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ORIGINAL ARTICLES

ETIOLOGICAL SPECTRUM AND ANTIMICROBIAL RESISTANCE OF THE MOST FREQUENTLY ISOLATED PATHOGENS, ASSOCIATED WITH URINARY TRACT INFECTIONS IN AMBULATORY PATIENTS

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ABSTRACT

INTRODUCTION: Urinary tract infections (UTIs) are among the most common infections both in the community and in the hospital setting.

AIM: The aim of this study is to investigate the etiological spectrum and antimicrobial resistance of most frequently isolated pathogens, associated with UTIs in ambulatory patients in Varna city, Bulgaria during a seven-month period (October 2020–April 2021).

MATERIALS AND METHODS: A total of 1600 urine samples, collected from patients with suspected UTIs were tested. Screening for bacterial growth was done by HM&L Uroquattro instrument (ALIFAX, Italy). Species identification and antimicrobial susceptibility testing were performed by VITEK 2 Compact System (bioMerieux) and Kirby-Bauer disk diffusion method.

RESULTS: *E. coli* was the most commonly isolated uropathogen regardless of the age group (48%, n=61), followed by *Enterococcus faecalis* (33%, n=42) and other representatives of order *Enterobacterales* (13.3%, n=18). The resistance rates in the group of Gram-negative isolates (n=79) in decreasing order were as follows: ampicillin, 64.5% > trimethoprim/sulfamethoxazole, 36% > ciprofloxacin, 29.1% > amoxicillin-clavulanic acid, 27.8% > cefuroxime, levofloxacin, 21.5% > fosfomycin, 13.1% > ceftriaxone, 12.6% > ceftazidime, 10.1% > gentamicin, nitrofurantoin, 6.5% > nitroxoline, 4.9%. The resistance rates among the isolates of *E. faecalis* (n=42) were as follows: ciprofloxacin, 28.6% > gentamicin, 23.8% > levofloxacin, 19% > nitrofurantoin, 4.7% > amoxicillin, 2.4%. No resistance to meropenem, amikacin, vancomycin, and teicoplanin was found in the studied collection of isolates (n=127).

CONCLUSION: The etiological spectrum of UTIs in ambulatory patients was dominated by *E. coli*, followed by *E. faecalis*. In the group of Gram-negative uropathogens, high resistance rates to ampicillin, trimethoprim/sulfamethoxazole and quinolones were detected. Third-generation cephalosporins, fosfomycin, nitrofurantoin and nitroxoline retained very good activity. Among *Enterococcus faecalis* isolates, the second most commonly isolated bacterial species, a decreased activity of quinolones was found too, but the aminopenicillins and nitrofurantoin remain highly active.

Keywords: *urinary tract infection, etiology, antimicrobial resistance*

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common infections both in the community and in the hospital setting. Regardless of the age group, the most important pathogen of UTIs is *Escherichia coli*, followed by other representatives of order *Enterobacterales* (*Proteus* spp., *Klebsiella* spp., etc.), *Enterococcus* spp., and *Staphylococcus saprophyticus* (1). Adult women are 30 times more likely to develop a UTI than men, with almost half of them experiencing at least one episode of UTI during their lifetime (2, 3). Nowadays UTIs caused by multiple-drug resistant Gram-negative bacteria are a growing concern due to limited therapeutic options. These organisms easily acquire genes that encode a variety of antibiotic resistance mechanisms, including production of extended-spectrum beta-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases (4). The assessment of a patient's previous urinary pathogen and susceptibility profile is essential in selecting appropriate empiric antibiotic therapy.

AIM

The aim of this study is to investigate the etiological spectrum and antimicrobial resistance of most frequently isolated pathogens, associated with community-acquired UTIs in Varna city, Bulgaria during a seven-month period (October 2020–April 2021).

