Frequency and antibiotic resistance of bacteria in urinary tract infections in south Serbia

Nikola Stanković*, Nataša Joković, Tatjana Mihajilov Krstev, Milica Pejčić, Marina Dimitrijević

University of Niš, Faculty of Science and Mathematics, Department of Biology and Ecology, Višegradska 33, Niš, Serbia
* E-mail: nikola.stankovic@pmf.edu.rs

Abstract:

Urinary tract infection (UTI) refers to the presence of microbial pathogens within the urinary tract. The aim of this study was to determine frequency and antibiotic resistance of bacteria that cause urinary tract infections in south Serbia during the year 2015. From 4784 analyzed urine samples 2367 isolates of pathogenic bacteria were obtained. The most frequent cause of UTI was Escherichia coli (43.0%) followed by Enterococcus spp. (31.0%). Also, there was a significant number of Proteus mirabilis (11.0%) and Klebsiella spp. (7.0%) isolates. In addition, remaining 8.0% of isolated bacteria belonged to genera Staphylococcus spp., Enterobacter spp., Citrobacter spp., Providencia spp., Acinetobacter spp. and to species Pseudomonas aeruginosa and Proteus vulgaris. Gram-negative bacterial isolates were the most resistant to penicillin antibiotics, and the most sensitive to cephalosporins. Enterococcus spp., which are Gram-positive bacteria showed sensitivity to ampicillin in significant percentage and also to vancomycin (glycopeptide antibiotic), while fosfomycin (quinolone antibiotic) showed the lowest potency against these isolates.

Key words: antibiotic resistance, urinary tract infections, Escherichia coli, Enterococcus spp.

Apstrakt:

Infekcije urinarnog trakta (UTI) predstavljaju prisustvo patogenih mikroorganizama u urinarnom traktu. Cilj ovog rada je bio da se utvrdi učestalost i rezistencija na antibiotike uzročnika urinarnih infekcija na teritoriji južne Srbije u toku 2015. godine. Ispitivanje je vršeno u mikrobiološkoj laboratoriji poliklinike “Human” u Nišu. Iz ukupno analiziranih 4784 uzoraka urina bilo je 2367 bakterijskih izolata. Najčešći uzročnik urinarnih infekcija je bila bakterija Escherichia coli (43.0%), a zatim Enterococcus spp. (31.0%). U značajnijem broju je bilo i izolata Proteus mirabilis (11.0%) i Klebsiella spp. (7.0%). Pored navedenih bakterija izolovane su i Staphylococcus spp., Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter spp., Citrobacter spp., Providencia spp., Acinetobacter spp., koje zajedno čine 8.0% izolata iz analiziranih uzoraka. Gram-negativni
bakterijski izolati su bili najrezistentniji na penicilinske antibiotike, dok su najveću osjetljivost pokazali na cefalosporine. Enterococcus spp., koje su inače Gram-pozitivne bakterije, su u velikom procentu pokazale osjetljivost na ampicillin, kao i vankomicin (glikopeptidni antibiotik), dok je primećena najveća rezistencija na fosfomicin (hinolonski antibiotik).

Ključne reči: antibiotska rezistencija, infekcije urinarnog trakta, Escherichia coli, Enterococcus spp.

Introduction
Urinary tract infections (UTI) are among the most common infectious diseases occurring in either the community or healthcare settings. UTI are caused by bacteria which, in case that they are present in significant number in urinary system, result by occurrence of different symptoms in patients (Hooton, 2000; Nicolle, 2005). According to Foxman (2000), UTIs are up to 10 times more frequent in women than in men. Among the bacterial species Escherichia coli account to 80% to 85% of the infection (Vasudev, 2014). Except of E. coli, other bacteria can be the cause of UTI including Klebsiella spp., Proteus spp., Enterococcus spp., Staphylococcus spp., Pseudomonas aeruginosa (Linhares et al., 2013).

Resistance to antibiotics is a rising problem worldwide and many studies reported results on the main causative agents and their antibiotic resistance patterns in different regions (Chow, 2000; Aibinu et al., 2004; Rudy et al., 2004; Veličković-Radošević et al., 2009; Heintz et al., 2010; Swaminathan & Alangaden, 2010; El Bouamri et al., 2014). Local antimicrobial susceptibility patterns of urinary isolates should be known in order to achieve a satisfactory therapeutic effect (Huang et al., 2014).

