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Pathogen Bacteria of the Urinary Tract Isolated from Urine Cultures and Their Susceptibility

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: The present study was aimed to determine the distribution and antimicrobial susceptibility of strains isolated from urinary tract infections in our region.

Materials and Methods: In this study, the distribution and antibiotic resistance profiles of microorganisms isolated from the urine cultures of urology outpatient clinic and ward patients between December 2011 and May 2013 were retrospectively evaluated.

Results: The most commonly isolated microorganisms in outpatient clinic patients were; *E. coli* (71%), *K. pneumoniae* (8.8%), *P. aeruginosa* (6.3%), and the most commonly isolated microorganisms in hospitalized patients were *E. coli* (61.3%), *P. aeruginosa* (12.3%) and *K. pneumoniae* (5.8%). Amikacin, gentamicin, ceftazidime, cephalothin, ciprofloxacin and trimethoprim-sulfamethoxazole resistance rates of *E. coli* strains and cefotaxime and cephalothin resistance rates of *K. pneumoniae* were higher in hospitalized patients than that in outpatient clinic patients ($p<0.05$). While *E. coli* resistance to ampicillin, amoxicillin-clavulanate, gentamicin, nitrofurantoin, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin and trimethoprim-sulfamethoxazole was significantly higher among male patients who were admitted to the outpatient clinic, ceftazidime and trimethoprim- sulfamethoxazole resistance was significantly higher among hospitalized male patients, in comparison to that in the female patients ($p<0.05$).

Conclusion: As antibiotic resistance rates vary across centres, it will be beneficial that each region perform surveillance studies to determine local antibiotic resistance rates for developing treatment protocols.

Key words: Urine culture, urinary tract infection, antibiotic

INTRODUCTION

Urinary tract infections (UTI) are among the leading causes of nosocomial and community acquired infections and are defined as the presence of inflammation in the kidneys, collecting system and/or urinary bladder. This infection affecting all age groups and both genders is particularly more common in young adult females (1). Approximately 30% to 50% of the population is estimated to develop at least one urinary tract infection in their lifetime (2). Urinary tract infections account for 7 million physician visits annually in the United States of America and approximately 15% of the prescribed antibiotics are for UTIs (3, 4). While gram negative rods, which are part of the normal gut flora are responsible from the majority of UTIs, the most commonly isolated microorganism is *Escherichia coli* (5, 6). The isolation rates of bacteria including *Proteus, Klebsiella, Enterobacter, Pseudomonas* and *Serratia* in nosocomial and complicated urinary tract infections are gradually increasing (7). Due to the empirical use of antibiotics in infectious diseases and the lack of standardization in antimicrobial susceptibility tests, resistance to commonly used antimicrobial agents is increasing year by year (8, 9). Generally, community acquired UTIs are treated empirically, and urine culture/ antimicrobial susceptibility test is demanded in cases with no response to empirical treatment or those with recurrence. Therefore, knowing the common isolated uropathogens of each region and their antimicrobial susceptibility patterns is beneficial in planning treatment protocols (10, 11).

Our aim in this present study was to determine the distribution and antimicrobial susceptibility of the isolated strains from urinary tract infections in our region.

MATERIAL and METHODS

The present study retrospectively evaluated the distribution and antibiotic resistance profiles of isolated microorganisms from the urine cultures of patients treated in the urology outpatient clinic and ward of our hospital between December 2011 and May 2013. The midstream urine samples and/or samples collected in aseptic conditions were accepted by the Microbiology laboratory. Urine samples were inoculated on 5% sheep blood agar and Eosin Methylene Blue (EMB) agar medium using a standard loop, and were incubated at 37°C for 18-24 hours. Colonies that grow in culture plates were identified using gram staining, and the presence of catalase, coagulase,

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oxidase and urease enzymes and biochemical characteristics. For strains that could not be identified using conventional methods, BD Crystal ID kit (Becton, Dickinson and Company, New Jersey, USA) was used. Antimicrobial susceptibility of bacteria was evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) and available conditions using Müller Hinton Agar medium (Oxoid Limited, Hampshire, England) by Kirby Bauer disk diffusion technique. Resistance and susceptibility to the most commonly used antibiotics for empirical treatment including penicillin, ampicillin, amoxicillin clavulanic acid, piperacillin tazobactam, cefotaxime, ceftazidime, cefuroxime, cephalothin, ciprofloxacin, levofloxacin, amikacin, gentamicin, streptomycin, erythromycin, tetracycline, trimethoprim-sulfamethoxazole and nitrofurantoin were analysed.

