Analysis of bacterial resistance to antimicrobial drugs in the treatment of urinary tract infections

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Introduction: Non-critical and irrational use of antibiotics is the most important reason for the ever faster development of infectious diseases causative agents’ resistance to those drugs. The aim of this study was to determine the most frequent bacterial causative agents of urinary infections and their resistance to antibiotics. Methods: The study was carried out in the Microbiological Laboratory of the Institute for Public Health of Canton Sarajevo, during the period of January-March 2007, 2008, and 2009. The identification of the causative agents was conducted with classical biochemical series and the sensitivity test to antimicrobial drugs with the disc-diffusion method. The CLSI protocols that precisely define the kind of antibiogram discs used for particular bacteria were used. Results: The most common causative agents of urinary infections were Escherichia coli, Gr. Klebsiella-Enterobacter, Proteus mirabilis and Pseudomonas spp. The highest prevalence of the studied infections was at the age of 71-90 years for all four bacterial species. Women are more exposed to E. coli and Proteus mirabilis infections, and men to Pseudomonas spp. infections. The highest resistance of E. coli was to ampicillin and to trimethoprim-sulfamethoxazole, and the least towards cefixime. For Proteus mirabilis, there was significantly more nonresistant strains than resistant ones to all tested antibiotics except to nitrofurantoin. The least was shown in case of cefixime and gentamicin. Gr. Klebsiella-Enterobacter showed generally high resistance towards all antibiotics, the least to gentamicin. Documented resistance of Pseudomonas spp. to all antibiotics was also very high.

Key words: urine culture, antibiotics, antibiogram, sensitivity, resistance

Introduction
Antibiotics are amongst the most used drugs (1). In some countries it is estimated that 15-30% of the total health budget for drugs is being spent on them (2). However, up to 50% of all the antibiotics prescribed for people are not needed, or are not optimally effective as prescribed (3). Non-critical and irrational use of antibiotics is the most important reason for the ever faster development of infec-
tious diseases causative agents’ resistance to those drugs. The results of a certain study speak of the facts that from 41% up to even 91% of the prescribed antibiotics are prescribed irrationally (4). Their consumption is far greater than the consumption of the other groups of drugs, which is not a consequence of frequent bacterial infections, but the result of unsuitable prescribing in cases when there is no bacterial infection (5). The aim of this study was to determine the most frequent bacterial causative agents of urinary infections and their resistance to antibiotics.

**Material and methods**

Identification of the causative agents and the sensitivity tests to antimicrobial drugs were carried out in the Microbiological Laboratory of the Institute for Public Health of Canton Sarajevo. Data were recorded by examining the patients’ protocols, including data on their gender, age structure and resistance to certain antimicrobial drugs. Research encompassed the period of three years, exact periods being: January-March of 2007, January-March of 2008, and January-March of 2009. Only one sample, most often primary isolate, was processed from every patient.

The identification of the causative agents was conducted by classical biochemical series. The sensitivity test to antimicrobial drugs was conducted using the disc-diffusion method according to Kirby-Bauer (6), on Mueller-Hinton agar with antiobigram tablets (Torlak, Belgrade, Serbia). Antibiotic sensitivity of the causative agent was tested to the following antibiotics: ampicillin, cephalaxin, trimethoprim+sulfomethoxazole, ciprofloxacin, cefuroxime, gentamicin, cefixime, nalidixic acid, and nitrofurantoin.

**Disc-diffusion method**

The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. This method is carried out in the way that the paper disc is impregnated with standardized amount of an antibiotic; it is set on the surface of a rigid substrate that was earlier inoculated with a pure culture of tested bacteria in growth. An inoculated culture of bacteria grows at certain distance from the disc at which the concentration of the diffused antibiotic is too small and thus inefficient. Such absence of growth is termed the zone of inhibition. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism (7, 8), giving the required semi quantitative data on sensitivity to specific antibiotic as “sensitive”, “moderately sensitive” and “resistant” as listed in the Clinical and Laboratory Standards Institute (CLSI), document M2-A, Performance Standards for Antimicrobial Disk Susceptibility Tests (7).

