March 31st 2016. **Results:** Our study showed that *E. coli* had the highest antimicrobial resistance to trimethoprim / sulfamethoxazole (38.61%), followed by amoxicillin / clavulanic acid (19.62%), ciprofloxacin (9.49%), gentamicin (8.86%), cephalexin (8.23%), nitrofurantoin (8.23%), cefuroxime (7.52%), ceftazidime (6.33%), cefuroxime (89.87%), amikacin (4.43%). **Conclusions:** The isolated strains of *E. coli* showed the highest resistance to trimethoprim / sulfamethoxazole and amoxicillin / clavulanic acid. The isolated strains of *E. coli* showed the greatest susceptibility to amikacin and ceftazidime. Gender distribution of positive *E. coli* isolates showed statistically significant differences in favor of females.

Keywords: E. coli, antimicrobial resistance, susceptibility.

1. INTRODUCTION

Urinary tract infections are, after respiratory, the most common infection in humans (1). At least 80-90% of outpatient and 30-50% of inpatient urinary tract infections is caused by uropathogenic *Escherichia coli (E. coli)* (2). Specific human populations are at increased risk of developing urinary tract infection (UTI). These groups include children, pregnant women, the elderly, postmenopausal women, patients with spinal cord injuries and/or with catheters, patients with diabetes, multiple sclerosis, patients with human immunodeficiency virus (HIV) and patients with previous urological abnormalities (3). In children with a suspected urinary tract infection, the most common management strategy is to treat empirically with an antibiotic while waiting for results of culture and susceptibility testing. Young children are more vulnerable to immediate and long term complications, such as renal scarring and renal failure, and therefore require prompt appropriate treatment. *Escherichia coli* is the most common cause of bacteriaemia and also causes meningitis in neonates (4). Although urinary tract infections can occur in both men and women, it is more common in adult women than adult men.

In addition to frequency, urinary tract infections are also recurrent, and due to the resistance of uropathogenic bacteria to antibiotics, the access to alternative therapeutic procedures is growing (5).

The high frequency of infections, not only leads to major economic costs, but also to a decrease in labor productivity and high morbidity of patients (1). The aim of treatment for acute urinary tract infections is the eradication of pathogens, relief of symptoms, and reduction of the risk of permanent damage to the kidneys. The choice of medicaments for initial, empirical treatment (pending the outcome of susceptibility testing) is based on local sensitivities. The sensitivity of bacteria to antibiotics varies in relation to the geographical region, due to frequent use and misuse (6).

Since the discovery of antibiotics and their widespread use, many bacteria have developed mechanisms that make them resistant to some, but in some cases to almost all antibiotics (7). In Europe, the antimicrobial resistance of Gram-negative bacteria is increasing, especially *E. coli*, which accounts for the majority of invasive Gram-negative strains in European countries (8). Antimicrobial resistance is recognized threat to health, internationally. The contribu-

tion of primary healthcare is particularly important as this is where about 80% of all antibiotics used within the health service are prescribed (4).

Continuing education as well as the implementation of effective measures of activities to prevent and control infections may also reduce the occurrence of resistance (9). The aim of this study was to determine the frequency of outpatient and inpatient urinary tract infections caused by *E. coli* and to examine the presence of antimicrobial resistance / susceptibility strains of *Escherichia coli* in inpatients and outpatients.

2. MATERIAL AND METHODS

During the period from March 1st until March 31st, 2016, 3863 urine samples were tested in the region of Zenica-Doboj Canton, Bosnia and Herzegovina.

Midstream, clean-catch urine samples were sent to the laboratory for standard urinalysis and culture. The tested material was an urine sample of patients with symptoms of urinary tract infections processed in the Microbiological laboratory of the Cantonal Hospital in Zenica and the Microbiological laboratory of the General Hospital Tesanj, Bosnia and Herzegovina.

Bacteriological analysis of urine samples included microscopic identification, cultivation, standard biochemical testing and antimicrobial susceptibility testing. Urine samples were inoculated on blood agar and Endo agar, with incubation of 37°C for 24 hours. After the incubation period, we determined the number of bacteria in 1 ml of urine. Significant number of bacteria in urine (> 10^5 bacteria/ml) were tested to the basic biochemical reactions characteristic for *E. coli*, including double sugar, peptone water, mannitol, urea, and citrate. After the detection and identification of bacteria, we approached analyzing antimicrobial susceptibility by disc-diffusion method according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) standards. For susceptibility testing of Enterobacteriaceae it is used Mueller-Hinton (MH) agar by disc-diffusion method.