Statistical analysis

Data analysis was performed with SPSS 16 program using chi square test. In statistical analysis, statistical significance level was set at $p<0.05$.

RESULTS

In the study, culture growth was identified in 940 (494 female, 446 male) urine samples obtained from outpatient clinic patients and 308 (66 female, 242 male) urine samples obtained from hospitalized patients. The most commonly isolated microorganisms from outpatient clinic patients in the decreasing order of frequency were *E. coli* (71%), *K. pneumoniae* (8.8%), *P. aeruginosa* (6.3%), *Enterobacter spp* (3.4%), *Citrobacter* (2%), *P. Mirabilis* (1.9%), *Enterococcus faecalis* and *Streptococcus agalactiae* (1.2%), *Pseudomonas spp* (0.8%), *Staphylococcus aureus* and *Acinetobacter* (0.7%), and other bacteria (2%). The most commonly isolated microorganism in hospitalized patients was *E. coli* (61.3%), followed respectively by *P. aeruginosa* (12.3%), *K. pneumoniae* (5.8%), *Enterobacter spp* (4.5%), *E. faecalis* (3.2%), *Citrobacter* (2.9%), *Pseudomonas spp* (2.5%), *S. aureus* (1.9%), *P. mirabilis* and *Acinetobacter* (0.9%), *S. agalactiae* (0.6%) and other bacteria (3.2%). The distribution of isolated uropathogens in outpatient clinic and ward patients is shown in Table 1.

When the antimicrobial resistance rates of *E. coli* strains isolated from the urine cultures of outpatient clinic and ward patients were evaluated, it was found that amikacin, gentamicin, ceftazidime, cephalothin, ciprofloxacin and trimethoprim sulfamethoxazole resistance was significantly higher in hospitalized patients ($p<0.05$). In comparison of the antimicrobial resistance rates of *K. pneumonia,* cefotaxime and cephalothin resistance were again higher in hospitalized patients ($p<0.05$). The antimicrobial resistance rates of Gram negative and Gram positive bacteria isolated from the urine cultures of outpatient clinic and ward patients are given in Table 2 and Table 3.

The comparisons of resistance rates of *E. coli* strains isolated from female and male patients are presented in Table 4. When the antimicrobial resistance rates of *E. coli* grown in the urine cultures of outpatient clinic patients were evaluated, it was observed that ampicillin, amoxicillin-clavulanate, gentamicin, nitrofurantoin, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin and trimethoprim-sulfamethoxazole resistance was higher in male patients in comparison to females ($p<0.05$) (Table 4). There was also a statistically significant difference between hospitalized male and female patients in terms of ampicillin, ceftazidime and trimethoprim-sulfamethoxazole resistance. While ampicillin resistance (39.1%/18.4%; p=0.008) was higher in females, ceftazidime (30.4%/41.8%; p=0.01) and trimethoprim sulfamethoxazole (41.3%/63.1%; p=0.01) resistance was higher in males (Table 4).

