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Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran

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KEYWORDS
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Summary
Background: The empirical therapy of urinary tract infections (UTI) relies on the predictability of the agents causing UTI and knowledge of their antimicrobial susceptibility patterns.
Methods: In a prospective study undertaken over a 14-month period, 5136 samples from patients suspected of having a UTI were analyzed, of which 676 were culture-positive. Isolated bacteria were identified by standard tests, and antibiotic susceptibility was determined by disk diffusion method.
Results: According to our results, *Escherichia coli* was the most common etiological agent of UTI (74.6%), followed by *Klebsiella spp* (11.7%), *Staphylococcus saprophyticus* (6.4%), and *Pseudomonas aeruginosa* (2.2%). Analysis of the frequency of isolated bacteria according to the age of the patients revealed that Klebsiella infections are more prevalent in the older age groups (>10 years) and Pseudomonas infections are more prevalent in children and the elderly (<9 years and >60 years). Results of antimicrobial susceptibility analysis for *E. coli*, as the most prevalent cause of UTI, to commonly used antibiotics are as follows: amikacin (97.8%), gentamicin (97%), ciprofloxacin (94%), nitrofurantoin (87.1%), nalidixic acid (93.7%), trimethoprim—sulfamethoxazole (48.2%), cephalexin (76%), and ampicillin (6.9%).
Conclusions: The results show that the antimicrobial resistance patterns of the causes of UTI are highly variable and continuous surveillance of trends in resistance patterns of uropathogens is important.

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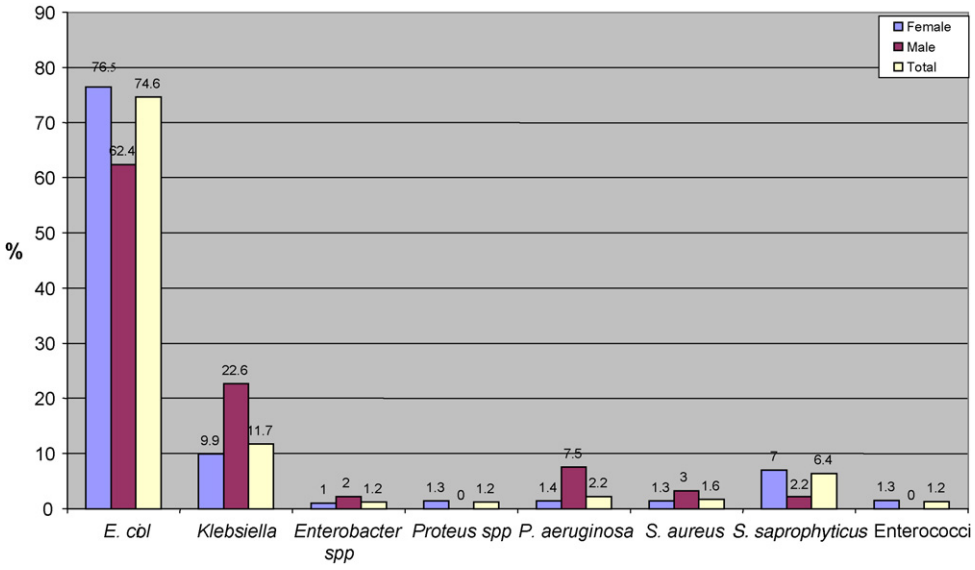

Figure 1 Frequency of isolated bacteria from positive urine samples according to patient gender.

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime.[1,2] UTIs are the most common infections after upper respiratory tract infections.[3] The infections may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if left untreated.[4] Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases.[5] *Escherichia coli* is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections. An accurate and prompt diagnosis of UTI is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure.[6]

Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy.[6–8] The aim of this study was to determine the causative agents of UTIs and their susceptibility patterns to commonly used antibiotics in patients from the East Azerbaijan province of Iran.

