DOI: 10.5455/msm.2016.28.121-124
Received: 03 January 2016; Accepted: 28 February 2016
Published online:25/03/2016 Published print:04/2016

ORIGINAL PAPER
Mater Sociomed. 2016 Apr; 28(2): 121-124

ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM URINE AT OUTPATIENT POPULATION: A SINGLE LABORATORY EXPERIENCE

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ABSTRACT

Objectives: The aim of this study was to examine antimicrobial resistance of Escherichia coli strains isolated from urine in outpatient population. **Material and methods:** We performed a retrospective study for tree months period, between January 1st and March 31st, 2015, at the Department of Microbiology and Parasitology, Faculty of Medicine, University of Sarajevo. We determined the E. coli antimicrobial resistance in 556 first urine samples from outpatient population of Hrasno community in Sarajevo, Bosnia and Herzegovina. E. coli is the most frequent agent causing urinary tract infections in outpatients as well. The standard methods of descriptive statistics were performed for data analysis. **Results:** We observed the highest antimicrobial resistance of E. coli for ampicillin (82,79%), followed by trimethoprim-sulfamethoxazole (40,86%), nalidixic acid (19,35%), cephazolin (7,52%), nitrofurantoin (5,37%), gentamicin (2,15%) and ciprofloxacin (4,30%). **Conclusions:** The results of study showed that E. coli has the highest resistance to ampicillin and trimethoprim-sulfamethoxazole in outpatient population of Hrasno community.
Key words: Escherichia coli, resistance, antibiotics, antimicrobial susceptibility.

1. INTRODUCTION

Urinary tract infections are the most common bacterial infections in population of all ages and also one of the most common reasons of antimicrobial prescribing in the general practice. Bacterial infections caused by gram negative bacterial strains are increased in the last fifty years, especially those that are caused by *Enterobacteriaceae*. *Escherichia coli* (*E. coli*) causes 80% of community-acquired infections while some strains show resistance as a result of wide and frequent inappropriate use of antibiotics (1). According to the results of the National Ambulatory Medical Care, urinary tract infections are the reason of approximately seven million visits to the general practitioners all over the world (2). These infections are more often in women of reproductive age by 30-50% than in men of the same age (3).

The main aim of the urinary tract infections therapy is disappearance of clinical symptoms and eradication of infection in order to prevent recurrence. It is necessary to treat all asymptomatic urinary infections and bacteriuria as well, applying the least toxic, and also the least expensive antimicrobial drug in appropriate doses and sufficiently long period (4).

Antimicrobial resistance is a major current problem. The frequency of resistance to antibiotics is correlated with their use (5).

Because of the increase of bacterial resistance to antibiotics is necessary to constantly monitor resistance rates for specific pathogens in their own environment. For each drug-resistant bacteria, there are wide variations between countries that range from 1% to more than 50% of resistant isolates (6). Number of mortality associated with bacterial resistance to certain antibiotics in the European Union is estimated at 25.000 per year, with two-thirds related to gram-negative bacteria (7). Treatment of urinary infections begins empirically, before the agent identification and susceptibility testing. The choice of antibiotics for further treatment depends on the cause and its sensitivity to certain

antibiotics. If the resistance to a particular antibiotic is higher than 20%, that antibiotic should not be prescribed in the empirical antimicrobial treatment (8).

E. coli resistance to major antibiotics is increasing in almost all countries in Europe (6-8). Monitoring the range of antimicrobial susceptibility of *E. coli* to antibiotics is important for the rational prescribing of antibiotics to treat infections of the urinary system, with the aim of controlling the current level of resistance and improve the etiological diagnostics (9). For this reason, in the world there are a plenty of organizations and committees, such as the CDC, ECDC and WHO, which follow the profile of antimicrobial resistance and with their activities try to reduce this global problem.