DISCUSSION

Although the most commonly isolated agents from urinary tract infections vary, almost all of them are caused by a single bacteria type, *E. coli* being the leading (12). In the study of Temiz et al the most frequently isolated microorganism was *E. coli* with a rate of

Table 1. Distribution of uropathogens isolated from outpatient clinic and ward patients

	Outpatient Clinic			Ward		
BACTERIA	**Female**	**Male**	**Total n (%)**	**Female**	**Male**	**Total n (%)**
E. coli	380	283	663 (71)	46	141	187 (61.3)
K. pneumoniae	46	37	83 (8.8)	2	16	18 (5.8)
P. aeruginosa	12	48	60 (6.3)	5	33	38 (12.3)
Enterobacter spp.	8	24	32 (3.4)	3	11	14 (4.5)
Citrobacter	13	6	19 (2.0)	2	7	9 (2.9)
P. mirabilis	10	8	18 (1.9)	-	3	3 (0.9)
Enterococcus faecalis	2	10	12 (1.2)	5	5	10 (3.2)
S. agalactiae	12	-	12 (1.2)	-	2	2 (0.6)
Pseudomonas spp.	1	7	8 (0.8)	-	8	8 (2.5)
S. aureus	4	3	7 (0.7)	1	5	6 (1.9)
Acinetobacter	1	6	7 (0.7)	-	3	3 (0.9)
Other	5	14	19 (2.0)	2	8	10 (3.2)
Total	494	446	940	66	242	308

Table 2. The types and antibiotic resistance rates of uropathogens isolated from outpatient clinic patients. (%)

BACTERIA	AMK	AMP	AMC	GEN	LVX	NIT	TZP	CTX	CAZ	SFR	CEF	CIP	SXT	STR	TET	ERY	PEN
E. coli	0.4	21.4	18.4	28.2	4.0	5.8	3.6	11.6	28.9	16.1	17.6	40.8	40.5	-	-	-	-
K. pneumoniae	0	16.8	22.8	15.6	2.4	27.7	7.2	3.6	26.5	20.4	10.8	20.4	31.3	-	-	-	-
P. aeruginosa	26.6	3.3	5	61.6	1.6	-	6.6	20.0	48.3	-	-	65.0	-	-	-	-	-
Enterobacter spp	-	15.6	-	31.2	25.0	-	-	-	-	-	-	53.1	3.1	18.7	62.5	28.1	9.3
Citrobacter	0	21.0	47.3	5.2	-	26.3	0	1.6	10.5	15.7	36.8	10.5	15.7	-	-	-	-
P. mirabilis	0	11.1	0	5.5	0	38.8	-	0	0	0	5.5	11.1	50.0	-	-	-	-
E. faecalis	-	25.0	0	33.3	-	8.3	-	-	-	-	-	41.6	-	25.0	50.0	25.0	8.3
S. agalactiae	-	25.0	0	0	-	-	-	-	-	-	-	8.3	8.3	0	66.6	41.6	8.3
Pseudomonas spp.	37.5	-	0	75.0	-	-	12.5	-	37.5	-	-	87.5	-	-	-	-	-
S. aureus	-	0	0	0	-	-	-	-	-	-	-	0	-	0	0	0	57.1
Acinetobacter	-	14.2	14.2	57.1	-	57.1	42.8	42.8	42.8	42.8	14.2	57.1	57.1	-	-	-	-
Other	0	10.5	10.5	21.0	5.2	15.7	0	5.2	5.2	10.5	15.7	36.8	36.8	-	-	-	21.0

AMK: Amikacin, AMP: Ampicillin, AMC: Amoxicillin clavulanic acid, GEN: Gentamicin, LVX: Levofloxacin, NIT: Nitrofurantoin, TZP: Piperacillin tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, SFR: Cefuroxime, CEF: Cephalothin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, STR: Streptomycin, TET: Tetracycline, ERY: Erythromycin, PEN: Penicillin

Table 3. The types and antibiotic resistance rates of uropathogens isolated from ward patients (%)