MATERIAL AND METHODS

A total of 1600 urine samples, collected prospectively from ambulatory patients with symptoms of UTI were studied. The following diagnostic criteria for UTIs were used: a microbial count of $\geq 10^5$ CFU/mL for clean catch midstream samples or any bacterial growth of a single pathogen for urine samples collected from patients with urethral catheterization. Gram microscopy was directly performed from the Uro-Quick screening broth of all positive samples after screening on HM&L instrument (Alifax, Italy). Bacterial isolates were identified using routine biochemical identification and were confirmed by VITEK (bioMérieux, France). Antimicrobial susceptibility testing to set of antimicrobial agents (ampicillin, amoxicillin-clavulanic acid, cefuroxime, ceftriaxone, ceftazidime, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, gentamicin, amikacin, meropenem, imipenem) was performed by VITEK 2 Compact System (bioMerieux) and for fosfomycin, nitroxoline and nitrofurantoin by Kirby-Bauer disk diffusion method. The results were interpreted according to EUCAST Guidelines 2021 (Version 11). The detection of extended-spectrum beta-lactamase production (ESBL) was done by the double-disk synergy test (DDST) using amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg) and ceftazidime (30 μg) discs (MAST group, UK) according to EUCAST Guidelines 2021.

The study was approved by the Ethical Committee of the Medical University of Varna (protocol No. 92/02.04.2020).

RESULTS

A total of 127 urine samples (7.9%) were positive for bacterial growth by HM&L. From these samples, 127 bacterial pathogens were isolated: Gram-negative bacteria were found in 62.2% (n = 79) and Gram-positive—in 37.8% (n=48). *E. coli* was the predominant species, associated with UTIs in the studied patient group (77%, n = 61). Other representatives of order *Enterobacterales* were also identified: *Klebsiella* spp. (n =7, 8.9%); *Enterobacter cloacae complex* (n=4,5%); *Proteus* spp. (n = 3, 3.7%); *Citrobacter* spp. (n=1, 1.26%); *Morganella morganii* (n=1, 1.26 %) and *Serratia marcescens* (n=1, 1.26%). A single isolate of *Pseudomonas aeruginosa* was also detected. The Gram-positive bacteria accounted for 37.8 % (n = 48), with *Enterococcus faecalis* being the leading pathogen in this group (87.5%, n=42). *Staphylococcus saprophyticus* and *Streptococcus agalactiae* were diagnosed in 8% (n=4) and 4% (n=2), respectively.

The resistance rates in the group of Gram-negative isolates in decreasing order were as follows: ampicillin, 64.5% > trimethoprim/sulfamethoxazole, 36% > ciprofloxacin, 29.1% > amoxicillin-clavulanic acid, 27.8% > cefuroxime, levofloxacin, 21.5% > fosfomycin, 13.1% > ceftriaxone, 12.6% > ceftazidime, 10.1% > gentamicin, nitrofurantoin, 6.5% > nitroxoline, 4.9%. No resistance was detected to amikacin and carbapenems (meropenem, imipenem). The rates of ESBL-producing isolates, confirmed by the phenotypic DDST, were as follows: 5% in *E. coli* (n=4), 2.5 % in *K. pneumoniae* (n=2) and *Enterobacter cloacae* (n=2). The resistance rates of *E. coli* as the dominant uropathogen are shown in Table 1.

Table 1. Antimicrobial resistance of 66 isolates of E. coli from urine samples, obtained from ambulatory patients with UTIs

Antimicrobial Agent	Susceptible n (%)	Resistant (R+I) n (%)
ampicillin	24 (39.34)	37 (60.6)
amoxicillin/clavulanic acid	47 (7)	14 (22.9)
cefuroxime	48 (78.7)	13 (21.1)
ceftriaxon	55 (90.1)	6 (9.8)
ceftazidime	57 (93.4)	4 (6.5)
ciprofloxacin	42 (68.8)	19 (31.1)
levofloxacin	44 (72.1)	17 (27.9)
trimethoprim/sulphamethoxazole	39 (63.9)	22 (36)
nitrofurantoin	57 (93.4)	4 (6.5)
5-nitroxoline	58 (95)	3 (4.9)
fosfomycin	53 (86.9)	8 (13.1)
gentamicin	57 (93.4)	4 (6.5)
amikacin	79 (100)	0
meropenem	79 (100)	0
imipenem	79 (100)	0

The resistance rates among the isolates of *E. faecalis* (n=42) were as follows: ciprofloxacin, 28.6% > gentamicin, 23.8% > levofloxacin, 19% > nitrofurantoin, 4.7% > amoxicillin, 2.4%. *Enterococcus faecalis* isolates, demonstrating high level aminoglycoside resistance (HLAR), were detected in 23.8% (n=10). We did not find enterococcal isolates resistant to glycopeptide antimicrobial agents (vancomycin, teicoplanin).