Usually, the first step in treating UTI is an empiric antibiotic selection, partially determined by local resistance patterns. Because of increasing resistance of bacteria to most common agents used in empiric therapy (trimethoprim/sulfamethoxazole), it is necessary to isolate, identify and make a susceptibility test on bacterial causative agent of infection for an adequate treatment of UTI (Nicolle, 2005; Hande et al., 2005).

The aim of this research was to isolate and identify bacterial species that are the most common causes of UTI in South Serbia, as well as to determine whether the incidence of infection is related to gender. Resistance to antibiotics of obtained isolates was also done in order to determine the local resistance patterns.

Material and methods
Samples
Urine samples from patients of polyclinic “Human” in Niš, were investigated in this study. A total of 4784 urine samples collected in the period January – December 2015, were analyzed.

Mediums and antibiotics
Cultivation and isolation of pathogenic bacteria from urine samples were performed on following microbiological culture media: UTI agar, Sheep blood agar, MacConkey agar, Mannitol salt agar base, Esulcin bile agar, Kliger iron agar, Peptone water, Simmons citrate agar, Christensen urea agar, Parisian mannitol, Mueller-Hinton agar (Titan Media, India). Kovacs reagent and Gram staining reagents were used for identification purposes.

Testing the resistance of isolates was done using antibiotic discs (Himedia, India), by list and concentration recommended by CLSI standards (CLSI, 2010). Gram-negative isolates were tested to following antibiotics: ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), cefixime (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), and trimethoprim/ sulphamethoxazole (25 µg). Isolates of Enterococcus spp. were tested to: ampicillin (10 µg), levofloxacin (15 µg), doxycycline (30 µg), fosfomycin (50 µg), gentamicin (120 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), and vancomycin (30 µg).

Isolation and identification of bacteria
Isolation of bacteria from urine samples was performed by plating samples on the UTI agar followed by 24 h incubation on 35–37 °C. Bacterial colonies with different macromorphological characteristics were further transferred on different selective and differential media in order to identify isolates on the basis of their biochemical characteristics.

Small blue-green colonies on UTI agar were plated on blood and esculin agar. Bacteria which had hemolytic activity on blood agar and decomposed bile salts on esculin agar were identified as
Enterococcus spp. Large white colonies on UTI agar were further transferred on mannitol salt agar. Isolates that formed yellow colored colonies and were positive on coagulase test (rabbit plasma coagulase test) were identified as S. aureus (Karakasović, 1989). Small white-light blue colonies on UTI agar were transferred on blood agar in order to examine hemolytic activity. Confirmation of Streptococcus spp. was done by using agglutination strepto-kit (Microgen, Bioproducts).

On UTI agar, Gram-negative bacteria form a large purple, light to dark blue, or white to cream colored, mostly slimy colonies. Identification of these bacteria was performed using standard biochemical tests.

Susceptibility testing of bacteria

Agar plates were inoculated with a standardized inoculum (McFarland standard 0.5) of the bacteria and antimicrobial disks were placed on the inoculated agar plate according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010). The disks used for a disk diffusion assay contains a standardized known amount of an antimicrobial agent (Table 1 and 2), which diffuses into the agar when is in contact with the agar surface. The plates were incubated inverted at 36±1°C for 18 to 24 h. Following incubation, the diameter of this zone was measured, and the results were interpreted as resistant (R), intermediate (I), or susceptible (S) using standard guidelines. The isolates that were intermediary sensitive to certain antibiotics were classified into sensitive, because intermediate represents the sensitivity to a particular antibiotic in a smaller extent.

Results

Frequency of bacteria in UTI

In order to examine the dominant groups of bacteria in patients with UTI, 2367 bacterial strains from the 4784 urine samples were isolated and identified. The most common pathogen in urine samples was E. coli (43.0%), while significant percentage of Enterococcus spp. (31.0%), Proteus mirabilis (11.0%) and Klebsiella spp. (7.0%) was observed. Also, Staphylococcus spp., Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter spp., Citrobacter spp., Providencia spp., Acinetobacter spp. were isolated but in smaller percentage (8.0% in total).

