ACTA MICROBIOLOGICA BULGARICA Volume 35 / 4 (2019)

Antimicrobial Resistance of the Most Prevalent Urinary Isolates from Outpatients of the Upper Vrbas Region in Bosnia and Herzegovina

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Abstract

The aim of the study was to determine the frequency and antibiotic susceptibilityof urinary pathogens isolated from outpatients of both genders divided into three age categories. A prospective study was carried out in the period from December 2017 to April 2018 of the Upper Vrbas Region in Bosnia and Herzegovina. Midstream samples of urine (1 444) were taken and sent to the microbiologic laboratory for further evaluation. Antibioticssusceptibility patternsaccording to EUCAST standards were observed. Data were analyzed by age and gender. The results showed that *E. coli* was the most prevalent cause of urinary infections in females (86%), followed by *Klebsiella pneumoniae* (16%). The most prevalent isolates in males were caused by *Enterobacter* spp. (37%), *K. pneumoniae* (33%), and besides that, strains of *Enterococcus* genus were reported. *E. coli* showed the highest resistance to ampicillin (77% in females vs. 100% in males) and gentamycin (100% in females vs. 45% in males) but also high resistance to cephalosporines (30-50%), fluoroquinolones (30%) and penicillin. *Enterococcus* spp. isolates showed resistance to trimethoprim/sulfamethoxazole and phosphomycin in males (100%), but were also highly resistant to fluoroquinolone activity. Isolates of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Hafnia alvei*, *Serratia marcescens* and *Acinetobacter* spp. showed low resistance percentages. In conclusion, *E. coli* is the most prevalent cause of urinary tract infections, especially in females. In addition to *E. coli*, a large number of female urinary infections are caused by *K. pneumoniae*, *Enterococcus* spp. and *Enterobacter* spp.

Keywords: antimicrobial resistance, *Escherichia coli*, ESBL strains, *Klebsiella pnumoniae*, EUCAST, urinary

Резюме

Целта на изследването е да се определи честотата и чувствителността към антибиотици на пикочните патогени, изолирани от амбулаторно болни от двата пола, разделени в три възрастови категории. Проведено е проспективно проучване в периода от декември 2017 г. до април 2018 г. в района на Горна Връбас в Босна и Херцеговина. Взети са средни проби от урина (1 444) и са изпратени в микробиологична лаборатория за допълнителна оценка. Наблюдавани са модели на чувствителност към антибиотици съгласно стандартите на EUCAST. Данните са анализирани по възраст и пол. Резултатите показват, че *Escherichia coli* е най-разпространеният причинител на уринарни инфекции при жени (86%), следвана от щамове *Klebsiella pneumoniae* (16%). Най-разпространените причинители при мъжете са *Enterobacter* spp. (37%) и *K. pneumoniae* (33%), като освен тях се съобщават щамове от род *Enterococcus*. *E. coli* показва най-високата резистентност към ампицилин (77% при жените срещу 100% при мъжете) и гентамицин (100% при жените срещу 45% при мъжете), но също така и висока устойчивост към цефалоспорини (30-50%), флуорохинолони (30%) и пеницилин. Изолатите от *Enterococcus* spp. от мъже показват 100% резистентност към триметоприм/сулфаметоксазол и фосфомицин и висока устойчивост към флуорохинолонова активност. Получените данни за изолати от *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Hafnia alvei*, *Serratia marcescens* и *Acinetobacter* spp. демонстрират ниска степен на резистентност. В заключение може да се отбележи, че *E. coli* е най-разпространеният причинител на инфекции по пикочните

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пътища, особено при жените. Освен това, голям брой пикочни инфекции при жените се причиняват от *Klebsiella pneumoniae*, *Enterococcus* spp. и *Enterobacter* spp.

Introduction

Urinary tract infections (UTI) are very prevalentand occur approximately in 150 million people per year (Flores-Mireles *et al*., 2015). They are more common in females than in males and are caused by bacterial pathogens with high adherence potential (Colgan and Williams, 2011). Most commonly, they occur in between the ages of 16 and 35, and reinfections are frequent. Healthy people with a strong immune response generally have no problem fighting an invasion of any bacteria. It should be known that the urine is naturally sterile and its environment is suitable for growth and division of bacteria, whether commensals or pathogens.