BACTERIA	AMK	AMP	AMC	GEN	LVX	NIT	TZP	CTX	CAZ	SFR	CEF	CIP	SXT	STR	TET	ERY	PEN
E. coli	2.1	23.5	24.0	37.4	3.7	8.5	3.2	17.1	39.0	19.7	29.4	65.2	58.2	-	-	-	-
K. pneumoniae	0	27.7	27.7	16.6	0	38.8	11.1	22.2	38.8	27.7	33.3	27.7	38.8	-	-	-	-
P. aeruginosa	23.6	-	-	57.8	0	-	2.6	15.7	57.8	-	-	63.1	-	-	-	-	-
Enterobacter spp	-	14.2	-	21.4	14.2	-	-	-	-	-	-	42.8	-	21.4	42.8	21.4	7.1
Citrobacter	11.1	22.2	55.5	33.3	-	33.3	11.1	11.1	44.4	22.2	33.3	22.2	22.2	-	-	-	-
P. mirabilis	0	0	0	0	0	100	-	0	0	0	0	0	33.3	-	-	-	-
E. faecalis	-	50.0	0	50.0	-	0	-	-	-	-	-	70.0	-	50.0	60.0	40.0	20.0
S. agalactiae	-	0	0	0	-	-	-	-	-	-	-	0	-	0	0	0	0
Pseudomonas spp.	12.5	-	12.5	37.5	-	-	25.0	-	25.0	-	-	62.5	-	-	-	-	-
S. aureus	-	0	0	0	-	-	-	-	-	-	-	33.3	-	0	0	0	50.0
Acinetobacter	-	33.3	33.3	33.3	-	66.6	33.3	0	66.6	0	66.6	100	66.6	-	-	-	-
Other	-	0	10.0	0	0	20.0	10.0	0	0	10.0	0	0	10.0	-	-	-	20.0

AMK: Amikacin, AMP: Ampicillin, AMC: Amoxicillin clavulanic acid, GEN: Gentamicin, LVX: Levofloxacin, NIT: Nitrofurantoin, TZP: Piperacillin tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, SFR: Cefuroxime, CEF: Cephalothin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, STR: Streptomycin, TET: Tetracycline, ERY: Erythromycin, PEN: Penicillin

* The underlined resistance rates show a statistically significant difference when compared with the resistance rates of outpatient clinic patients ($p<0.05$)

71%, followed by *Klebsiella* strains with 13% (13). In the study of Yılmaz and colleagues in which they evaluated the results of three-year urine culture-antimicrobial susceptibility tests, similarly *E. coli* and *Klebsiella* were on the first two ranks (14). Rifaioğlu et al evaluated the urine cultures of outpatients and hospitalized patients separately, and found that the first three most commonly isolated microorganisms in outpatient clinic patients were *E. coli* (67.2%), *P. mirabilis* (7.5%) and *K. pneumoniae* (3.9%), while the corresponding order for hospitalized patients was *E. coli* (49.6%), *P. aeruginosa* (10.5%) and *K. pneumoniae* (5.3%) (15). Urbarlı et al also demonstrated that the most commonly isolated microorganism in both outpatient clinic and ward patients was *E. coli* (75%), followed by *P. aeruginosa*(8%) and *Klebsiella* (5%) (16). Consistent with the results of the other studies, the present study demonstrated that *E. coli* (outpatient clinic: 71%, ward: 61.3%) was the most common pathogen isolated in both outpatient clinic and ward patients, followed by *K. pneumoniae* (8.8%) and *P. aeruginosa* (6.3%) in outpatient clinic patients, and *P. aeruginosa* (12.3%) and *K. pneumoniae* (5.8%) in ward patients. No significant difference was determined between the outpatient clinic patients and hospitalized patients regarding the isolated bacteria type (Table 1).

Table 4. Antibiotic Resistance Rates of *E. coli* strains according to gender

	Outpatient Clinic			Ward		
ANTIBIOTIC	**Female (%)**	**Male (%)**	**p value**	**Female (%)**	**Male (%)**	**p value**
Amikacin	0.2	0.7	0.57	4.3	1.4	0.254
Ampicillin	17.3	27.9	0.002	39.1	18.4	0.008
Amoxicillin-clavulanate	12.1	27.2	<0.001	15.2	26.9	0.117
Gentamicin	18.4	40.9	<0.001	30.4	40.4	0.294
Levofloxacin	2.8	5.3	0.15	2.1	4.2	0.98
Nitrofurantoin	3.4	9.8	0.001	13.0	7.0	0.22
Piperacillin tazobactam	2.6	4.5	0.20	4.3	2.8	0.63
Cefotaxime	6.5	18.7	<0.001	17.3	17.0	0.66
Ceftazidime	19.4	42.0	<0.001	30.4	41.8	0.01
Cefuroxime	12.6	20.8	0.005	26.0	18.4	0.29
Cephalothin	14.4	21.9	0.14	19.5	31.9	0.13
Ciprofloxacin	26.8	59.7	<0.001	60.8	66.6	0.48
Trimethoprim-sulfamethoxazole	29.2	53.0	<0.001	41.3	63.1	0.01