Considering that gender of the patients is the most common factor that affects the urinary infections, an analysis of the frequency of isolation of the pathogen from urine in relation to this factor was performed. Analyses related to the gender of the patients were done for four most frequent bacteria causing UTI and the results are presented in Tab. 1.

From a total of 2367 isolates from urine samples, 1737 (73.4%) isolates were isolated from female patients, 630 (26.6%) isolates from male patients. E. coli was significantly represented in females than males (82.4% and 17.6% respectively). Other pathogens were also more common in females than in males (Tab. 1). In male patients, Enterococcus spp. were most frequent (n=225, 35.7%), followed by E. coli (n=181, 28.7%), P. mirabilis (n=128, 20.3%) and Klebsiella spp. (n=37, 5.9%).

When it comes to female patients, E. coli was most frequent (n=846, 48.7%), followed by Enterococcus spp. (n=503, 29.1%), P. mirabilis (n=142, 8.2%), and Klebsiella spp. (n=127, 7.3%).

Antibiotic resistance of isolates

Antibiotic susceptibility testing was performed for E. coli, P. mirabilis, Klebsiella spp., and Enterococcus spp. (Tab. 2). The following groups of antibiotics were tested: penicillins (apicillin and amoksicillin/clavulanic acid), sulphonamides (trimethoprim/sulphamethoxazole), quinolones (ciprofloxacin and levofloxacin), cephalosporins (cefixime, cefotaxime and cefuroxime), aminoglycosides (gentamicin), nitrofururan derivates (nitrofurantoin), glycopeptide (vancomycin), tetracyclines (doxycycline), and fosfomicin.

Table 1. Number and frequency of isolates considering gender of the patients

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Total number</th>
<th>Frequency</th>
<th>Male</th>
<th>Female</th>
<th>% of isolates by gender</th>
<th>% of isolates by gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>1027</td>
<td>43.0%</td>
<td>181</td>
<td>17.6</td>
<td>846</td>
<td>82.4</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>728</td>
<td>31.0%</td>
<td>225</td>
<td>30.9</td>
<td>503</td>
<td>69.1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>270</td>
<td>11.0%</td>
<td>128</td>
<td>47.4</td>
<td>142</td>
<td>52.6</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>164</td>
<td>7.0%</td>
<td>37</td>
<td>22.6</td>
<td>127</td>
<td>77.4</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>178</td>
<td>8.0%</td>
<td>59</td>
<td>33.1</td>
<td>119</td>
<td>66.8</td>
</tr>
</tbody>
</table>
Among the penicillins all isolates were more resistant to ampicillin in comparison to amoxicillin/clavulanic acid. Klebsiella species showed the highest resistance to ampicillin (98.1%), followed by E. coli (72.7%) and P. mirabilis (58.2%). The same order of frequencies for amoxicillin clavulanic acid was observed, but with a much smaller percentage of resistant isolates (53.4%, 36.5%, and 24.9% respectively).

It was noticed that resistance to trimethoprim/sulfamethoxazole was significant. Resistance to trimethoprim/sulfamethoxazole for P. mirabilis was 49.6%, for Klebsiella spp. 48.4%, and for E. coli 41.8%.

Resistance to cefixime for Klebsiella spp. was 40.7%, for E. coli 25.0%, and for P. mirabilis 16.5%. In Enterococcus spp. resistance to cefixime was 35.7%.

Table 2. Antibiotic resistance of Gram-negative pathogens, (%) Resistant

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>E. coli</th>
<th>P. mirabilis</th>
<th>Klebsiella spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>72.7</td>
<td>58.2</td>
<td>98.1</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>36.5</td>
<td>24.9</td>
<td>53.4</td>
</tr>
<tr>
<td>Trimethoprim/sulphamethoxazole</td>
<td>41.8</td>
<td>49.6</td>
<td>48.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
<td>16.5</td>
<td>40.7</td>
</tr>
<tr>
<td>Cefixime</td>
<td>18.5</td>
<td>13.4</td>
<td>41.4</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>15.6</td>
<td>16.2</td>
<td>44.4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>14.7</td>
<td>16</td>
<td>45.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>22.9</td>
<td>25</td>
<td>41.4</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>7.7</td>
<td>47.6</td>
<td>53.7</td>
</tr>
</tbody>
</table>

In relation to cephalosporins resistance was: to cefixime in Klebsiella spp. 41.4%, in E. coli 18.5%, and P. mirabilis 13.4%; to cefuroxime in Klebsiella spp. 44.4%, in P. mirabilis 16.2%, and in E. coli 15.6%; to cefotaxime in Klebsiella spp. 45.3%, in P. mirabilis 16.0%, and in E. coli 14.7%.