All *Staphylococcus saprophyticus* isolates (n=4) were obtained from urine samples of female patients (median age of 23.8 years). No resistance to ciprofloxacin, levofloxacin, and nitrofurantoin was detected. Only a single isolate was methicillin and gentamicin resistant.

The isolates of *Streptococcus agalactiae* (n=2) showed no resistance to all tested antimicrobial agents (amoxicillin, amoxicillin/clavulanate, ciprofloxacin, levofloxacin, clindamycin, erythromycin).

DISCUSSION

The major scope of this study was to investigate the etiology of UTIs in ambulatory patients in Varna city, Bulgaria and to demonstrate the trends in antimicrobial resistance of the leading uropathogens to commonly used agents in the ambulatory practice. According to the reports of BULSTAR (Bulgarian Surveillance Tracking Antimicrobial Resistance), *E. coli* is the most commonly isolated etiological agent of community-acquired UTIs in Bulgaria, representing more than 50% of the urine isolates in both genders (71% in female and 51% in male patients). *Klebsiella pneumoniae* (9–14%) and *Proteus* spp. (6–11%) occupy the second and third place, respectively (5). Our results are in concordance with these data. We found that *E. coli* accounted for 77% of all isolates, dominating as a causative agent in all age groups.

The data regarding the Gram-positive bacterial species (*Staphylococcus* spp., *Enterococcus* spp.) in the etiological spectrum of UTIs vary significantly between different reports because these pathogens are very often gender-, age-, and risk factor-associated (pregnancy, catheterization, transplantation, surgical procedures). The two clinically most important enterococcal species *E. faecalis* and *E. faecium* are responsible for a minority of community-acquired UTI, but both together cause between 15 and 30% of the catheter-associated UTIs and are the third leading cause of hospital-acquired UTIs (6). In our

study *Enterococcus faecalis* was identified as the most important Gram-positive uropathogen and the second most commonly isolated bacterial species after *E. coli*. This is in contrast with the findings of the BULSTAR system that reports *Enterococcus* spp. isolates taking fourth place in the etiological structure of community-acquired UTIs after *E. coli*, *Klebsiella* spp., and *Proteus* spp. (5).

The activity of aminopenicillins against Gram-negative bacteria, incl. *E. coli* isolates, in this study is significantly reduced, reaching 64.5%. In comparison to ampicillin, the activity of amoxicillin/clavulanic acid is more preserved, but unfortunately the resistance rates are above 20%. This agent is considered an adequate empiric treatment for UTIs in specific patient groups like children, elderly patients, or during pregnancy. Similar to our results were reported by Martínez-Casanova and Gaspari (7,8).

The cephalosporins with their broad spectrum of activity are among the preferred and frequently used antimicrobials for treatment of UTIs, both community- and hospital-acquired. According to the Bulgarian Guidelines of Nephrology and Dialysis, these agents are still recommended for empirical treatment of UTIs in children, elderly, as well as for operation prophylaxis (9). We detected a relatively high resistance rate to second-generation cephalosporins (cefuroxime) (> 20%) in *E. coli*. Marhova et al. reported similar resistance rates to cefuroxime in urine isolates of *E. coli* (25%), obtained from patients in Plovdiv region (10). A large Polish study from 2016 detected 10% resistance to cefuroxime in *E. coli*, associated with uncomplicated UTIs and 18% in isolates from complicated infections (11). Recent research from Romania shows 12.6% cefuroxime resistance in urine isolates of *E. coli* (12). Other neighboring countries report 36% resistance in 2018 (13).

