Risk factors for healthcare-acquired urinary tract infections caused by multi-drug resistant microorganisms

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SUMMARY
Introduction Healthcare-acquired urinary tract infections (HAUTI) make up to 40% of all healthcare-acquired infections and contribute significantly to hospital morbidity, mortality, and overall cost of treatment.

Objective The aim of our study was to investigate possible risk factors for development of HAUTI caused by multi-drug resistant pathogens.

Methods The prospective case-control study in a large tertiary-care hospital was conducted during a five-year period. The cases were patients with HAUTI caused by multi-drug resistant (MDR) pathogens, and the controls were patients with HAUTI caused by non-MDR pathogens.

Results There were 562 (62.6%) patients with MDR isolates and 336 (37.4%) patients with non-MDR isolates in the study. There were four significant predictors of HAUTI caused by MDR pathogens: hospitalization before insertion of urinary catheter for more than eight days (ORadj = 2.763; 95% CI = 1.352–5.647; p = 0.005), hospitalization for more than 15 days (ORadj = 2.144; 95% CI = 1.547–2.970; p < 0.001), previous stay in another department (intensive care units, other wards or hospitals) (ORadj = 2.147; 95% CI = 1.585–2.908; p < 0.001), and cancer of various localizations (ORadj = 2.313; 95% CI = 1.255–4.262; p = 0.007).

Conclusion Early removal of urinary catheter and reduction of time spent in a hospital or in an ICU could contribute to a decrease in the rate of HAUTI caused by MDR pathogens.

Keywords: urinary tract infections; nosocomial infections; multiple antibacterial drug resistance; risk factors

INTRODUCTION
Healthcare-acquired urinary tract infections (HAUTI) make up to 40% of all healthcare-acquired infections and contribute significantly to hospital morbidity, mortality and overall cost of treatment [1]. Major risk factor for HAUTI is an indwelling urinary catheter. Bacteriuria develops in up to 25% of patients who carry urinary catheter for one week or more, with a daily risk of 5–7% [2, 3]. It was estimated that there are about one million cases of HAUTI in hospitals and nursing homes annually associated with bladder catheter [4].

Bacteria are the primary organisms that cause HAUTI. Among gram-negative microorganisms, Enterobacteriaceae are predominant pathogens (Escherichia coli, Klebsiella, Proteus and Enterobacter). However, non-fermenting organisms (e.g. Pseudomonas aeruginosa) and gram-positive cocci (e.g. Staphylococci and Enterococci) may also play an important role, depending on the underlying conditions [5].

There are numerous recent reports coming from various hospitals around the world about increasing incidence of HAUTI caused by multi-drug resistant (MDR) microorganisms [6, 7]. Emergence of resistant strains is becoming a serious health problem because it limits the number of available antibiotics with potential to successfully treat these infections, and increases the costs of treatment. Knowledge of local and national antimicrobial resistance trends is of utmost importance for translation of evidence based recommendations to empiric antibiotic treatment of HAUTI.

Previous epidemiological studies identified indwelling urinary catheters, prior exposure to broad-spectrum antimicrobial therapy, advanced age of patients and male sex as risk factors for the development of HAUTI caused by MDR pathogens [8, 9, 10]. However, there is a whole spectrum of other characteristics of patients or hospital environments that were not investigated, and yet are candidates for risk factors for emergence of these infections.

OBJECTIVE
The aim of our study was to investigate possible risk factors for development of HAUTI caused by MDR pathogens, and to reveal their resistance patterns to various antibiotics. Good knowledge of the risk factors is the prerequisite for devising effective infection control strategies.
for HAUTI relevant to local settings, which may reduce the burden of healthcare-acquired infections.

METHODS

Study design

We conducted a prospective case-control study in a large tertiary-care hospital in Kragujevac, Serbia (1,183 beds and 50,000 inpatients per year) from January 2009 to December 2013 (five years).

The patient population

The study enrolled all patients with HAUTI according to standard definition established by the Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States, who were hospitalized during the study period for more than five days [11]. The exclusion criteria were isolation of a causative agent within the first 48 hours from the admission, and patients younger than 18 years. In patients who had several episodes of HAUTI during hospitalization, only the first episode was included in the analysis. The study was approved by the local Ethics Committee.

The cases consisted of patients with HAUTI caused by MDR agents. The controls were patients with HAUTI caused by non-MDR isolates.

For each participant a special epidemiological questionnaire was completed containing the following information: age, sex, hospital ward, dates of the patient’s admission and discharge from the hospital, date when the HAUTI was diagnosed, dates of insertion and removal of urinary catheter, stay in another hospital ward before emergence of the HAUTI, and co-morbidities (diabetes mellitus, injuries, cancer of various locations). The data were obtained from both the patients’ files and interviews with the patients and their physicians.