In our study, the susceptibility of *E. coli* was tested on the following antimicrobials: amoxicillin / clavulanic acid (AMC) 30 mg (ratio 20:10), cephalexin (CN) 30 mg, cefuroxime (CXM) 30 mg, ceftazidime (CAZ) 30 mg, amikacin (AK) 30 mg, gentamicin (GEN) 10 mg, ciprofloxacin (CIP) 5 mg, nitrofurantoin (NIT) 100 mg, trimethoprim / sulfamethoxazole (STX) 5 mg.

For the statistical analysis we used SPSS software program (Statistical Package for Social Sciences) version 24.0. Results of descriptive statistical analysis were broken down according to frequency and presented in absolute numbers and percentages. Differences between groups were tested by Mann-Whitney U test. The Shapiro-Wilk test was used for testing the significance of differences deviations from the normal distribution. Results are presented in tabular form and graphically, and accepted statistical level of significance difference was $p<0.05$.

3. 3. RESULTS

The research showed that out of 3863 urine culture examined, 452 (11.70%) was positive and 3411 (88.30%) of tested urine culture was negative.

Bacteria	Number and percentage of positive urine culture
Escherichia coli	255 (56.42%)
Proteus mirabilis	60 (13.27%)
Klebsiella pneumoniae	24 (5.31%)
Enterococcus spp	21 (4.65%)
Citrobacter spp	18 (3.98%)
Enterococcus faecalis	14 (3.10%)
Pseudomonas spp	13 (2.88%)
Acinetobacter	9 (1.99%)
Enterobacter spp	9 (1.99%)
Citrobacter freundii	7 (1.55%)
Klebsiella oxytoca	6 (1.33%)
Proteus vulgaris	6 (1.33%)
Escherichia coli ESBL	4 (0.88%)
Streptococcus spp	4 (0.88%)
Morganella morganii	1 (0.22%)
Proteus mirabilis ESBL	1 (0.22%)

Table 1. Distribution of bacteria in positive urine culture

Table 1. shows the frequency of causes for positive urine culture and it can be seen that from a total of 452 samples of positive urine culture, 56.42% of samples were positive on *E. coli*. There were 13.27% of samples with positive urine culture on *Proteus mirabilis*, followed by 5.31% of samples positive on *Klebsiella pneumoniae*, *Enterococcus spp* (4.65%), *Citrobacter spp* (3.98%), *Enterococcus faecalis* (3.10%) and *Pseudomonas spp* (2.88%). Other bacteria had incidence of less than 2% of positive urine culture samples.

Urine cultures positive for *E. coli* (255) were isolated in 158 patients. Out of 158 patients, 123 (77.85%) were outpatients, while 35 (22.15%) were inpatients. Based on gender distribution positive test for *E. coli* was recorded in 142 (89.87%) female patients and 16 (10.13%) male patients.

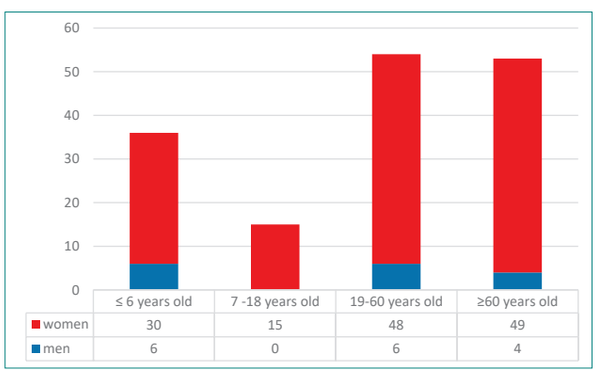

Figure 1. The ratio of gender and age groups of patients with isolated E. coli

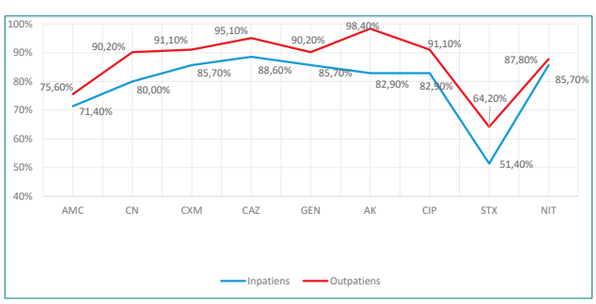

Figure 2. The susceptibility of E. coli strains to antibiotics of inpatients and outpatients