A positive result from our study is the low rate of third-generation cephalosporin resistance (ceftazidime, cefotaxime, ceftriaxone) detected among the representative of order *Enterobacterales* (10.1%) and especially *E. coli* (6.5%), a result that reflects the proportion of the ESBLs producers among the Gram-negative isolates, confirmed by the DDST in all isolates. The overall rate of ESBLs production among *E. coli* and *K. pneumoniae* isolates from different studies and countries vary significantly: 25.6% in Israel (10), 35.7% in Iran (14), 10% for USA (15), and 4.2% for France (16). The third-generation cephalosporins are strategic broad spectrum antimicrobial agents, which should be restricted for use only for complicated UTIs or UTIs in the hospital setting. Currently, carbapenems (meropenem, imipenem) are considered the drugs of choice for treatment of infections caused by ESBL-producing organisms (18). In our study no carbapenem-resistant isolates from order *Enterobacterales* were identified. This result can be explained with the fact that most of the patients included in this study were presented with community-acquired UTIs.

Fluoroquinolones are broad-spectrum antimicrobial agents used for a long period of time as a preferred empiric treatment option. Nowadays they are no longer first-line agents for uncomplicated UTIs and are preferred for more severe and complicated infections in order to avoid induction of bacterial resistance (19). A relatively high resistance rate to fluoroquinolones in the major uropathogen *E. coli* was detected in our study (above 25%). This is in concordance with the results reported by BULSTAR regarding the fluroquinolone resistance in urine isolates during the period 2015–2020, demonstrating a trend to increase from 16.5% to > 20.5% (5).

The combined antimicrobial agent trimethoprim/sulfamethoxazole is one of the recommended agents for uncomplicated UTIs as well as for prophylaxis in cases of chronic UTIs. In many guidelines, trimethoprim/sulfamethoxazole is recommended as initial therapy only if *E. coli* resistance to this agent does not exceed 20% (20). In our study a high level of trimethoprim/sulfamethoxazole resistance (36%) in the leading pathogen *E. coli* was detected, a result that demonstrates that the agent is not an appropriate choice for empiric therapy of community-acquired UTIs in our region.

Our study demonstrates preserved activity of nitrofurantoin, nitroxoline, and fosfomycin against the major uropathogens. We detected 6.5% and 4.7% resistance to nitrofurantoin in *E. coli* and *E. faecalis,* respectively, making this drug an ideal agent for initial empiric therapy of uncomplicated community-acquired UTIs or in cases with limited treatment alternatives, usually associated with multidrug resistant pathogens. Nitroxoline is an agent with broad

spectrum activity against different Gram-negative and Gram-positive bacteria, as well as yeasts. It is recommended as one of the drugs of first choice for therapy of uncomplicated UTIs in adults and for a long-lasting prophylaxis of recurrent UTIs (21). In comparison to nitrofurantoin and nitroxoline, fosfomycin resistance rate in *E. coli* isolates is higher, but the level is under 20%. Taking into consideration the resistance to trimethoprim/sulfamethoxazole in our region, fosfomycin might be a good alternative for oral therapy of UTIs in ambulatory patients, even in clinical cases with limited treatment options.

The aminoglycosides gentamicin and amikacin are among the antimicrobials of choice for parenteral and combined therapy of more severe or complicated UTIs or infections with limited alternatives for therapy (4,8). The synergistic effect between aminoglycosides and β-lactams is well documented. A positive result from this study is the low level of gentamicin resistance in the studied collection of isolates (6.5%) and the fully preserved activity of amikacin. In the present study a relatively high proportion of HLAR enterococci (23.8%) was found. Such isolates are identified predominantly in the hospital settings, but their significance increases as many of these patients are also subjects of ambulatory care (nursing homes, presence of post operation catheters, permanent catheters).

CONCLUSION

The etiological spectrum of UTIs in Varna ambulatory patients was dominated by *E. coli*, followed by *Enterococcus faecalis*. In the group of Gram-negative uropathogens, high resistance rates to ampicillin, trimethoprim/sulfamethoxazole and quinolones were detected, but relatively low resistance rate to third-generation cephalosporins was found. Fosfomycin, nitrofurantoin, and nitroxoline retained very good activity and are adequate choice for antimicrobial treatment of uncomplicated community-acquired UTIs or UTIs when other alternatives are lacking. The study highlights the importance of the periodic surveillance of the resistance rates to most commonly used antimicrobial agents in the ambulatory practice at local level to guide the empiric treatment recommendations. The good medical practice should always include microbiological examination of the urine sample in patients with suspected UTIs to identify the pathogen and to determine its susceptibility to antimicrobial agents.

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