Bacterial adhesion is a key role in any pathogenesis of UTI. Urinary tract infection usually begins with periurethral contamination of the urinary pathogen originating from the intestine, followed by urethra colonization and later migration of the pathogen into the bladder, an event requiring the presence of flagellas or pilots. In the urinary bladder, the consequence of a complex interaction of the pathogen-host is either a successful colonization of the uropathogen or its elimination. A large number of bacterial adhesins recognize receptors on the bladder epithelium (also known as uroepithelium) and mediate colonization. Uropathogens such as uropatogenic *Escherichia coli*-UPEC have survived the invasion of the bladder epithelium, creating toxins and proteases to release nutrients from the host cell and synthesizing siderophores for iron supply. Uropathogens may subsequently reach the kidney safter frequency division and recapturing through adhezine or colonization of the kidney epithelium, producing toxins that damage the tissue. As a result, uropathogens can cross the intestinal epithelial barrier to access the bloodstream, initiating bacteraemia. Uropathogens that cause uncomplicated urinary infections, including UPEC, *Klebsiella pneumoniae* and *Staphylococcus saprophyticus*, have the ability to bind directly to the kidney epithelium.

UPEC and *K. pneumoniae* bind to the uroplakins, the main protein components of the apical membrane surface cells (Khandelwal *et al*., 2009), which form a crystal sequence that protects mammalian urinary bladder tissue from harmful urinary agents (Lee, 2011). In addition to uroplakins, α3β1 integrins expressed on the surface of uroepithelial cells can also serve as receptors for UPEC (Eto *et al*., 2007). In contrast, complicated urinary infections are initiated when the bacteria bind to the urinary catheter, kidney stone or bladder stone or when they are retained in the urinary tract by physical obstruction. Some pathogens (UPECs) may also cause uncomplicated and complicated urinary infections. However, others such as *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Enterococcus* spp. mainly cause complicated urinary infections. They often form biofilms that are responsible for colonization and persistence (Jacobsen and Shirtliff, 2011; Niveditha *et al*., 2012).

Uropathogenic *E. coli* from the intestine causes 80-85% of the urinary tract infections, together with *S. saprophyticus* as a causative agent of 5-10% of infections (Nicolle, 2008; Abraham and Miao, 2015). Urinary infections can be caused by a huge number of pathogens: *E. coli* (27%), *Klebsiella* (11%), *Pseudomonas* (11%), *Candida albicans* (9%), *Enterococcus* (7%) (Bagshaw and Laupland, 2006; Salvatore *et al*., 2011; Sievert *et al*., 2013). Urogenital tract infections caused by *Staphylococcus aureus* usually occur as secondary infections that are transmitted by blood (Lane and Takhar, 2011).

Antimicrobial resistance (AMR or AR) is the ability of particular microorganisms (bacteria, fungi, viruses or parasites) to resist the effects of drugs (antibiotics, antifungicides, antiviral drugs) that could successfully fight such microorganisms (CDC, 2017). The term antibiotic resistance (AR or ABR) is a subset of antimicrobial resistance, and refers to resistance to antibiotic resistant bacteria. Resistant microorganisms are more difficult to cure, require alternative treatments or higher doses of antimicrobial drugs. These approaches can be more expensive, more harmful, or both. Microorganisms resistant to multiple antimicrobial drugs are termed multi-resistant microorganisms or MDRs (multidrug-resistant). Those that are extensively resistant to antimicrobial drugs are referred to as extensively drug-resistant-XDR, and those that are fully resistant to all antimicrobial drugs are labeled totally drug-resistant-TDR and in science they are termed "superbugs". New resistance mechanisms appear constantly and are widespread globally, threatening the treatment of contagious diseases, resulting in longer-lasting illness, disability, and even death. Without effective antimicrobial agents for the prevention and treatment of infections, medical procedures such as organ transplantation, cancer chemotherapy, diabetes treatment,

and even surgical procedures, become a major risk of infection. Antimicrobial resistance increases the cost of health care, prolonging hospital stay and the need for more intensive care. Drug-resistant microorganisms are found in humans, animals, food, and even in the environment (water, soil and air). They can spread between humans and animals by eating animal food, but also from person to person. Poor infection control, inadequate sanitation, and inadequate handling of food stimulate the spread of antimicrobial resistance (WHO, 2018).