2. MATERIAL AND METHODS

This study included 556 urine samples which were collected and analyzed in the period between January 1st and March 31st, 2015, at the Department of Microbiology and Parasitology, Faculty of Medicine, University of Sarajevo. We tested *E. coli* antimicrobial resistance in the first urine sample at outpatient population of Hrasno community in Sarajevo, Bosnia and Herzegovina. In the study, isolation and identification of pathogens were performed routinely, which were taken over several days. In the laboratory, samples were tested using standard methods of diagnostics that includes: microscopic identification, cultivation, biochemical and serological tests, as well as examining the profile of sensitivity/resistance to an appropriate antibiotic. All samples were obtained and cultivated on blood and McConkey agar, and were incubated in aerobic conditions at 37°C for 24 hours. *E. coli* was identified using standard biochemical tests.

Testing the resistance of *E. coli* to antibacterial drugs was done using disk diffusion method, according to Kirby-Bauer on Muller-Hinton agar (Himedia, India) by CLSI standards (Clinical and Laboratory Standards Institute) (10). This method is one of the oldest approaches to antimicrobial susceptibility testing. It is based on the principle of diffusion of antibiotics/chemotherapeutics in the disc or tablet environment and growth inhibition of inoculated bacteria. The following antibiotics were tested: ampicillin (10μg), cephazolin (30μg), gentamicin (10μg), ciprofloxacin (5μg), nalidixic acid (30μg), nitrofurantoin (300μg), and trimethoprim-sulfamethoxazole (1.25μg/23.75μg) (Becton-Dicinson, USA).

Results were analyzed using standard statistical methods, using the computer program Excel (Microsoft Excel, 2003) and SPSS computer program for statistical analysis (SPSS Statistical Package for Social Sciences) version 13.0. Results are presented as absolute numbers (N) and percentage values (%). For nominal and ordinal variables was used chi-square test. Results are presented in tables and graphs. P value ($p < 0.05$) was considered statistically significant.

3. RESULTS

Out of all tested, 72.84% were women (N = 405) while men was 27.16 % (N= 151). The results showed that out of total number of 556 tested samples of urine, 141 were positive (25.36%), while 415 were negative (74.64%) (Table 1). There was a statistically significant difference in favour of negative urine samples ($p < 0.05$).

Isolate	N	%
Positive urine cultures	141	25.36
Negative urine cultures	415	74.64
Total	556	100.00

Table 1. The ratio of positive and negative urine cultures. $\chi^2 = 92.516$; $p = 0$.

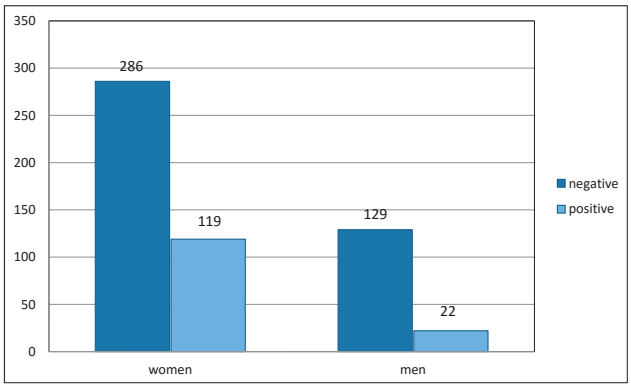

Figure 1. Gender distribution according to the number of positive/negative samples

Out of 405 samples from the women population, 119 (29.4%) were positive while 286 (70.6%) were negative (Figure 1). When it comes to the men population, out of all 151 samples, positive findings were detected in 22 (14.6%), while the negative results were recorded in the other 129 (85.4%). There was a statistically significant difference in favour of negative urine samples ($p = 0.00035$).