The increasing antimicrobial resistance throughout the world make the treatment of UTIs difficult every passing day. The reasons for antibiotic resistance may be the improperly adjusted treatment doses or frequent use of antibiotics in the treatment of various infections, as well as the acquisition of resistance in bacteria with low susceptibility by selection / spontaneous mutation or development of resistance in enteric bacteria by R plasmids responsible from multiple drug resistance (17). In previous studies, there is a discrepancy in antibiotic resistance rates of *E. coli* strains isolated from UTIs.

Ağca et al, in their study performed in two centres, reported that *E. coli* isolated from outpatient clinic patients and hospitalized patients were mostly susceptible to imipenem and amikacin, respectively (7). In this study, *E. coli* strains showed the lowest resistance rate to amikacin and piperacillin tazobactam; these rates were 0.4% and 3.6% in outpatient clinic patients and 2.1% and 3.2% in hospitalized patients. The low resistance rates detected for these antimicrobials may be attributed to the uncommon use of amikacin, piperacillin tazobactam and carbapenem group antibiotics in the empirical treatment of UTIs, and the use of these antibiotics only in hospitalized patients according to culture results.

Fluoroquinolones are wide spectrum antibiotics that are prescribed frequently for the treatment of complicated and uncomplicated urinary system infections; hence, resistance rates to these antibiotics are quite high. Various studies reported different quinolone resistance rates for *E. coli*. Yaşar and colleagues, in their study in which they evaluated the effects of extended spectrum beta lactamase (ESBL) production on antibiotic resistance in *E. coli* strains, determined ciprofloxacin resistance as 52.2% (18). Rifaioğlu et al reported ciprofloxacin susceptibility in *E. coli* strains isolated from outpatient clinic patients as 15.4% and ward patients as 40.2% (15). Ağca et al in their study performed in two centres found that ciprofloxacin resistance of one of the centres was 19% in outpatient clinic patients and 63% in ward patients, while the corresponding figures were 23% and 24% in the other centre (7). In this present study, ciprofloxacin resistance in *E. coli* strains in outpatient clinic and ward patients were 40.8% and 65.2%, respectively. Resistance to levofloxacin, a third generation fluoroquinolone which is commonly used in empirical treatment, was 4% and 3.7%, respectively.

Trimethoprim-sulfamethoxazole resistance in *E. coli* strains are reported between 22.1% and 60% in many studies performed in our country (19). In this study, co-trimoxazole resistance in *E. coli* strains was 40.5% in outpatient clinic patients and 58.2% in ward patients.

The *E. coli* resistance rates to ampicillin, amoxicillin-clavulanate in the present study show a wide difference from the rates reported in the literature. Temiz et al. reported ampicillin and amoxicillin-clavulanate resistance as 76.1% / 65.7% in outpatient clinic patients and 79.3% / 68.8% in hospitalized patients (13). These rates were 69% / 36% and 82% / 58%, respectively, in the study of Bayraktar et al. (20). In the present study, the resistance rates to ampicillin were 21.4% and 23.5% in outpatient clinic patients and that to amoxicillin-clavulanate were 18.4% and 24% for ward patients. According to these results, it should be bear in mind that aminopenicillins may be a good option for treatment of community acquired UTI caused by *E. coli* in our region.