Each study participant was then analyzed by the Expert Group, comprising an epidemiologist, an infectologist, and a clinical pharmacologist, formed with the purpose of this study, and patients with colonization of the urinary tract were excluded from further analysis.

Antibiotic sensitivity

The isolation and identification of causative agents was performed in the hospital microbiology laboratory, using conventional biochemical methods [12]. An antimicrobial susceptibility test (AST) was performed using disk-diffusion method on Mueller-Hinton agar (bioMérieux, Marcy l’Etoile, France), by measuring the diameter of the zones of inhibition. The results were interpreted in accordance with the guidelines of The Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory [13]. The susceptibility of isolates to the following antibiotics was analyzed: ampicillin (up to 25 µg/mL), cefotaxime (up to 30 µg/mL), gentamicin (up to 10 µg/mL), amikacin (up to 30 µg/mL), ciprofloxacin (up to 5 µg/mL), trimethoprim-sulfamethoxazole (up to 2.5 µg/mL). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Statistical analysis

Primary analysis of collected data was made by descriptive statistics (arithmetic mean, standard deviation, and percentages), by testing statistical hypotheses, and by analysis of relations between outcomes and potential predictors.

The differences among the study groups were tested by the Student’s t-test for continual variables (after confirming normal distribution of data) and by the χ2 test for categorical variables. The variables which turned out to be significant predictors of HAUTI caused by MDR pathogens after univariate logistic analysis were included in a multivariate binary logistic regression analysis. The hypotheses were tested at 0.05 level of statistical significance. The statistical software SPSS version 18 for Windows (SPSS Inc., Chicago, IL, USA) was used for all calculations.

RESULTS

During the study period, 775 patients met all eligibility criteria for HAUTI. The average age of the patients was 67.6 ± 13.4 years. Participation of male (n = 389; 50.2%) and female subjects (n = 386; 49.8%) was similar.

The majority of patients (n = 664; 85.7%) suffered from HAUTI caused by a single organism, whereas the rest were with two or three isolated pathogens. A total of 841 isolates were gram-negative agents (93.7%) (Table 1). Generally, the most common pathogen was Klebsiella spp. (32.4%), followed by Proteus mirabilis (16.8%), E. coli (12.8%), Enterobacter spp. (12.7%), and Pseudomonas aeruginosa (11.4%), all accounting for over 85% of total isolates.

A statistically significant difference in the frequency of isolation between the patients with a bladder catheter and those without one was found for only one of the top five most common agents – Pseudomonas aeruginosa (p = 0.034).

The percentage of MDR isolates by types of bacteria varied between 0% and 82.4%, and showed higher values in gram-negative than among gram-positive isolates (64.2% and 38.6%, respectively). Pseudomonas aeruginosa and Acinetobacter spp. were the most common MDR uropathogens (Table 1).

There were 562 (62.6%) patients with MDR isolates (cases) and 336 (37.4%) patients with non-MDR isolates (controls) in the study. The results of univariate analysis of risk factors for HAUTI caused by MDR pathogens are shown in Table 2. According to the univariate analysis, age 65 years and above (p = 0.019), insertion of a urinary catheter (p = 0.005), hospitalization before the insertion...
of a urinary catheter longer than eight days ($p < 0.001$), hospitalization longer than 15 days ($p < 0.001$), previous stay in another department (other wards, intensive care units or other hospitals) ($p < 0.001$), patients in the surgical department, cancer of various localizations ($OR_{adjusted} = 2.313; 95\% CI = 1.255–4.262; p = 0.007$), The Hosmer–Lemeshow goodness-of-fit test for this logistic regression model was $\chi^2 = 6.032; df = 7; p = 0.536$.

Resistance of the isolates from the patients with HAUTI is shown in Table 4. The isolates of Klebsiella spp. were highly resistant to trimethoprim-sulfamethoxazole, cephalosporins of the third and fourth generation, and ciprofloxacin (over 90%), whereas 199 (68.4%) isolates were multiresistant. The highest level of sensitivity was retained toward carbapenems (around 13% of isolates were resistant). Proteus mirabilis isolates showed the highest resistance to ampicillin (96.4%), followed by resistance to trimethoprim-sulfamethoxazole (91.3%), gentamicin (90.8%), third-generation cephalosporins (89.5–91.0%), and ciprofloxacin (84.1%). Isolates of E. coli showed high degree of resistance to trimethoprim-sulfamethoxazole (84.1%) and ciprofloxacin (80.8%), while the resistance to aminoglycosides was somewhat lower (77.5% to gentamicin, and 48.1% to amikacin). The percentage of isolates...
resistant to carbapenems was rather low (10%). The other isolated gram-negative bacteria showed high degree of resistance to cephalosporins (85–98%), aminoglycosides (65–100%), fluoroquinolones (90%), while resistance to carbapenems was lower (10–45%).