Isolated bacteria	Frequency
Echerichia coli	93
Proteus spp.	17
Enterococcus faecalis	11
Pseudomonas spp.	7
Enterobacter spp.	6
Klebsiella spp.	3
Acinetobacter	2
Streptococcus spp.	2

Table 2. Distribution of isolated bacteria at outpatient population

Out of all positive samples, the most frequent pathogen was *E. coli*, with prevalence 0.17 (n=93, 65.95%), followed by *Proteus spp.* (n=17, 12.05%), *Enterococcus faecalis* (n=11, 7.80%) and *Pseudomonas spp.* (n=7, 4.96%) (Table 2). In 6 samples (4.25%), *Enterobacter spp.* (n=6, 4.25%), and *Klebsiella spp.* (n=3, 2.12%) were isolated. The lowest frequency was observed in *Acinetobacter* and *Streptococcus spp.* There were isolated in 2 samples (1.41%). There was a statistically significant difference in terms of the distribution of isolated pathogens ($p < 0.05$).

Out of 93 positive samples of *E. coli*, 83 samples (89.24%) were isolated in women (Table 3). There was a

	Men		Women	
Isolated *E. coli* strains	N	%	N	%
	10/93	10.75	83/93	89.24

Table 3. Presence of E. coli strains at outpatient population with positive urine cultures. $\chi 2 = 41.0081$; $p = 0$.

Tested antibiotics	R		S		I	
	Number	%	Number	%	Number	%
Ampicillin	77/93	82.79	14/93	15.05	2/93	2.15
Cefazolin	7/93	7.52	69/93	74.19	17/93	18.27
Gentamicin	2/93	2.15	77/93	82.79	14/93	15.05
Ciprofloxacin	4/93	4.30	89/93	95.69	0/93	0.0
Nalidixic acid	18/93	19.35	75/93	80.64	0/93	0.0
Nitrofurantoin	5/93	5.37	88/93	94.62	0/93	0.0
Trimethoprim-sulfamethoxazole	38/93	40.86	55/93	59.14	0/93	0.0

Table 4. Antimicrobial resistance of E. coli strains isolated from urine at outpatient population in Hrasno community, Sarajevo. $\chi 2 = 341{,}53$; $p < 0{,}005$.

statistically significant difference in favour of women ($p < 0.05$). Table 4. showed profile of antimicrobial resistance of *E. coli* strains isolated from urine at the outpatient population in Hrasno community in Sarajevo.

4. DISCUSSION

Results of our research showed that *E. coli* had major antimicrobial resistance to ampicillin and trimethoprim-sulfamethoxazole, while minor resistance to gentamicin. Also frequency of *E. coli* and other bacteria was higher in women.

Excessive use of antibiotics, especially in situations when it is not justified, presents the most important factor that contributes to the development of bacterial and it is also one of the major global public health problems (11). Antibiotics are a group of effective and commonly used drugs. Unfortunately, bacteria have developed extremely genetic mechanisms of antibiotic resistance. Infections of the urinary tract are one of the most common bacterial infections and one of the most common reasons for prescribing antimicrobial drugs (2).

E. coli is the most common cause of uncomplicated and community acquired urinary tract infections. However, as a dominant pathogen primarily occurs in uncomplicated urinary tract infections of young women, where constitutes at least 80% of the agents (12).

In the last fifteen years, in Europe and North America, scientific studies were conducted in order to investigate and monitor the occurrence of resistance to antimicrobials. Firstly, the appearance of resistance is monitored for certain bacterial groups, such as family *Enterobacteriaceae*. The resistance of bacteria isolated from this family have been occurring in 30% of isolated strains since 2001, with a tendency to increase (13).

Out of 556 first urine samples which were tested, 141 were positive. Results pointed to a higher incidence of urinary tract infections in women than men. This is primarily due to differences in the urogenital system (14). In fact, women have much shorter urethra than men, and its opening is much closer to the outlet of colon and vagina, where it can be more easily contaminated by pathogenic mucosa and cause an infection (14). In consistence with our results, Prakasam et al. (15), in his outpatient population based study also found higher prevalence in women.

Ngwai et al. in their study (16), have included outpatient population of 400 respondents, presented that 20% of the urine samples showed significant bacteriuria, with a higher incidence in women (25%) comparing to (15%) in men.