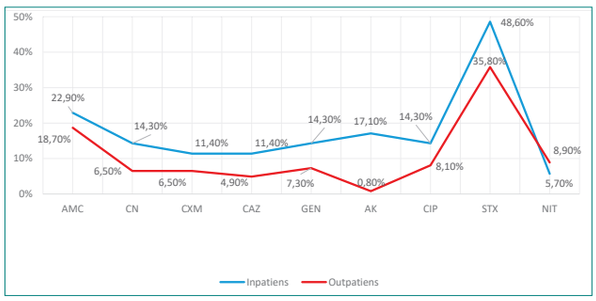

Figure 3. The resistance of E. coli strains to antibiotics of inpatients and outpatients

There was a statistically significant difference in favor of females ($p<0.05$).

Based on the age distribution, patients with isolated *E. coli* were split in four age groups: age 0 to 6, age 7 to 18, age 19 to 60 and age group over 60. It has seen that 54 patients (34.18%) were in the age group 19 to 60 years, 53 patients (33.54%) were in the age group over 60 years, 36 patients (22.78) were in the age group 0 to 6 years and 15 patients (9.49%) were in the age group 7 to 18.

Based on gender distribution in each age group (Figure 1.) There is a significant difference ($p=0.001$). Within females, significantly lowest incidence is in the age group from 7 to 18 years ($p<0.001$). Within males, there are no significant differences by age group ($p=0.751$).

There was no significant difference in susceptibility between the groups any of the antibiotics.

The greatest resistance of *E. coli* strains was observed to trimethoprim / sulfamethoxazole, in both, inpatients (48.60%) and outpatients (35.80%). The lowest resistance of *E. coli* strains of inpatients was observed to nitrofurantoin (5.70%), while the lowest resistance of *E. coli* strains of outpatients was observed to amikacin (0.80%). Amikacin resistance in inpatients amounted to 17.10%, while in outpatients was 0.80%, therefore there was a statistically significant difference ($p <0.001$).

4. DISCUSSION

Urinary tract infections are one of the most common bacterial infection that can affect the bladder, urethra or kidneys. *Escherichia coli*, as gram-negative bacteria, is the dominant cause and can be easily grown in the laboratory.

E. coli causes more than 80% of urinary tract infections in previously healthy women. First, there is fecal contamination of periurethral area, then the bacteria spreads on ascending through the bladder and causes cystitis. These infections of the lower urinary tract, in some cases, can affect the kidneys and cause acute pyelonephritis, which consequently may result in bacteremia and sepsis (10).

According to our study, of 158 patients with *E. coli* 142 (89.87%) patients were female and 16 patients (10.13%) were males. There was a statistically significant difference in favor of females ($p <0.05$). Malmartel (11) also in its research conducted in France proved the prevalence of female gender, where 86.4% of *E. coli* positive urine culture were women. The reason for this may be that, anatomically, women have a shorter urethra which facilitates the ascending spread of bacteria (5).

By examining the frequency of *E. coli* in hospital and outpatients, we came to the result that 77.85% were outpatients and 22.15% were hospital patients. Sahuquillo-Arce et al. (12) with his study in Spain, which included 25 microbiological laboratories also proved that the isolates from the community are more common than the hospital ones.