The resistance of *K. pneumoniae* to carbapenem antibiotics has spread to all regions of the world. *K. pneumoniae* is the major cause of hospital infections such as lung inflammation, blood infections and infections in newborns and intensive care patients. Resistance of *E. coli* to fluoroquinolone antibiotics (one of the most common antibiotics for urinary tract infections) is very widespread. There are countries in many parts of the world where this treatment is ineffective in more than half of the patients.

According to the ECDC report for 2016, in Europe *E. coli* has shown resistance to quinolones of 59.8%, ciprofloxacins 64%, ampicillin 58%, sulphamethoxazole 49.9%, tetracycline 47.1% and trimethoprim/sulfamethoxazole 47.1 %. It could also be established that there are 24 significantly decreasing and 10 increased levels of ampicillin resistance among the 11 EU member states.

The aims of the study were to obtain the following: to determine the frequency of pathogens in the etiology of UTI of the Upper Vrbas Region in BiH in outpatient population in relation to gender and age distribution; to determine the difference in the frequency of isolated pathogens in the etiology of urinary infections in the time interval of the study, and investigate the antimicrobial sensitivity of isolated strains to applied antibiotics and chemotherapeutics.

Material and Methods

The prospective study was carried out investigating the outptient population of different gender and age in the period from January 2017 to April 2018.

The study included 1 444 urine specimens. Antibiotics susceptibility patterns according to EUCAST standards were observed. Midstream urine specimens of all patients were evaluated by the microbiologists in the laboratory of the hospital of Bugojno, Upper Vrbas Region. The disc diffusion test was applied to determine antimicrobial susceptibility, as described by the National Committee for Clinical Laboratory Standards. The Hospital Microbiology Laboratory in Bugojno used two methods to prove ESBL strains of bacteria: the double disk method (DDST) and the combined disk method. For the method of combined discs, the microbiology laboratory Hospital used MASTDISCSTM ID ESβL tests (Mast Group, UK).

Statistical data analysis was performed based on the collected data from medical documentation (referrals, findings, protocols) and laboratory analyses. Microsoft Office Excel 2010 and SPSS 2.0 (Statistical Package for the Social Sciences) programs were used. The results of the research and statistical analysis are presented in text, by tables and charts, as absolute numbers (N) and percentages (%).

Results

E. coli, *K. pneumoniae*, *Acinetobacter* spp., *H. alvei*, *S. marcesecens*, yeast *Candida* spp., bacteria from the genera *Enterococcus*, *Enterobacter*, *Streptococcus*, *Staphylococcus*, *Proteus*, *Pseudomonas*, were isolated from urine cultures of outpatients of different gender and age. Out of 1 444 urine cultures, 358 were positive isolates during the period of testing from December, 2017 to April, 2018. The statistical difference was established in meaning of the percentage of positive urine cultures by months of research (34% in December; 22% in January; 25% in February, 21% in March and 26% in April) and the arithmetic mean was 25%.

The percentage of all isolated microorganisms according to gender distribution for the period from December to April is presented in Figs. 1-5.

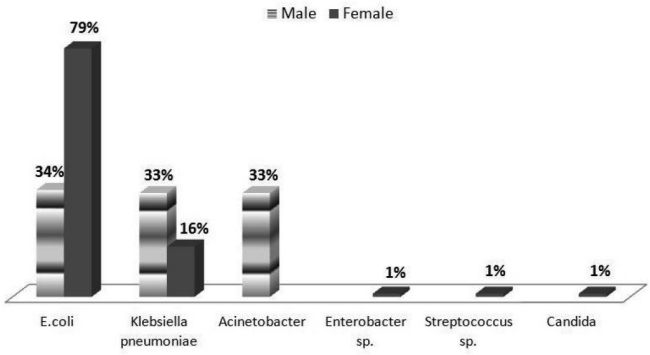

Fig. 1. The percentage of all isolated pathogens according to gender distribution (December, 2017)

According to sample size, patient age data were divided into three age categories asfollows: up to 14 years, from 14 to 65, and over 65 years of age. High resistance rates were recorded after antibiotic susceptibility testing. The resistance profile of *E. coli* in Figs. 6 and 7 reveal a high resistance rate in females and males, divided by age categories.