Resistance to gentamicin in Klebsiella spp. was 41.4%, in P. mirabilis 25.0%, and in E. coli 22.9%. Resistance to nitrofurantoin in Klebsiella spp. was 53.7%, in P. mirabilis 47.6%, and in E. coli 7.7%. Resistance to pipemidic acid was identified in 47.4% of Klebsiella spp., in P. mirabilis 41.0%, and in E. coli 36.3%.

Enterococcus spp. isolates were also tested to fosfomycin, doxycycline, levofloxacin, and vancomycin, and resistance to named antibiotics were 79.5%, 32%, 29%, and 1.1% respectively (Tab. 3).

Discussion

Infections of the urinary tract are one of the most common bacterial infections and one of the most common reasons for prescribing antimicrobial drugs. Antibiotics are a group of effective and commonly used drugs. Unfortunately, bacteria have developed extremely genetic mechanisms of antibiotic resistance. The most important factor that leads to the development of bacterial resistance to antibiotics is their overuse, especially in cases where their use is not necessary (Foxman, 2010).

In our study, of total 2378 isolated bacterial strains from urine samples obtained of patients from south Serbia, the most common cause of UTI was E. coli, as expected. Our study showed that E. coli was less frequent than in most European regions (Schito et al., 2009; Malmar et al. & Gasarossian, 2015) but more frequent than in Ivory Coast (Moroh et al., 2013). The frequency of P. mirabilis was 11%, which is similar to Bosnia (Mahmutović-Vranić & Uzunović, 2016). Unlike the other members of Enterobacteriaceae, P. mirabilis is not a common pathogen that causes
urinary tract infections in normal hosts (Chen et al., 2012). The prevalence of Klebsiella spp. infection was similar to studies carried out in Portugal (6.0-13.45%), reported by Limares et al. (2013). Other pathogens (Staphylococcus spp., P. aeruginosa, P. vulgaris, Enterobacter spp., Citrobacter spp., Providencia spp., Acinetobacter spp.) that we isolated were not investigated because they all together accounted 8 percent of the total number of isolates.

Our research has shown that urinary infections are three times more common in women. That was expected because studies worldwide reported that UTI is more common in females (Foxman et al., 2000; Ayegoro et al., 2007; Omorogie et al., 2008). These data on the frequency of the gender coincide with the data obtained in the other regions worldwide (Cunha et al., 2016).

The lowest in vitro efficiency on pathogens has been shown by ampicillin, where only 1 of 164 Klebsiella spp. isolates were susceptible to this antibiotic. Escherichia coli, as the most prevalent pathogen had also high rate of resistance to ampicillin, followed by P. mirabilis. Resistance of Gram negative pathogens to ampicillin observed in this study was like those observed in South Africa, Israel, Hong-Kong, Philippines, Iran and Bosnia, where range of resistance were 62.0 – 84.0% (Desenclos et al., 1988, Kazemnia et al., 2014; Mahmutović-Vranić & Uzunović, 2016). Minor resistance of Enterococcus spp. to ampicillin was observed. That was expected, because enterococci are typically susceptible to ampicillin, because of the lack of beta-lactamase (Holzbach & Rice, 2012).

Resistance of E. coli and other Gram-negative pathogens to trimethoprim/sulphametho-xazole was similar, in the range of 41.8 – 49.6%. High percentage of resistance to this antibiotic was reported in European countries and Brazil, in the range of 38.9% - 50.6% (Nickel, 2007; Cunha et al., 2016). The reason for this high resistance may be due to the wide use of these antimicrobials in the treatment of community-acquired infections (Cunha et al., 2016). Guidelines of the American Infectious Diseases Society and the European Society for Microbiology and Infectious Diseases suggest that antimicrobials with a resistance rate above 20% should not be prescribed empirically to patients with uncomplicated cystitis, unless susceptibility is determined by priorisolation in culture (Gupta et al., 2011).