Treatment of urinary tract infections is becoming more complicated with an increase of the number of resistant strains to antibiotics and prevalence of antibiotic resistance mechanisms. The majority of strains of *E. coli* were identified as resistant to the antibiotics such as ampicillin, amoxicillin / clavulanic acid, norfloxacin, cefuroxime, ceftriaxone, and trimethoprim/sulfamethoxazole (13). The emergence of antibiotic resistance is a major threat to public health, which is driven by excessive use of antibiotics. Antibiotics are among the most frequently prescribed medications in hospitalized patients, which are often prescribed inappropriately. The positive urine cultures are the trigger for antibiotic therapy in hospitalized patients. The guidelines recommend avoiding antibiotic therapy in bacteriuria in the absence of symptoms, with a few exceptions, such as pregnancy. Despite the recommendations, antibiotic treatment is prevalent and every day contributes to increasing antibiotic resistance, increased costs, and antibiotic side effects, such as *Clostridium difficile* infection (14).

According to the results of our study, *E. coli* strains showed the highest antimicrobial susceptibility to amikacin (94.94%) and ceftazidime (93.67%). The sensitivity of *E. coli* to cefuroxime was 89.87%, ciprofloxacin 89.24%, gentamicin 89.24%, cefalexin 87.97%, nitrofurantoin 87.97%, amoxicillin / clavulanic acid 74.68%, trimethoprim / sulfamethoxazole 61.39 %, indicating that the *E. coli* is significantly sensitive to all tested antibiotics ($p<0.01$; $p <0.05$).

The greatest resistance strains of *E. coli* to antibiotics in our study showed trimethoprim/sulfamethoxazole (38.61%) and amoxicillin / clavulanic acid (19.62%). *E. coli* resistance to ciprofloxacin was 9.49%, gentamicin 8.86%, cephalexin 8.23%, nitrofurantoin 8.23%, cefuroxime 7.52%, ceftazidime 7.52% and amikacin 4.43%.

In research conducted at the Department of Urology of the University Clinical Centre in Sarajevo, the highest level of resistance of *E. coli* found on trimethoprim / sulfamethoxazole (55%) and ampicillin (53%), followed by ciprofloxacin 17%, amoxicillin / clavulanic acid and gentamicin 16.6% (15).

The in vitro activities of trimethoprim / sulfamethoxazole and amoxicillin / clavulanic acid found in our study suggest that they would provide adequate alternative therapy in locations where trimethoprim / sulfamethoxazole use is no longer prudent because of elevated (>10 to 20%) rates of resistance.

Bacteria have developed mechanisms of genetic adaptation, and a result of the use of antibiotics is always faster or slower development of resistance. Because of the increase in bacterial resistance to antibiotics it is necessary to consistently monitor and be aware of resistance rates for specific pathogens in their own environment. If the resistance to the antibiotic is higher than 20%, the antibiotic should not be prescribed in an empirical antimicrobial therapy (16). The current global threat of antimicrobial resistance, an urgent need for it to be controlled, and the discovery of

new antibacterial products has prompted many scientists to take measures such as rational use of antibiotics, infection control in health care, the formation of a strategy to reduce risk factors in the environment, the development of rapid diagnostic tests, promoting research on the prevention and control of antimicrobial resistance, the development of new antimicrobial and antibacterial agents strategies, improving awareness of population on the use of antibiotics and the risk of increasing their resistance in order to prevent the development and spread of resistance to antibiotics in the world (17). It is not a new problem, but it is becoming increasingly dangerous and requires urgent investment of effort and resources into its resolution (18).

5. CONCLUSIONS

Our research has shown that the largest number of *E. coli* isolates was found among outpatient population. Considering the highest resistance percentages of trimethoprim / sulfamethoxazole and amoxicillin / clavulanic acid, in both, outpatients and inpatients, we can conclude that these agents are not suitable for the empirical treatment of urinary tract infections. From our study, it is evident that unbiased surveillance of pathogens is important for antimicrobial decision-making. Unfortunately, such a database is nonexistent in Zenica-Doboj Canton, as in other Cantons of Bosnia and Herzegovina. The results of our study indicate the existence of a great need for prevention of urinary tract infections rational use of antibiotics, as well as the multidisciplinary approach to further control the development of resistance.

- **Author's contribution:** Mufida Aljicevic and Velma Rebic – collecting data from primary care and hospital and processing to Excel. Sabina Mahmutovic-Vranic – collection of literature on antibiotic resistance. Kadrija Abduzaimovic and Sabina Sestic – collecting medical history and send samples to the laboratory.
- **Declaration of interest:** There is no conflict of interest.

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