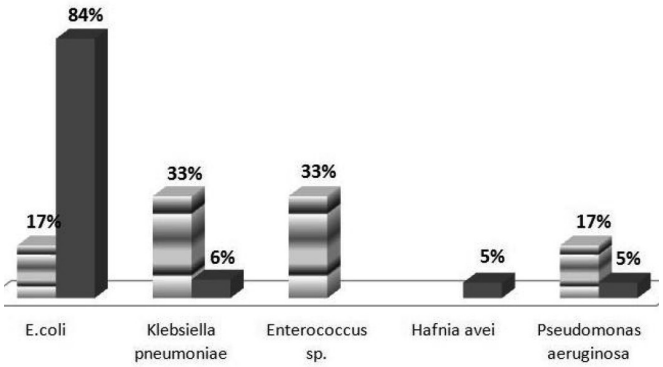

Fig. 2. The percentage of all isolated pathogens according to gender distribution (January, 2018)

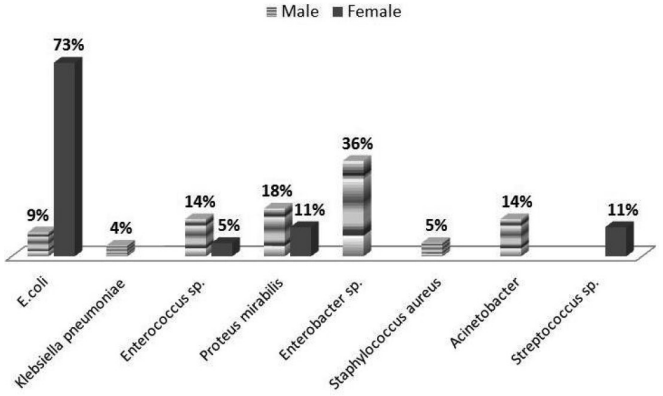

Fig. 3. The percentage of all isolated pathogens according to gender distribution (February, 2018)

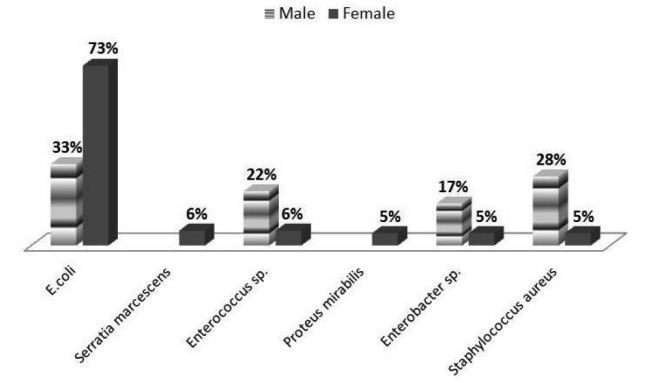

Fig. 4. The percentage of all isolated pathogens according to gender distribution (March 2018)

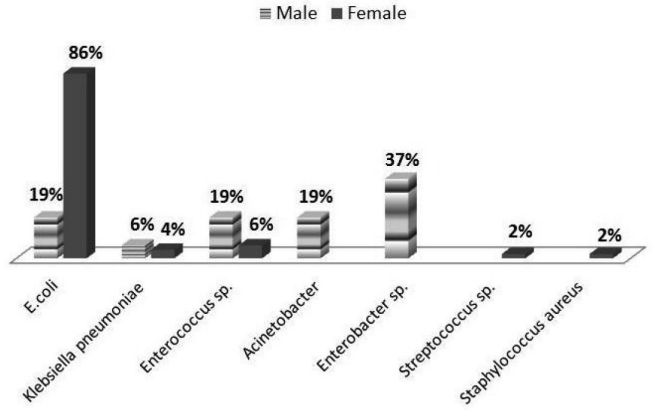

Fig. 5. The percentage of all isolated pathogens according to gender distribution (April 2018)

