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Antimicrobial Susceptibility/Resistance of *Escherichia coli* among the Outpatients with Urinary Tract Infections in Mostar

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Abstract

Urinary tract infections (UTIs) are considered to be the most common bacterial infection. *Escherichia coli* is the most common uropathogen in uncomplicated upper and lower urinary tract infections (70–95% of cases). The aim of this study was to examine the presence of antimicrobial susceptibility/resistance among *E. coli's* strains in outpatients. This retrospective study was carried out from January1st to December 31th 2017, at the Department of Microbiology, Faculty of Medicine, University of Sarajevo, in cooperation with the Microbiological laboratory of the Cantonal Hospital „Dr. Safet Mujić", Mostar. During the research, a total of 3 148 urine samples of outpatients were examined. Our study showed that *E. coli* had the highest resistance to ampicillin (58%), followed by trimethoprim-sulfamethoxazole (38.4%) and amoxicillin-clavulanic acid (38.4 %), and the lowest resistance to nitrofurantoin (2.7%) and cefuroxime (4.1%). The isolated strains of *E. coli* showed the highest resistance to ampicillin, and the highest susceptibility to nitrofurantoin. Gender distribution of positive *E. coli* isolates showed statistically significant differences in favor of females.

Keywords: *E. coli*, urinary tract infections, resistance, susceptibility, infection, uropathogen.

Резюме

Инфекциите на пикочните пътища (ИПП) са сред най-разпространените бактериални инфекции, а *Escherichia coli* е най-често срещаният уропатоген при неусложнени инфекции на горните и долните пикочни пътища (70–95% от случаите). Целта на настоящото проучване е да се изследва наличието на антимикробната чувствителност/резистентност сред щамовете на *E. coli*, изолирани от амбулаторно болни. Това е ретроспективно проучване, проведено от 1 януари до 31 декември 2017 г. в Катедрата по микробиология при Медицинския факултет на Университета в Сараево в сътрудничество с Ммикробиологичната лаборатория на кантоналната болница „Д-р Сафет Муджич" в Мостар. Изследвани са общо 3148 проби урина от амбулаторни пациенти. Резултатите показват, че *E. coli* има най-висока резистентност към ампицилин (58%), следвана от тази към триметоприм-сулфаметоксазол (38.4%) и амоксицилин-клавулонова киселина (38.4%), а най-ниската резистентност - към нитрофурантоин (2.7%) и цефуроксим (4.1%). В същото време те показват най-висока устойчивост на ампицилин и най-висока чувствителност към нитрофурантоин. Разпределението по пол на положителните изолати на *E. coli* показа статистически значими разлики в полза на жените.

Introduction

Urinary tract infections (UTIs) are the most common infections worldwide accounting for nearly 25% of all infections. There are different etiological agents, but *Escherichia coli* is the most common, with a frequency of 70 to 95% (Flores-Mireles *et al*., 2015). Relapses and recurrent infections are very common. Strains of *E. coli* that cause UTI act as opportunistic intracellular pathogens that exploit the sensitivity of the host using the spectrum of various virulence factors for the colonization of the urinary tract (Foxman, 2003). By entering into the urinary tract of the host, uropathogenic strains of *E. coli* (UPEC) generally

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colonize the bladder mucous membrane causing cystitis. Ascending spread through the ureter reaches the kidney and can lead to the development of pyelonephritis. UPEC ability to bind to host tissue is one of the most important factors that facilitate the colonization of the urinary tract, allowing the bacteria to withstand the higher flow of urine and support cell invasion (Eto *et al*., 2007).