The disc-diffusion method gives the required semi quantitative data on sensitivity to a specific antibiotic as “sensitive”, “moderately sensitive” and “resistant”. The bacterial strain sensitivity was determined by measuring the zones of growth inhibition, according to the manufacturer’s instructions. If the causative agent is sensitive (zone of growth inhibition 16 mm or more; zone of inhibition corresponds to the concentration of antimicrobial drug achieved in serum after the application of the standard dose) the clinical effect is achieved with the application of the standard doses of the antimicrobial drug. If the causative agent is resistant, the inhibition zone is small or nonexistent (inhibition zone 10 mm or lower), i.e. the concentrations of the antimicrobial agent achieved in blood by application of the usual doses do not affect the causative agent. Moderate sensitivity (inhibition zone 11-15 mm), means that much higher doses of the antimicrobial drug than the standard ones need to be applied in order to achieve a clinical effect. Clinically, moderately sensitive strain of a causative agent towards the
antimicrobial drug needs to be considered resistant until proven otherwise (5).

**The statistical analysis**

The statistical analysis was processed using statistical software SPSS (Statistical Package for Social Sciences©, March 2004), version 11.0, for Windows. The results of the study are presented as arithmetic mean. Frequencies were expressed as percentages. For statistical testing, p value less than 0.05 was considered statistically significant. Results are shown in tables and graphs.

**Results**

According to the protocol of the Microbiological Laboratory of the Institute for Public Health of Canton Sarajevo, during the period of January-March 2007, 2008, and 2009, 15,012, 17,124 and 17,501 samples were processed for urine culture and antibiogram in the adult population, respectively. The number of causative agents isolated in the study period of year 2008 was 1,184, of year 2009 2,334 and of year 2010 2,548. The four most frequently isolated bacteria were: *Escherichia coli*, *Gr. Klebsiella-Enterobacter*, *Proteus mirabilis* and *Pseudomonas spp* (Figure 1) and the share of infections of adult urine is shown in Table 1.

Upon the analysis of prevalence of the considered infections by age groups during the three years analysed (Figure 2), it can be perceived that the highest prevalence of infections caused by all four considered bacterial species was documented in the age range of 71-90 years. It was thus determined that the average number of patients in that age group infected by *E. coli* was 1,515 (29.38%); 157 (34.97%) by *Gr. Klebsiella-Enterobacter*; 119 (40.07%) by *Proteus mirabilis* and 99 (40.24%) by *Pseudomonas spp*. The number of infected patients generally decreased with

Table 1. The share of microorganisms isolated in the adult urine cultures

<table>
<thead>
<tr>
<th>Causative Agent</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>989 (83.53)</td>
</tr>
<tr>
<td><em>Gr. Klebsiella-Enterobacter</em></td>
<td>95 (8.02)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>69 (5.83)</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>31 (2.62)</td>
</tr>
</tbody>
</table>
age, except for *Pseudomonas* spp, where in the age group of 31-40, an average number of 25 patients (15.24%) were registered, and in the age group of 18-30, an average number of 30 patients (18.29%) were registered.

Analysis of gender exposure showed a higher exposure of women to the infections caused both by *E. coli* (91.32 vs. 8.68%) and *Proteus mirabilis* (73.74 vs. 26.26%). For infections caused by *Gr. Klebsiella-Enterobacter*, significant gender differences in exposure was observed only in 2009 with the women significantly more exposed to infection than men (70.16 vs. 29.84%). In the case of *Pseudomonas* spp. men were more exposed to infection during the three years of the study (59.76 vs. 40.24%).

Statistical analysis of the antibiogram results in outpatients (Table 2) in all studied years showed significantly more strains of *E. coli* to be resistant than sensitive to ampicillin (p<0.01). Through the whole considered period *E. coli* showed higher sensitivity than resistance towards all other tested antibiotics (p<0.01).