The difference in hospital mortality between the patients with MDR infection [94 (19.6%) patients died from 479 in total] and those without it [56 (18.9%) died from 296 in total] was not significant (χ² = 0.058, p = 0.809).

**DISCUSSION**

There are various reports on HAUTI causative agents in medical literature. In a study similar to ours nearly 95% of all isolates were gram-negative pathogens: *Klebsiella* spp. making one third (32.4%), followed by *Proteus mirabilis* (16.8%) and *E. coli* (12.8%) [14]. In a prospective study of HAUTI which included 29 European countries it was found that six most common causative agents are *E. coli*, *Enterococcus*, *Candida* spp., *Klebsiella*, *Proteus* and *Pseudomonas aeruginosa* [15]. This result is not surprising since these bacteria belong to normal flora of the human intestine and therefore easily colonize urinary tract. This study showed significant difference in prevalence of only one of the gram-negative uropathogens in relation to presence of urinary catheter: *Pseudomonas aeruginosa* was isolated more frequently from patients with a catheter, which is consistent with the results of other authors [16]. Previous studies have also indicated that patients with HAUTI caused by *Pseudomonas aeruginosa* were more likely to have history of urinary tract procedures, to have a neurogenic bladder, to be male, and to have received recent antibiotic therapy [17].

There are many different definitions of MDR in medical literature which characterize different patterns of resistance found in healthcare-associated, antimicrobial-resistant bacteria. However, a group of international experts came together through a joint initiative by the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention, to create a standardized international terminology for describing acquired resistance profiles of all bacteria often responsible for healthcare-associated infections and prone to multidrug resistance. Epidemiologically significant antimicrobial categories were determined for each bacterium. Lists of antimicrobial categories proposed for antimicrobial susceptibility testing were created using documents and breakdowns from the Clinical Laboratory Standards Institute, the European Committee on Antimicrobial Susceptibility Testing, and the United States Food and Drug Administration. The experts reached consensus that MDR organisms are those which acquired non-susceptibility to at least one agent in three or more antimicrobial categories [18]. This definition has practical value because it allows differentiation of sensitive and MDR strains in clinical settings.

In accordance with this definition, we separated patients with MDR isolates and observed that there was a high percentage of MDR particularly among gram-negative isolates (64.2%). Increase in the prevalence of MDR isolates is being registered all over the world, and these microorganisms have become a global public health problem. Extremely rapid development of antimicrobial resistance is probably the result of the ability of uropathogens to quickly adapt to antibiotics, together with the widespread overuse of antibiotics in the hospital environment. In addition, broad-spectrum antibiotics cause suppression and eradication of competing microorganisms and facilitate selection of the MDR strains [19]. Infections caused by MDR pathogens are difficult to treat and control, leading to prolonged hospital stay, increased mortality, and higher hospitalization costs [20].

Our study showed that hospitalization before the insertion of a urinary catheter for more than eight hours increases the risk of developing HAUTI caused by MDR pathogens 2.7 times. Some other recent studies have shown that unnecessary catheterization is widely prevalent (30–50%), even in tertiary care referral centers. Large proportion of these patients who did not need a catheter in accordance with accepted indications subsequently went on to develop HAUTI, especially if the catheter was kept for longer time period [21]. These findings also emphasize the need for more stringent implementation of aseptic techniques while inserting a catheter and therefore better infection control.

Catheters and other foreign bodies in the urinary tract disrupt natural protective barriers (urethral sphincter) and provide a nidus for infections by offering surface for formation of biofilm [22]. Several studies have shown that

<table>
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<tr>
<th>Antibiotic</th>
<th><em>Klebsiella</em> spp</th>
<th><em>Proteus mirabilis</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Enterobacter</em> spp</th>
<th><em>Pseudomonas aeruginosa</em></th>
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<tr>
<td>Ampicillin</td>
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<td>90.4</td>
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<td>Ceftriaxone</td>
<td>97.6</td>
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<td>98.1</td>
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<td>Gentamicin</td>
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<td>93.3</td>
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<td>Amikacin</td>
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<td>Ciprofloxacin</td>
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<td>Trimethoprim-sulfamethoxazole</td>
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most uropathogens are able to form biofilm over urinary catheter shortly after its placement. Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms and hence are difficult to eradicate. Biofilms contribute to virulence of the pathogens as these often cause persistent and recurrent infections [23, 24]. In our study, carrying a urinary catheter was frequent in both groups (cases 83.3% vs. 75.6% of control). However, multivariate analysis excluded wearing of urinary catheter as a risk factor for HAUTI caused by MDR pathogens, although in univariate analysis it was significant (p = 0.005).