Most urinary infections are caused by the ascending spread of microorganisms through the urethra, although some microorganisms can reach the urinary tract through the blood and lymph (Krkić-Dautović, 2011). The largest number of urinary infections is caused by a single pathogen. In 70-95% of cases, the causative agent is *E. coli*, and in 5-10% of cases the cause is *Staphylococcus saprophyticus*, and slightly less *Proteus mirabilis* and *Klebsiella spp*. In complicated infections, strains of *Pseudomonas*, *Staphylococcus*, *Serratia* and *Providencia* may also be isolated (Dąbrowski *et al*., 2016). Urinary tract infections can range from asymptomatic bacteriuria, which is characterized by the presence of bacteria in the urine in the absence of symptoms, cystitis, in which the infection is restricted to the bladder, and pyelonephritis, in which the kidneys are involved (Bauman, 2015). It is common that cystitis subsides without consequences, while pyelonephritis can cause permanent damage and even lead to death (Marrs *et al*., 2005; Barišić 2011). Around the world, antimicrobial resistance among UPEC infection is a major health problem because of the increasing development of resistance to different classes of antibiotics. Bacteria have developed excellent mechanisms of genetic adaptation and the consequences of the use of antibiotics are the development of bacterial resistance to them. In the treatment of infection, antibiotics do not distinguish the pathogenic bacteria that cause the infection from the non-pathogenic bacteria of the normal flora, so resistance also develops into bacteria of the normal flora, thus creating reservoirs of the resistance gene in nature (Brumbaugh and Mobley 2012; Sanchez *et al*., 2012; Al-Badr and Al-Shaikh 2013; Mohseni *et al*., 2013; Picozzi *et al*., 2014). Antibiotics remain, despite antibiotic resistance, crucial drugs in medicine, where their use reduces child mortality and extends life expectancy. New bacterial resistance mechanisms are constantly being described, and new ways of transferring resistant genes are discovered daily. Due to the insufficient development of new antibiotics and the increasing levels of resistance, many countries have become aware of the problem and therefore, at the request of the Council of the European Union, the fight against bacterial resistance to antibiotics is one of the priorities of the World Health Organization (Paphitou, 2013).

Material and Methods

The study included 3148 urine samples of outpatients collected at Hospital "Dr. Safet Mujić", Mostar, of which 295 samples were found positive for *E. coli*. The research was conducted in the period from January 1st to December 31th 2017.

Bacteriological analysis of urine samples included cultivation, standard biochemical testing and antimicrobial susceptibility testing. Midstream, clean-catch urine samples were processed in the laboratory for standard urine analysis and culture. Urine samples were inoculated on blood agar and Endo agar, with incubation at 37°C for 24 hours. Significant numbers of bacteria in urine ($>10^5$/ml) were tested with the basic biochemical reactions characteristic of *E. coli*, such as double sugar, peptone water, mannitol, urea, citrate and 10% lactose.

Susceptibility testing of isolates to antibiotics and interpretation of the results was carried out according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) standards. Antimicrobial susceptibility/resistance of *E. coli* isolates was tested by the disc-diffusion method. The pre-prepared suspension of the tested strain was inoculated onto the surface of Mueller-Hinton (MH) agar, after which the following antibiotic discs were applied: ampicillin (AMP) 10 mg, amoxicillin-clavulanic acid (AMC) 20/10 mg, gentamicin (GARA) 10 mg, nitrofurantoin (FURA) 100 mg, trimetoprim-sulfometoksazol sulfametoksazol (TSH) 1.25-23.75 mg, cefazolin (CZ) 30 mg, cefuroxim (CXM) 30 mg, ciprofloxacin (CIP) 5 mg, norfloxacin (NOR) 10 mg, ceftriaxone (CRO) 30 mg, moxifloxacin (MOX) 5 mg, cefotaxime (CTX) 5 mg, ceftazidime (CAZ) 10 mg, levofloxacin (LEV) 5 mg, meropenem (MRP) 10 mg, imipenem (IMP) 10 mg, tobramycin (TOB) 10 mg, amikacin (AN) 30 mg, piperacillin (PIP) 30 mg, piperacillin/tazobactam (PIP/IT) 30/6 mg, cefepime (FEP) 30 mg and fosfomycin (FOSF) 30 mg.

For the statistical analysis we used SPSS software program (Statistical Package for Social Science version 23.0).

The results were shown by the number of cases, percentage, arithmetic means with standard deviation and range values. Differential testing was done using the chi-square and Student's t=test. The results of these tests were considered statistically significant at a confidence level of 95% or with $p < 0.05$.