Fluoroquinolones are widely used for empirical treatment of UTI (Rocha et al., 2012). It can be noticed that resistance to ciprofloxacin in our study is dramatically high comparing with studies from region and another European countries. In Bosnia, the most common pathogen E. coli was resistant to ciprofloxacin in 4.3% of cases, comparing with our study where 25% of E. coli isolates were resistant. In 9 European countries its reported that E. coli was resistant in 8.8% of cases (Nickel, 2007). The highest percentage of resistance to ciprofloxacin was reported in Nigeria, with 65.7% (Olu runmola et al., 2013). It is considered that ciprofloxacin has only modest activity against enterococci (Perry et al., 1994). Results for the resistance of Enterococcus spp. isolates are in accordance with with results from other studies (Abdulla & Abdulla, 2006; Gilho, 2013). Overuse of one of the fluoroquinolone leads to the development of resistance to the whole group of quinolone antibiotics (Mahmutović-Vranić & Uzunović, 2016).

Resistance of Gram-negative pathogens to cephalosporins of second (cefuroxime) and third generation (cefixime and cefotaxime) was relatively low comparing with other antibiotics. There was no significant difference in a resistance pattern within bacterial species based on cephalosporin classification. The most resistant were Klebsiella spp. isolates, and the least resistant were P. mirabilis. The most common pathogen E. coli was resistant to cephalosporins in range of 14.7-18.5%. Meyer et al. (2010), reported dramatic increase of third generation cephalosporin-resistant E. coli in German intensive care units in a period of 8 years, where this pathogen developed resistance from 1.2% to 19.7%.

Gentamicin was only aminoglycoside antibiotic used in this study. Klebsiella spp., P. mirabilis and E. coli have shown significant resistance. Rate of resistance presents double value comparing with Brazil (Cunha et al., 2016). Compared with region, resistance rate of E. coli in Bosnia was 2.15%, which presents 10 times lower value (Mahmutović-Vranić & Uzunović, 2016). Higher rate of resistance in E. coli to gentamicin was observed in Egypt and Iran, 40% and 36% respectively (Gad et al., 2011; Kazemnia et al., 2014).

In our study, nitrofurantoin was the most efficient antibiotic against two most common pathogens, E. coli and Enterococcus spp. Similar data were obtained in Bosnia, Brazil, Nigeria, and Portugal (Olu runmola et al., 2013; Limares et al., 2013; Mahmutović-Vranić & Uzunović, 2016; Cunha et al., 2016).

The rising prevalence of vancomycin-resistant enterococci (VRE) is of particular concern within many institutions because of its association with increased mortality and health care costs, as well as limited treatment options (Heintz et al., 2010).
Present study detected VRE but in a very small percentage. Fosfomycin (Monural) is very frequently prescribed at first symptoms of UTIs, but high resistance to this agent was observed, showing unjustified use of this treatment. Resistance to gentamicin was also significant, and explanation of rising level of resistance to aminoglycosides at general is that enterococci have acquired aminoglycoside resistance genes that mediate production of aminoglycoside-modifying enzymes (Chow, 2000).

Conclusion

The results obtained in this study suggest that *E. coli* is the most common cause of urinary tract infections, followed by *Enterococcus* spp. Urinary tract infections occur more often in women than in men. Nitrofurantoin is the most efficient agent against bacterial uropathogens and represent effective option for empirical therapy. Ampicillin presents the least effective antibiotic against Gram-negative uropathogens, but still effective against *Enterococcus* spp. Maximum resistance to all applied antibiotics showed *Klebsiella* spp. isolates, wherein the resistance to ampicillin was almost absolute.

It can be concluded that the cephalosporins *in vitro* are more efficient than other groups of antibiotics to Gram-negative UTI pathogens, and it can be assumed that cephalosporins are the most effective in the treatment of urinary tract infections. Also, conclusion is that ampicillin (penicillins) is still highly efficient to *Enterococcus* spp., while fosfomycin and aminoglycosides cannot be used as the first-choice treatment due to low efficiency against these isolates.

We reported unique local pattern of frequency and resistance to antibiotics of bacterial uropathogens in south Serbia, which can help in definition of empirical treatment for UTI. Besides, it is more efficient to perform culture and susceptibility tests on isolated pathogen prior to treat, in order to avoid failure of therapy.

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