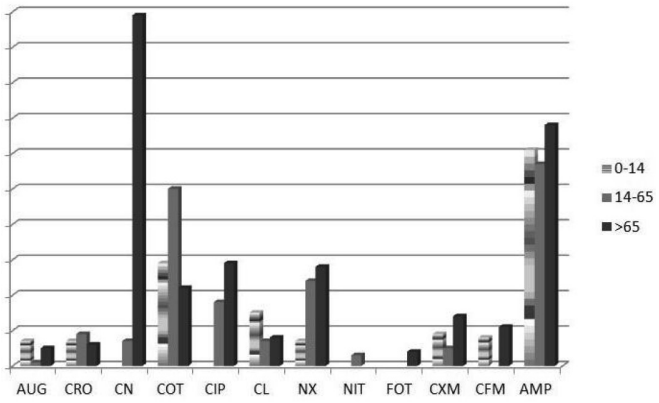

Fig. 6. Resistance profile of *E. coli* in females by age groups

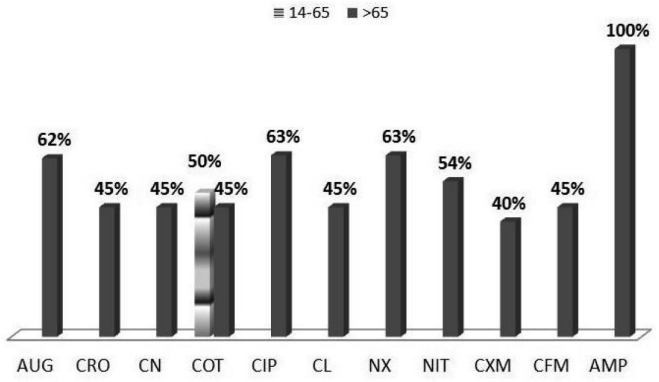

Fig. 7. Resistance profile of *E. coli* in males by age groups

Discussion

During this prospective study, for a period of five months a total of 1 444 urine cultures from 358 isolated pathogens of UTI in different genders and age groups of outpatients of Upper Vrbas Region were evaluated. The most common cause of urinary infections was primarily *E. coli*, but other bacterial species from the *Enterobacteriaceae* family were also isolated. Isolation and identification of UTI pathogens was performed based on their biochemical properties, but also with visible morphological characteristics on nutrient media. By analysing the data the presence of isolated pathogens in patients of different genders was revealed. The most prevalent UTI pathogen in females during the test period was certainly *E. coli*, followed by high rates of *K. pneumoniae*.

Pathogens such as *H. alvei*, *S. marcescens*, *Candida* spp., and *Streptococcus* strains were identified only in females. For comparison, the most prevalent cause of UTI in males was *K. pneumoniae*, but also bacteria from the genus *Enterococcus* and *Enterobacter* were recorded at high rates, showing greater prevalence in relation to females. *Acinetobacter* spp. was reported as a causative agent in males, without isolation in females. In

addition to these pathogens, *P. aeruginosa*, *P. mirabilis* and *S. aureus* were also isolated, almost equally in both genders. Since *E. coli* was the most prevalent cause in females, it was found that this bacterium caused most UTI in the age group 14 to 65 years, but a high rate was also observed in the group over 65 years of age. *E. coli* in males is not a common cause of urinary infections, and in this study it was mostly represented in the age group 14 to 65 years. *K. pneumoniae* was observed at high rates in females in age groups 14 to 65 years, and over 65 years, while in males in the age group 14 to 65 years. *Enterococcus* spp. is a cause of urinary infections in males, mainly in the age group 14 to 65 years, while in females it is almost the same in all age groups, but at a low rate.

Resistance to antibiotics is a major problem in the treatment and prevention of urinary infections. Bacteria have the ability to continuously develop new ways and types of antimicrobial resistance, as well as the ability to spread the resistance gene by exchanging it with other bacteria. Resistance to antibiotics is increased by excessive antibiotic use, but also by the absence of newly synthesized compounds with antimicrobial activity. During this study, sensitivity to a large number of antibiotics was tested in different groups, and high percentages of resistance rates were recorded.

E. coli and *K. pneumoniae* showed high resistance rates to a wide spectrum of antibiotics and the reason was the isolation of extended-spectrum beta-lactamases (ESBL) of these bacteria.

E. coli was most resistant in females and males in the elderly group over 65 years. The highest resistance rates were observed for ciprofloxacin and norfloxacin (about 30%), ampicillin (69%) and gentamicin (100%) in females in the above mentioned group. A high rate of resistance to 11 antibiotics and the highest rates of resistance to nitrofurantoin above 50%, amoxicillin-clavulanic acid, ciprofloxacin and norfloxacin (over 60%) and ampicillin (100%) were recorded in males in this group.

In the age group 14 to 65 years, the highest percentage of resistance of about 50%was observed in both genders to trimethoprim/sulfamethoxazole. High rates of resistance to cephalosporins have been reported especially in males, where they exceed 40%.