Through all three years there were significantly more resistant strains of *Gr. Klebsiella-Enterobacter* to ampicillin compared to the nonresistant ones (p<0.01). In case of trimethoprim+sulfomethoxazole during 2007 and 2009 significantly more nonresistant strains were recorded (p<0.01), while in year 2008 there were significantly more resistant strains (p<0.01). During 2007 and 2009, significantly more nonresistant strains compared to the resistant ones were also recorded towards ciprofloxacin (p<0.01), while in year 2008 there was no significant difference between the mentioned categories (p=0.034). Concerning cefuroxime, during 2007 and 2008, significantly more resistant strains compared to nonresistant ones were recorded (p<0.01), while in year 2009 there was no statistically significant difference between the categories (p=0.61). Through all three years there were more nonresistant strains of *Gr. Klebsiella-Enterobacter* to gentamicin compared to the resistant strains (p<0.01). In case of cefixime in 2009, significantly more nonresistant strains compared to the resistant ones were recorded (p=<0.01). In 2008, there was no significant difference between those categories (p=0.69), while in 2007 there were no data for cefixime. Concerning nalidixic acid, during 2007 and 2009 significantly more nonresistant strains compared to the resistant ones were recorded (p<0.01), while in 2008 the inverse was true, i.e. there were more resistant strains (p<0.01). During 2008
significantly more strains resistant to nitrofurantoin were recorded compared to the nonresistant ones (p<0.01), while in 2007 and 2009 there was no significant difference between the mentioned categories (p=0.47 and p=0.82).

During the years of 2007 and 2008 there were significantly more nonresistant strains of *Proteus mirabilis* than ones resistant to ampicillin (p<0.05) and trimethoprim+sulfamethoxazole (p<0.05), while in 2009, there was no significant difference between the mentioned categories (p=0.2 and p=0.92). There were significantly more sensitive strains of *Proteus mirabilis* (p<0.01) than strains resistant to cephalaxin, ciprofloxacin, cefuroxime, cefixime, gentamicin and nalidixic acid during all three years (there were no data for cefixime for year 2007.). Concerning nitrofurantoin, through all three years significantly more resistant strains of *Proteus mirabilis* compared to the nonresistant ones were recorded (p<0.01).

Significantly more resistant than nonresistant strains of *Pseudomonas spp.* were documented during all three years to ampicillin, cephalaxin, trimethoprim+sulfamethoxazole, cefuroxime, cefixime, nalidixic acid and nitrofurantoin (p<0.01), and to ciprofloxacin (p<0.05) (there were no data for cefixime for 2007). Significantly more resistant than nonresistant strains of *Pseudomonas spp.* were documented in year 2008 to gentamicin (p<0.05), while in years 2007 and 2009 no significant difference between those categories was recorded (p=0.1).

Analysis of resistance of *E. coli*, *Gr. Klebsiella-Enterobacter*, *Proteus mirabilis* and *Pseudomonas spp.* to antibiotics recommended according to the CLSI protocols in outpatients is given in Figures 3, 4, 5, and 6. The prevalence of resistance of *E. Coli* or *Pseudomonas spp.* towards each antibiotic was relatively similar during the observed period, while *Gr. Klebsiella Enterobacter* or *Proteus mirabilis* showed significant variations among the years observed.

### Table 2. Analysis of the antibiogram results in outpatients (S-significantly higher sensitivity than resistance; R-significantly higher resistance than sensitivity; ns-no statistically significant difference between resistance and sensitivity; x-no data or insufficient data available for statistical analysis)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Escherichia coli</th>
<th>Gr. Klebsiella-Enterobacter</th>
<th>Proteus mirabilis</th>
<th>Pseudomonas spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'07.</td>
<td>'08.</td>
<td>'09.</td>
<td>'07.</td>
</tr>
<tr>
<td>ampicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>cephalexin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>TMP+SMX</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>cefixime</td>
<td>x</td>
<td>S</td>
<td>S</td>
<td>x</td>
</tr>
<tr>
<td>nalidixic acid</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>nitrofurantoin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ns</td>
</tr>
</tbody>
</table>
Discussion

The analysis of the prevalence of microorganisms isolated from urine cultures showed *E. coli*, *Gr. Klebsiella-Enterobacter*, *Proteus mirabilis* and *Pseudomonas spp* to be the most frequently isolated ones. The share of *E. coli* infections of adult urine was between 83.53-85.40%, *Gr. Klebsiella-Enterobacter* 7.50-8.02%, *Proteus mirabilis* 4.12-5.83% and *Pseudomonas spp* 2.62-2.98% (Table 1). Earlier studies documented the representation of *E. coli* to be 31%, *Gr. Klebsiella-Enterobacter* 12.30%, *Proteus mirabilis* 12.90%, *Pseudomonas spp* 4.30% and *Pseudomonas aeruginosa* 13.80% (9). To prevent antimicrobial resistance in the healthcare system, the Center for Disease Control and Prevention (CDC) led a campaign which comprised of 4 core actions: preventing infections and preventing the spread of resistance, tracking resistant bacteria, improving the use of today’s antibiotics, promoting the development of new antibiotics and developing new diagnostic tests for resistant bacteria (3).