In our study, *E. coli* was isolated with a frequency of 65.95%, followed by *Proteus spp.* (12.05%). The frequency of *Enterococcus faecalis* was 7.80% and *Pseudomonas spp.* 4.96%. *Enterobacter spp.* was isolated in 4.25% of samples, while in 2.12% was isolated *Klebsiella spp. Acinetobacter* and *Streptococcus spp.* had a lowest frequency. Prior studies of *E. coli* resistance profile have been reported at outpatient population as well (17, 18, 19). Similarly to our results, in their studies *E. coli* was the most common isolated uropathogen while the presence of other bacteria was significantly lower.

According to our study, *E. coli* showed the highest antimicrobial resistance to ampicillin (82.79%) and trimethoprim-sulfamethoxazole (40.86%). The reason for this is common practice of prescribing ampicillin in the empirical treatment of urinary tract infections, and the fact that ampicillin (82.79%) and trimethoprim-sulfamethoxazole were the most commonly prescribed antibiotics in the war and post-war period in Bosnia and Herzegovina. A similar situation was observed in Serbia and in other less developed countries, where the WHO donated large doses of these antibiotics in the treatment of many infectious diseases (12).

The rate of *E. coli* resistance to trimethoprim-sulfamethoxazole in the world, has largely increased in recent years. In all studies performed in Europe and North America, it is noticeable that it exceeds the limit of 15% while in developing countries the rate of resistance is between 30 and 40% (20).

In our study, the resistance of *E. coli* to cephazolin was found in 7.52% of isolates, to gentamicin in 2.15%, ciprofloxacin 4.30%, nalidixic acid 19.35% and nitrofurantoin in 5.37% of isolates.

It is evident increase in resistance to nalidixic acid. Before 1990, *E. coli* resistance to fluoroquinolones was rarely noticed. During the period when they were introduced on the market, it was thought that resistance to them would be rare because they belong to xenobiotics, and actually they are not found naturally in the human body. However, the excessive and uncontrolled use of fluoroquinolones in clinical and veterinary medicine led to the increasing resistance of bacteria, which reduces the effectiveness of these drugs for future use (3). Research in Split-Dalmatia County, showed that the rate of resistance in the five year period to ciprofloxacin was increased from 2.48% in 1999 to 7.28% in 2004 (21).

Overuse of one of the fluoroquinolone leads to the development of resistance to the whole group of quinolone antibiotics.

Fluoroquinolones which were added to the animal

feed select *E. coli* strains that are resistant to these drugs and used to treat humans. In general, countries of the European Union spend in veterinary purposes 3494 tons of antimicrobial agents per year, and out of this, 43 tons are fluoroquinolones (22).

In our study, *E. coli* species is increasingly resistant to ampicillin and trimethoprim-sulfamethoxazole. Also other isolated uropathogens, that were observed, show a higher frequency in women than in men, but this difference is not statistically significant, as that was the case with *E. coli.*

Geographic differences in the resistance of some bacteria, related to the family *Enterobacteri aceae*, can be explained by different practices of physicians in prescribing certain antimicrobials. Appearance of resistant strains of bacteria is a major therapeutic problem. It is multifactorial and can be explained by several hypotheses (23).

The impact of excessive and/or inappropriate use of antibiotics, especially broad-spectrum antibiotics prescribed empirically, has resulted in some negative consequences such as (multiple-resistant profile, a limited number of therapeutic options...).

Antimicrobial resistance is not a new problem, but it is becoming increasingly dangerous and requires urgent investment of effort and resources into its resolution.

Strengths

There are limitations in our study. We only analyzed the resistance to a few antibiotics and relatively small numbers of isolates. Additionally, our tested population were not clinical followed (we did not have their diagnosis!).

5. CONCLUSION

Results of our research have proved that *E. coli* showed major antimicrobial resistance to ampicillin and trimethoprim-sulfamethoxazole, and minor resistance to gentamicin. Also frequency of *E. coli* and other bacteria was higher in women than in men.

- -Author's contribution: all authors in this paper have contributed in all phases in it's preparing. First author made final proof reading.
- - Declaration of Interest: There is no conflict of interest.

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