It has been known for some time that longer hospitalization of patients increases the risk of nosocomial infections [25], and our study has also shown that the risk of HAUTI caused by MDR pathogens is 2.1 times higher if patients were hospitalized for longer than 15 days. Longer stay in a hospital is associated with invasive medical procedures, and with increased contact with the bacteria from hospital environment, which are often multi drug-resistant.

Our study shows that a previous stay in another department (intensive care unit, other wards or hospitals) increased the risk of HAUTI caused by MDR pathogens by 2.1 times. Our patients usually stayed in intensive and semi-intensive care units (65%) where MDR pathogens are common causes of nosocomial infections [26, 27]. The obtained result is not surprising if one considers that the majority of patients in these departments are those with severe underlying diseases, the elderly, the immunocompromised, and patients with many comorbidities, who require large number of medical procedures (e.g. placement of urinary catheters), which further violate epithelial barriers.

In addition to horizontal transmission of pathogens, there was a vertical one, because our study site is an institution of tertiary care which receives patients from secondary care hospitals within the region. In hospitals without consistent antibiotic policy and with practice of injudicious utilization of antibiotics, patients rapidly become potential sources of infection, particularly of MDR bacteria. A recent study of Falagas and Kopterides [28] found higher rate of MDR bacterial isolates in patients with a history of previous hospitalizations. Increased vigilance and complete implementation of infection control policies and practices in these hospitals could be one part of the solution.

After taking into account individual characteristics of the patients, multivariate analysis showed that only cancer of various localizations increases the risk of HAUTI caused by MDR pathogens by 2.3 times. Generally speaking, patients with cancer frequently have HAUTIs, due to immunosuppression caused by the malignancy itself or by cytotoxic therapy. Also, information on previous catheterization, and previous hospitalization, which are often found in the history of patients with malignant tumors, may explain increased prevalence of perineal colonization with potential MDR pathogens in oncology patients, which may be important for the development of HAUTI [29].

A disturbing result of our study was that isolates of *Klebsiella* spp. showed high resistance (over 90%) to trimethoprim-sulfamethoxazole, third- and fourth-generation cephalosporins, and ciprofloxacin, antibiotics that are commonly used to treat infections caused by these microorganisms in hospitals. Such high rates of resistance limit their use in empirical therapy. The results were significantly worse than in other countries [30, 31], and are even more significant given the high proportion of *Klebsiella* spp. causing HAUTIs in our study (32.4%). This could be explained by a wide use of these antibiotics for treatment of HAUTIs and community-acquired urinary tract infections over the past decade in this region. Further efforts of the entire community are necessary in order to maintain sensitivity of urinary pathogens to these antibiotics in the future.

The degree of *Proteus mirabilis* resistance to antibiotics in our study was generally high for all tested antimicrobials. Thus, the resistance to trimethoprim-sulfamethoxazole was 91.3%, to cephalosporins of the third generation 89.5–91.0% and to ciprofloxacin 84.1%, which are much higher rates than those observed in other studies [29, 30].

Isolates of *E. coli* in our study showed high degree of resistance to trimethoprim-sulfamethoxazole (84.1%) and ciprofloxacin (80.8%), while the resistance to aminoglycosides was somewhat lower (77.5% to gentamicin, and 48.1% to amikacin). Such high resistance rates are two to three times higher than in other recent studies [31, 32], but it was already reported in the study conducted in the same hospital during the previous period [9]. Particularly worrying is resistance of *E. coli* isolates to broad-spectrum antibiotics such as fluoroquinolones and cephalosporins due to over-utilization of these two groups and parallel development of co-resistance to other antibiotics (collateral damage) [33].

Carbapenems were increasingly used during the 1980s for treatment of serious nosocomial infections. However, the emergence of resistant gram-negative bacilli to these antibiotics is nowadays depriving doctors of these very active antibiotics against nosocomial infections. However, in our study, resistance to carbapenems was relatively low (around 10%, except that of *Pseudomonas aeruginosa*, which was around 45%), which is encouraging. According to the recommendations of European Association of Urology, carbapenems should be used as therapy for complicated cases of HAUTIs [5]. Our results emphasize the importance of optimizing the use of carbapenems, in order to preserve their activity in the future. Hospitals that achieved at least some control over the use of carbapenems halted further increase in resistance to these antibiotics [34].