Results

The research showed that out of the 3148 urine cultures examined, 295 (9.40%) were positive and 2 853 (90.60%) tested negative. The incidence of *E. coli* in the observed period was 9.37%. Table 1 gives data based on gender distribution, positive test for *E. coli* was recorded in 274 (92.9%) female patients and 21 (7.1%) male patients. There was a statistically significant difference in favor of females ($p < 0.05$). Higher incidence of positive female patients is evident.

Table 1. Gender structure

Gender	N	%
Male	21	7.1
Female	274	92.9
Total	295	100

$\chi^2 = 16{,}980$; $p = 0{,}0001$

As seen in Table 2 and Fig. 1, the average age of the observed sample was 55.9 ± 23.8 years with the youngest patient aged 1 and the oldest aged 95 years old.

Table 2. The age of patients

Age		
N	Correct	263
	Missing	32
Average		55,9316
Std. deviation		1,46495
Median		63,0000
Std. deviation		23,75742
Minimum		1,00
Maximum		95,00

T=38,180; p=0,0001

Statistical analysis by Student's t-test shows that there is a significant ($p < 0.05$) deviation from the expected distribution in terms of greater representation of older patients - 60 years and older.

The etiological agents of urinary tract infections are different. Table 3 clearly shows that *E. coli* was isolated in 275 cases (93.2%), but in 20 cases (6.8%) patients with *E. coli* had another cause of infection (co-infection). The second most common isolate was *Proteus mirabilis* with 11 (3.7%), followed by *Pseudomonas aeruginosa* with 4 (1.4%), *Klebsiella pneumoniae* with 3 (1%), and *Enterobacter clocae* and *Citrobacter diversus* in 1 case (0.3 %).

Table 3. Presence of co-infection

Co-infection		
	N	%
E. coli	275	93,2
E. coli + C. diversus	1	0,3
E. coli + E. cloacae	1	0,3
E. coli + K. pneumoniae	3	1,0
E. coli + P. aeruginosa	4	1,4
E. coli + P. mirabilis	11	3,7
Total	295	100,0

Figure 2 shows the results of the antibiotic resistance of *E. coli* in non-hospitalized patients' samples tested throughout the research period.

The highest resistance of *E. coli* strains was observed to ampicillin (58%). Out of a total of 5 antibiograms, resistance to moxifloxacin (MOX) was observed in 80%, however due to the small number of performed antibiograms the resistance is not statistically significant. A slightly lower resistance was manifested to trimethoprim-sulfamethoxazole (38.4%),