An aminoglycoside derivative, gentamicin, has an important place in antimicrobial treatment, primarily in Gram negative infections. When the studies on gentamicin susceptibility in *E. coli* are evaluated, it is observed that there is an increasing trend of resistance in the last ten years. Kaya et al. reported that gentamicin resistance in *E. coli* strains increased from 4% to 16% within four years; Kurutepe and colleagues also reported that gentamicin resistance in *E. coli* strains from outpatient clinic patients increased from 7% to 13.8% in a six years period (19, 21). In this study gentamicin resistance rates of *E. coli* isolates (outpatient clinic: 28.2%, ward: 37.4%) were consistent with the increasing trend of resistance in the literature. This situation emphasizes the necessity of regular monitoring of antimicrobial susceptibility of uropathogens.

The problem of increasing resistance is also observed in studies evaluating the effects of cephalosporin group antibiotics on *E. coli*. Kaya et al reported that cefuroxime resistance increased from 9.6% to 32.1%, cefotaxime resistance from 1% to 27.5%, and ceftazidime resistance from 1% to 24.6% between 2000 and 2003 (19). Among the cephalosporins included in antimicrobial susceptibility test, *E. coli* strains isolated in the present study exhibited higher rates of resistance to ceftazidime, compared to literature (outpatient clinic: 28.9%, ward: 39%). Cefuroxime and cefotaxime resistance rates were consistent with the results of other studies (Table 2-3).

In comparison of the resistance rates of *E. coli* strains isolated from UTI between female and male patients, it was found that male patients had a higher resistance to numerous antibiotics. Linhares et al, in their ten-year surveillance study determined that strains isolated from male patients were more resistant to fluoroquinolones, penicillin, nitrofurantoin and to first and second generation cephalosporins (22). Another surveillance study in USA and Canada reported that ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole resistance was higher in males (23). Mc Gregor et al, in their study in which they evaluated sex- and age-specific antibiotic resistance patterns found that *E. coli* isolated from male patients was more resistance to ampicillin, amoxicillin-clavulanate, ciprofloxacin and nitrofurantoin (24). In this study, ampicillin, amoxicillin-clavulanate, gentamicin, nitrofurantoin, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin and trimethoprim sulfamethoxazole resistance was statistically significantly higher in male outpatient clinic patients compared to that in females. While ampicillin resistance was higher in female hospitalized patients, ceftazidime and trimethoprim-sulfamethoxazole resistance was higher in males.

In the present study, amikacin, levofloxacin and piperacillin tazobactam were the most effective antibiotics whereas trimethoprim sulfamethoxazole, ceftazidime and cephalothin had the highest resistance rates to *K. pneumoniae* strains, the second most commonly isolated microorganism in outpatient clinic patients and the third most commonly isolated microorganism in hospitalized patients. In the study of Abdullah and colleagues where only *Klebsiella* isolates were investigated, amoxicillin (0.1%) and nitrofurantoin (15.5%) showed the lowest susceptibility, while imipenem (97.7%) and piperacillin tazobactam (95.7%) were reported as the most effective antibiotics (25).

In our study, when the resistance profiles of the isolated *P. aeruginosa* strains were assessed, it was observed that ciprofloxacin, gentamicin and ceftazidime resistance rates were high in both outpatient clinic and ward patients (Table 2-3). Our results were similar to the results of the study of Temiz et al, in which ciprofloxacin, gentamicin and ceftazidime resistance were found as 68.1%/38.8%, 54.5%/50%, and 59%/44.4%, respectively in outpatient clinic and ward patients (13).

CONCLUSION

The selection of antibiotics for treatment of urinary tract infections is important for both treatment success and prevention of resistance development. Urine culture should be performed in every outpatient clinic and ward patient before starting empirical treatment, antibiotic susceptibility of the isolated microorganism should be determined and empirical treatment should be rearranged according to antimicrobial susceptibility results. As antibiotic resistance rates show variations across centres, it will be beneficial that every region perform surveillance studies to determine local antibiotic resistance rates for the development of treatment protocols.

Ethics Committee Approval: Ethics committee approval was received for this study from the local ethics committee of Bozyaka Training and Research Hospital.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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