Nowadays, approximately 50% of *E. coli* has gained resistance to ampicillin in many parts of the world. Since resistance to ampicillin in *E. coli* is mediated by the production of broad-spectrum beta-lactamase, belonging to class A and sensitive to inhibitors such as clavulanic acid, the addition of clavulanate greatly protected ampicillin and reversed its efficacy. Consensus about UTI is difficult to reach, and the European Committee on Antimicrobial Sensitivity Testing (EUCAST) several times changed the border concentrations and interpretation of the sensitivity of enterobacteria to this antibiotic.

In our study, resistance to ciprofloxacin, levofloxacin and norfloxacin by *Enterococcus* spp. was found at 75% in males in the ages 14 to 65, and over 65 years, and exceptionally high resistance rates (100%) to trimethoprim/sulfamethoxazole and phosphomycin in the age group above 65 years. In this age group resistance to ciprofloxacin and norfloxacinwas also found in females.

Lower resistance rates (25%) in males were found to ampicillin, vancomycin and amoxycillin-clavulanic acid. Similar research suggests that *Enterococci* are intrinsically resistant to some penicillins, all cephalosporins and in small amounts to aminoglycosides. In fact, they have the ability to develop resistance to most other classes of antibiotics (Linden, 2007; Chou *et al*., 2008). Because of the low affinity levels for penicillin binding proteins (PBPs), they can develop intrinsic resistance to most beta-lactam antibiotics. *P. aeruginosa* has shown resistance to the action of levofloxacin, piperacillin-tazobactam, cefepime and ceftazidime antibiotics in our isolates. Similar results were found during the study from 2005 to 2010, where resistance to cepefime, ceftazidime and piperacillin-tazobactam remained unchanged at a rate of 23 to 26%. *P. aeruginosa* is intrinsically resistant to numerous beta-lactam antibiotics including amoxicillin, first and second generation cephalosporins, cefotaxime, ceftriaxone and ertapenem. *P. aeruginosa* also has the ability to obtain beta-lactamase, including ESBL and carbapenemase (Ruppé *et al*., 2013). The reduced permeability of the outer membrane caused by qualitative or quantitative changes of the OprDporin, which controls the passage of imipenem through the outer membrane, gives *P. aeruginosa* basal resistance to carbapenems, especially to imipenem (Li *et al*., 2012). Resistance of *P. aeruginosa* to fluoroquinolone significantly increased over time, from about 22% in 2005 to 33% in 2010. Resistance to imipenem remained unchanged at a rate of 20%. However, the SMART study has shown that the activity of selected antimicrobial drugs varies in different regions of the world. In South Africa, during the 2004-2009 pe-

riod, resistance to piperacillin-tazobactam was approximately 8%, while resistance to cefepime, ceftazidime and imipenemwas approximately 25%, and 27% to amikacin.

During the relatively same time period (2002-2009), in China, *P. aeruginosa* resistance to amikacin was 12% and to piperacillin-tazobactam was 8% (Babinchak *et al*., 2010; Morrissey *et al*., 2013).

P. mirabilis was resistant to trimethoprim/sulfamethoxazole, nitrofurantoin, cefotaxime, gentamicin, ceftriaxone, ceftazidime, ciprofloxacin, levofloxacin and norfloxacin antibiotics according to our results. Resistance to beta-lactams (penicillin and cephalosporin), fluoroquinolones, nitrofurantoin, phosphomycin, aminoglycosides, tetracycline and sulphonamides (Schito *et al*., 2009; Ma and Wang, 2013; Adamus-Bialek *et al*., 2013) is described. These results coincide with ours. *P. mirabilis* is also highly resistant to antimicrobial peptides, including polymyxin B, protegrin, LL-37 and defensin (McCoy *et al*., 2001; Belas *et al*., 2004). This resistance relies on LPS modification and extracellular protease such as ZapA. The issues of the increasing antibiotic resistance to this organism are largely similar to those to UPEC.