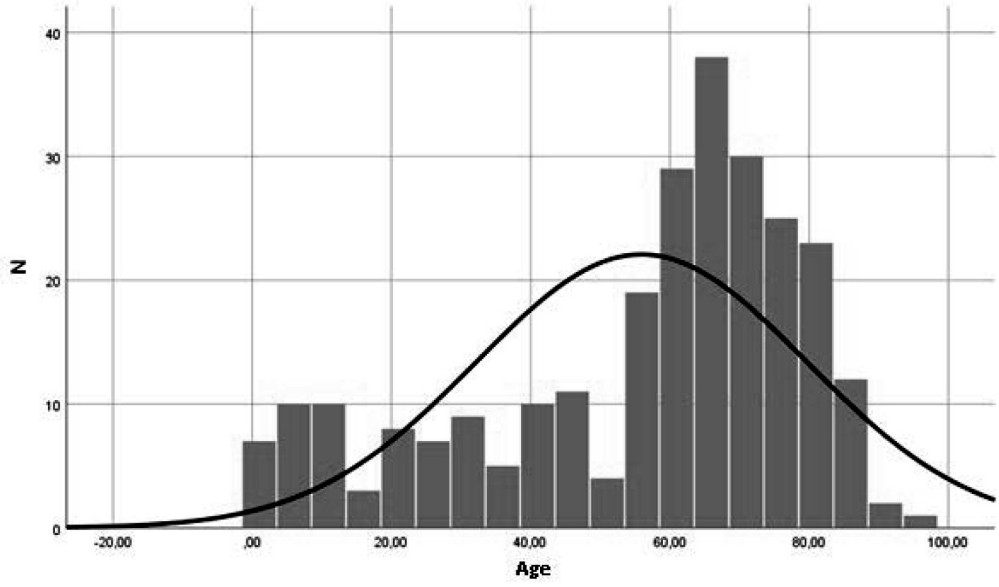

Fig. 1. The age of patients

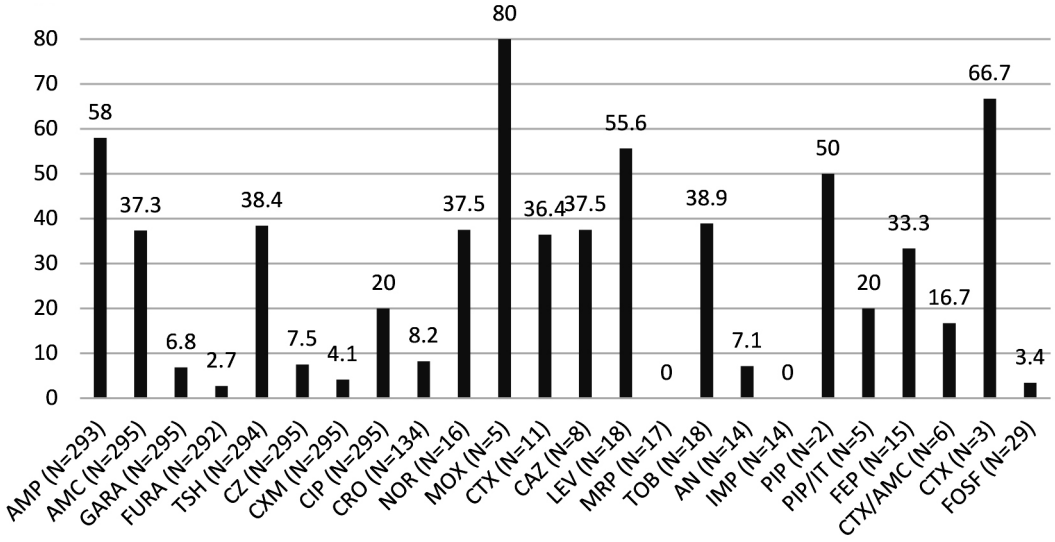

Fig. 2. The resistance of *E. coli* strains to antibiotics

followed by amoxicillin-clavulanic acid (37.3%). The lowest resistance of *E. coli* was observed to nitrofurantoin (2.7%) and cefuroxime (4.1%).

Discussions

Urinary tract infections are the most frequent acute bacterial infections, mostly in women. Females are more susceptible to UTI, because women have a shorter urethra, which enables the ascending spread of bacteria (O'Brien *et al*., 2015).

Bacteria may trigger inflammation and pain in any or all of the urinary tract, including the urethra, urinary bladder, or kidneys – conditions called urethritis, cystitis, and pyelonephritis (Bauman, 2015). A major problem is the inadequate use of antibiotics, which results in increased incidence of antibiotic resistance. This is a serious problem that leads to the development of severe urinary infections. UTI is the most common reason for prescribing antibiotics in primary care (Masajtis-Zagajewska and Nowicki, 2017). During our study, 3148 urine samples of outpatients were tested, of which 296 were positive for *E. coli*, whereas 2852 samples were negative. In terms of gender, there were more positive samples in women 274 (92.9%) than men (21.1%). There was a statistically significant difference in favor of females ($p < 0.05$). Wadekar and Sathish (2017) in their research conducted in India also proved the prevalence of the female gender (75%). A similar result was obtained in a cross-sectional study conducted in Pakistan, where of the 458 positive urine samples, 69.2% came from female patients (Ali *et al*., 2017).

In our study, co-infection was recorded in 20 (6.8%) cases. The most common other isolate was P. mirabilis (3.7%), followed by *P. aeruginosain* (1.4%), *K. pneumoniae* (1.0%), and *C. diversus* (0.3%). Abduzaimovic *et al*. (2016) and Mahmutovic *et al*. (2017) in their separate studies in Bosnia had similar results regarding co-infection: *Proteus* spp. 13.27%/9.83%; *Pseudomonas* spp. 2.88% / 2.45%; *Klebsiella pneumoniae* 5.31%/1.63%.