Having taken into account the incidence of each bacterium (nearly 90% of all isolates were gram negative) and rate of resistance in our study, it can be concluded that the role of fluoroquinolones and aminoglycosides in the empiric treatment of HAUTIs is becoming more and more limited. Available antimicrobials with good activity against majority of pathogens include cephepe and carbapenems. The therapy should be adjusted according to local data on bacterial susceptibility to antibiotics. Whenever possible, empirical therapy should be replaced by a therapy targeted to the specific infective organisms identified in the urine culture. Appropriate antimicrobial selection, surveillance systems, and effective infection control procedures are key measures for limiting antimicrobial-resistant pathogen occurrence and spread.
The overall mortality rate in our study was similar in the groups with and without MDR infections; we were not able to compare the rates of urinary tract infections – attributed mortality, which is reported to be low [35]. Only 5% of patients with bacteriuria develop bacteremia, but mortality rate in these patients is almost 10% [35]. There are certain limitations of our study. First, the study was conducted in a single center, reflecting the possibility of institutional bias either in the selection of patients or routine medical practices. Second, we were not able to conduct molecular epidemiological research in order to discover the mechanisms by which the drug resistance developed. In addition, we did not determine the minimum inhibitory concentration of the studied antibiotics.

CONCLUSION

The results of our study point to hospitalization for more than eight days before insertion of urinary catheter, to prolonged hospitalization, to previous stay in another department, and to cancer of various localizations as important risk factors associated with HAUTI caused by MDR pathogens. Early removal of urinary catheter and reduction of time spent in a hospital or an ICU could contribute to a decrease in the rate of these infections. Moreover, good knowledge of the susceptibility profile of isolated pathogens should help physicians when prescribing empiric therapy. Our study emphasizes the need for aggressive infection control strategies to prevent urinary tract infections with MDR pathogens.

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REFERENCES


Фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним микробионима

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КРАТАК САДРЖАЈ
Увод Болничке инфекције уринарног тракта чине и до 40% свих болничких инфекција и значајно доприносе болничком морбидитету, морталитету и расту укупних трошкова лечења.

Циљ рада Циљ нашег истраживања био је да утврдимо по-

tенцијални фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним микробионима (МР патогенима).

Методе рада Спроведена је клиничка студија типа случај

 kontrola у периоду од пет година у здравственој установи терцијарног нивоа здравствене делатности. Групу случајева чинили су болесници са болничким инфекцијама уринарног тракта узрокованим МР патогенима, док су контролну групу чинили пациенти са болничким инфекцијама уринарног тракта узрокованим бактеријама које нису припадале претходно наведеној групи уринарних инфекција.

Резултати Укупно било је 562 (62,6%) болесника са верифику-нанима МР изолатима, односно 336 (37,4%) пацијена-
tа са изолатима који нису припадали МР групи патогена.

Идентификована су четири значајна предиктора који могу допринети развоју болничких инфекција уринарног тракта узрокованих мултирезистентним патогенима: хоспита-
lизация присуствујућих болничких инфекција, односно код лица од осам дана (OR \textsubscript{adjusted} = 2,763; 95% CI = 1,352–5,647, p = 0,005), укратко боравак на другом нивоу медицинске услуге (OR \textsubscript{adjusted} = 1,547–2,970, p < 0,001), претходна хоспитализација од 15 и више дана (OR \textsubscript{adjusted} = 2,144; 95% CI = 1,585–2,908, p < 0,001) и карцином различних локализација (OR \textsubscript{adjusted} = 2,313; 95% CI = 1,255–4,262; p = 0,007).

Закључак Правовремено (рано) укидање уринарног катетера и смањење времена проведеног у болници или у ин-

tезивној зони могли би значајно да допринесу редукцији стопе болничких инфекција уринарног тракта узрокованих мултирезистентним патогенима.

Кључне речи инфекције уринарног тракта; болничке инфекције; уринарни катетер; МР патогенима.

Фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним микробионима

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tенцијални фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним патогенима (МР патогенима).

Методе рада Спроведена је клиничка студија типа случај
kontrola у периоду од пет година у здравственој установи терцијарног нивоа здравствене делатности. Групу случајева чинили су болесници са болничким инфекцијама уринарног тракта узрокованим МР патогенима, док су контролну групу чинили пациенти са болничким инфекцијама уринарног тракта узрокованим бактеријама које нису припадале претходно наведеној групи уринарних инфекција.

Резултати Укупно било је 562 (62,6%) болесника са верифику-


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