Candida spp. is actually a commensal member of the gastrointestinal microbiota and homeostasis with the host. However, this homeostasis can be disturbed, where the yeast can pass through the intestinal mucous barrier and cause dissemination (Yan *et al*., 2013; Schulte *et al*., 2015). Consequently, invasive candidiasis is considered to be predominantly from this reservoir (Miranda *et al*., 2009). *H. alvei* ESBL has been recorded as a cause of urinary infection in a female patient over the age of 65. Thus, the bacterium was only susceptible to imipenem and medium sensitive only to amikacin, while resistance was recorded to a variety of antibiotics including amoxicillin-clavulanic acid, ceftriaxone, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, cefalecin, cefuroxime, cefixim, norfloxacin, phosphomycin, ceftazidime, cefotaxime, cefepime, cefoxite, levofloxacin, moxifloxacin and piperacillin/tazobactam. *H. alveolus* is often a cause of urinary infections, and a number of studies are being discussed. It is isolated from the community of microorganisms that cause urinary system and hemolytic-uremic syndrome infections (Crandall *et al*., 2006). This bacterium can cause urinary tract infection, but also sepsis in infants (Laupland *et al*., 2006). The Laboratory Survey of 2006 by the Institute for Clinical and Laboratory Standards showed that *H. alvei* was isolated at a rate of 81% as a cause of urinary tract infection. In the Auda Al-Grawistudy (2008) of a total of 250 bacterial isolates 220 *H. alvei* isolates were reported in urinary infections patients. The bacteria showed sensitivity to cefotaxime, ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole and doxycycline, and resistance to penicillin, oxacillin and amoxicillin. Many studies of antimicrobial sensitivity have shown the same results as this study (Girlich *et al*., 2000; Janda and Abbott 2006).

S. marcescens was also reported as a cause of UTI, in the age group of females above 65 years of age. The bacterium was only sensitive to the action of three antibiotics, namely amikacin, imipenem and piperacillin/tazobactam. On the other hand, bacteria have shown resistance to a variety of antibiotic agents including ceftriaxone, gentamicin, trimethoprim/sulfamethoxazole,ciprofloxacin, levofloxacin, phosphomycin, norfloxacin, cefixim, cefepim, cefotaxim and ceftazidim.

Many of the clinical isolates of this microorganism carry chromosomal and plasmid-encoded determinants that determine resistance to a broad spectrum of antibiotics (Mahlen, 2011), producing ESBL and MBL. For example, a study in Poland between 1996 and 2000 in two hospitals showed a rate of 19% (67/354) *S. marcescens* isolates produced by ESBL (Naumiuk, 2004). Similarly, in Taiwan, from 2001 to 2002, the rate of 12% (15/123) indicated the strains of this bacterium produced by ESBL, resulting in mortality of 33% (Cheng *et al*., 2006). During the second half of the 1990s, an increasing number of epidemics of this imipenem-resistant microorganism (Troillet, 1999) and extended spectrum beta-lactamases (Pagani *et al*., 1994; Luzzaro *et al*., 1998) were reported. During the research that lasted 12 years, more precisely in the period from 1991 to 2002, 4 972 urinary tract infections were caused by *S. marcescens*.

According to the Croatian research by Pastuović *et al*. (2008), a total of 28 097 samples of urine were processed in 2007. The most common isolate was *E. coli* (46.5%). Out of 3 367 individuals with *E. coli* isolates, 2 595 were female (79.98%) and 674 were male (20.02%). *E. coli* is highly resistant to amoxicillin (57.8%), followed by sulfamethoxazole/trimethoprim (33.8%) and cephalexin (10.8%).

According to research in Europe for the past twenty years, the resistance of isolated bacteria from the family of *Enterobacteriaceae* has occurred in about 30% of isolated strains with a constant tendency of growth, which was officially confirmed in

2001. Then, extreme resistance of certain bacterial isolates to antimicrobial agents ranged from 66.6 to 92.3%. According to the European Survey on Antibiotic Sensitivity (ESGNI-003), 54.8% of *E. coli* isolates showed resistance to ampicillin, 14.2% to amoxicillin, and 2.8% to trimethoprim sulfamethoxazole (Buoza *et al*., 2001).

Conclusion

E. coli is the most prevalent cause of urinary infections, especially in females. Additionally, a large number of female urinary infections are caused by *K. pneumoniae*, *Enterococcus* spp. and *E. aerogenes*.The largest number of urinary infections in males are caused by *Enterobacter* spp. and *K. pneumoniae*. Individual infections caused by *P. mirabilis*, *P. aeruginosa*, *S. aureus*, *Streptococcus* spp., *Acinetobacter* spp. and *H. alvei* are documented. *E. coli* is the most common cause in the age group 14 to 65 years in both genders. The highest resistance rates of *E. coli* have been reported in the activity of ampicillin (70% females, 100% males) and gentamicin (100% females, 45% males), but high resistance rates to a large the number of cephalosporins (30 to 50%, depending on the antibiotic), as well as to the fluoroquinolones (30%).

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