Our research shows that the highest resistance of *E. coli* was manifested to ampicillin (58%). A slightly lower resistance was exhibited to trimethoprim-sulfamethoxazole (38.4%), followed by amoxicillin-clavulanic acid (37.3%). The lowest resistance of *E. coli* was observed to nitrofurantoin (2.7%). Hegazy *et al*. (2018) in their study in Egypt proved the highest resistance of isolated strains of *E. coli* to ampicillin (100% of the total 98 antibiograms), cefazolin (100%) and trimethoprim-sulfomethoxazole (87.9%). The results of our research on the resistance to these antibiotics are consistent with this study. In the same study, the highest susceptibility of isolated strains showed amycotine (77.55%), imipenem (76.53%), nitrofurantoin (77.5%) and gentamicin (71.43%). In a retrospective analysis of the antimicrobial susceptibility of *E. coli*, followed over 11 years in Ireland, ampicillin and trimethoprim were observed to be the least effective in therapy. The resistance rates for ampicillin and trimethoprim were 60.8 or 31.5%, respectively, andfor trimethoprim, amoxicillin-clavulanic acid, cefuroxime, and gentamicin significant trends of increasing resistance were identified over the 11-year period (Cullen *et al*., 2012). The results of the research conducted by Abduzaimovic and colleagues (Abduzaimovic *et al*., 2016) in Bosnia showed *E. coli* susceptibility to trimethoprim sulfamethoxazole was 38.61%, amoxicillin-clavulanic acid 19.62%, ciprofloxacin 9.49%, gentamicin 8.86%, cephalexin 8.23%, nitrofurantoin 8.23%, cefuroxime 7.52%, ceftazidime 6.33%, amikacin 4.43%. Yilmaz *et al*. (2016) in Turkey conducted a study of *E. coli* susceptibility to antibiotics and ob-

tained the following results: ampicillin 66.9%, cefazolin 30.9%, cefuroxime 30.9%, ceftazidime 14.9%, cefotaxime 28%, cefepime 12%, amoxicillin-clavulanic acid 36.9%, trimethoprim-sulfamethoxazole 20%, ciprofloxacin 49.9%, amikacin 0.3%, gentamicin 24%, nitrofurantoin 0.9% and phosphomycin 4.3%. In Croatia, in the period from 2003 to 2007, tested *E. coli* strains showed the highest resistance to amoxicillin ranging from 37% to 41%. High resistance was also noted for trimethoprim-sulfamethoxazole, ranging from 19% to 30% (Farkaš *et al*., 2008). In a retrospective analysis done in India (Shanthi, 2018), isolated *E. coli* strains showed the highest resistance to cotrimoxazole (75.8%) and cefotaxime (78.27%). The highest sensitivity was observed for nitrofurantoin (85.89%) and amikacin (68.89%). From the above studies it can be concluded that the strains of *E. coli* showed the greatest resistance to the compositions of penicillin (β-lactam antibiotics), and trimethoprim-sulfametaxazolu cephalosporins 1 and 2 generation. Resistance to penicillin preparations in all studies, as well as in our study, is the most pronounced. In our study, there was no marked resistance to cephalosporins first and second generation (cefazolin and cefuroxime), which was the case with the above studies. The highest sensitivity of *E. coli* in those studies was observed in aminoglycoside antibiotics, nitrofurantoin and carbapenems. The highest sensitivity in our research is to nitrofurantoin and cefuroxime.

Conclusions

Considering the increasing problem of bacterial resistance to antibiotics all over the world, it is extremely important to know how susceptible certain causal agents are in order to approach the therapy as rationally as possible. The results of our study suggest the necessity of rational use of antibiotics and the need for multi-disciplinary control of the development of resistance. Monitoring the shift in antimicrobial susceptibility of *E. coli* is important because of the rational prescription of antibiotics in the treatment of urinary tract infection, with the aim of controlling the current level of resistance and making etiological diagnosis.

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