Our results showed 70.31% resistance of *E. coli* to ampicillin recorded in 2009 and to trimethoprim+sulfamethoxazole of 36.26% in year 2008. Other reports show that the resistance of *E. coli* and the other agents causing urinary tract infections to beta-lactam antibiotics, such is ampicillin, is in continual rise reaching as high as 40% (10, 11). Similar to our results, analysis of antibiotic sensitivity showed that on average 35.32% of isolates of *E. coli* were not sensitive to trimethoprim+sulfamethoxazole, and according to the guidelines for treat-
ment of uncomplicated urinary infections, trimethoprim+sulfomethoxazole cannot be applied in the treatment of urinary tract infections in areas where the resistance of isolated strains of *E. coli* is higher than 10-20% (12, 13). Monitoring the fluctuations of resistance of *E. coli* isolated from urine of female outpatients in the time period of 1995-2001, showed that the resistance of *E. coli* to ampicillin was 36.00-37.40%; to trimethoprim+sulfomethoxazole 14.80-17.00%, ciprofloxacin 0.70-2.50% and nitrofurantoin 0.40-0.80% (14). In our study *E. coli* showed the least resistance towards cefixime: 3.06% in year 2008, and in the 2009 5.28%. Cephallexin, gentamicin and nitrofurantoin had a resistance bellow 10%. On the other hand, a study conducted in Canada showed high sensitivity of *E. coli*, *Proteus mirabilis*, *Pseudomonas spp. aeruginosa* to third generation cephalosporins, as well as to quinolones, which was 89-99% (15). However, significant resistance to quinolones, as high as 89% was also shown (16).

In case of the *Gr. Klebsiella-Enterobacter*, our results showed generally high (up to 100% in case of ampicillin in all years studied, 73.68% for cefuroxime in year 2007.), resistance towards all antibiotics. The least resistance was to gentamicin, i.e. 21.05% in 2007, 38.04% in 2008, and 18.85% in 2009, during the period January-March).

Our results obtained for *Proteus mirabilis* show significantly more nonresistant strains than the resistant ones, through the whole considered time period, to all tested antibiotics except nitrofurantoin. The least resistance in our study was shown in case of cefixime (0.00% in 2008, and 19.05% in 2009) and gentamicin (7.25% in 2007, 4.07% in 2008). The results of the resistance study of Talbot *et al.* on strains of *Proteus mirabilis* showed 10-20% resistance to the first generation cephalosporins and ampicillin (17).

Through the whole observed period of our study, there were significantly more resistant strains of *Pseudomonas spp.* than nonresistant ones to all tested antibiotics except to cefixime, for which there was no data in 2007. Documented resistance to all antibiotics was very high (the least was in case of gentamicin 64.52% in 2007, 64.91% in 2008, and 59.21% in 2009). These data are in correlation with the earlier published studies that demonstrated that *Pseudomonas spp.* shows phenotypic resistance or hazard of small colonies, which can be important for antibiotic treatment of this microorganism (18). Results of the study of Japoni *et al.* also documented that almost all isolates of *Pseudomonas spp.* show very high resistance towards most antibiotics (19). Data on resistance of *Pseudomonas spp. aeruginosa* to imipenem and ceftazidime show a growth from 10-15% in 1986 to 20-25% in 2003. (20).

**Conclusions**

The results of our study confirmed the results of other studies on the existence and growth of resistance. Therefore, the request for introduction of public health measures of prevention, which implies informing and additionally educating health workers, as well as the whole population on the growing problem of bacterial resistance to antibiotics is justified.

**Acknowledgements**

Authors declared no financial support and sponsorship and no conflict of interest.

**References**