Methods

Study design

This study was conducted in the microbiology section of the Central Laboratory of Tabriz University of Medical Sciences. Urine samples were collected from 5136 outpatients suspected of having a UTI, who had not received antimicrobials within the previous two months, and referred to the Central Laboratory for urine culture. There were 3842 (74.8%) samples from female patients and 1294 (25.2%) from male patients. Patient age ranged from 1.5 to 65 years (mean age 28.2 years). All patients were from East Azerbaijan province in the northwest of Iran. Adult patients were

Table 1 Frequency of isolated bacteria from positive urine samples according to patient age

Organism	Age (years)							Total *n* (%)
	0–9 *n* (%)	10–19 *n* (%)	20–29 *n* (%)	30–39 *n* (%)	40–49 *n* (%)	50–59 *n* (%)	>60 *n* (%)	
Escherichia coli	60 (80)	61 (78.2)	153 (78.5)	77 (72.6)	59 (70.2)	48 (75)	46 (62.2)	504 (74.6)
Klebsiella spp	2 (2.7)	9 (11.5)	19 (9.7)	12 (11.3)	15 (17.9)	7 (10.9)	15 (20.3)	79 (11.7)
Enterobacter spp	2 (2.7)	-	4 (2.1)	-	1 (1.2)	1 (1.6)	-	8 (1.2)
Proteus spp	2 (2.7)	1 (1.3)	2 (1.0)	2 (1.9)	-	1 (1.6)	-	8 (1.2)
Pseudomonas aeruginosa	5 (6.7)	1 (1.3)	1 (0.5)	-	1 (1.2)	-	7 (9.5)	15 (2.2)
Staphylococcus aureus	1 (1.3)	-	4 (2.1)	-	2 (2.4)	2 (3.1)	2 (2.7)	11 (1.6)
Staphylococcus saprophyticus	3 (4)	5 (6.4)	10 (5.1)	11 (10.4)	5 (6.0)	5 (7.8)	4 (5.4)	43 (6.4)
Enterococci	-	1 (1.3)	2 (1.0)	4 (3.8)	1 (1.2)	-	-	8 (1.2)
Total (%)	75 (11.1)	78 (11.5)	195 (28.8)	106 (15.7)	84 (12.4)	64 (9.5)	74 (10.9)	676 (100)

sampled by clean catch midstream urine and children aged under 3 years were sampled using sterile urine bags.

Isolation and identification of organisms

Samples for urine culture were tested within an hour of sampling. All samples were inoculated on blood agar as well as MacConkey agar and incubated at 37 °C for 24 hours, and for 48 hours in negative cases. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/ml, or when a single organism was cultured at a concentration of 10^4 cfu/ml and ≥ 5 leukocytes per high-power field were observed on microscopic examination of the urine. Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram-negative bacteria were identified by standard biochemical tests.[5,6] Gram-positive microorganisms were identified with the corresponding laboratory tests: catalase, coagulase, CAMP test (for *Streptococcus agalactiae*), and esculin agar (for enterococci).[9]

Susceptibility testing

Antimicrobial susceptibility of isolates was tested by the disk diffusion method according to the National Committee on Clinical Laboratory Standards (NCCLS) recommendations, using Mueller–Hinton medium.[6] Antimicrobial agents tested were amikacin, gentamicin, ciprofloxacin, nitrofurantoin, nalidixic acid, trimethoprim–sulfamethoxazole, ampicillin, cephalexin, oxacillin, and vancomycin (BD BBL™ Sensi-Disc™).

Statistical analysis

Discrete variables were expressed as percentages and proportions were compared using the Chi-square test.[10]

Results

Isolation and identification of bacteria

Over a 14-month period, 5136 urine samples from outpatients were analyzed, of which 676 (13.2%) had significant bacteriuria. The rate of positive culture was 15.2% (583/3842) for female subjects and 7.2% (93/1294) for male subjects. Gram-negative bacilli were responsible for 85.6% of cases followed by Gram-positive cocci, responsible for 9.3% of cases.

Analysis of the results according to patient gender indicated that although *E. coli* is the predominant isolated pathogen from both sexes, it occurred significantly more frequently in women (76.5% in women compared to 62.4% in men; $p < 0.05$), whereas the prevalences of UTI due to *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were higher in men than in women (22.6% and 7.5% in men compared to 9.9% and 1.4% in women, respectively; $p < 0.01$) (Figure 1). The prevalence of UTI caused by *Staphylococcus saprophyticus* in women (7%) was higher than in men (2.2%). Analysis of the frequency of isolated bacteria according to patient age (Table 1) revealed that Pseudomonas infections are more prevalent in children and the elderly (<9 years and >60 years) and Klebsiella infections are more prevalent in the older age groups (>10 years).

Table 2 Resistance rates of isolated bacteria from positive urine samples to commonly used antibiotics

Organism	*N*	Antibiotic										
		AMK	GEN	CIP	NIT	NAL	SXT	CEP	AMP	CAR	VAN	OXA
Escherichia coli	504	11 (2.2%)	15 (3.0%)	30 (6.0%)	65 (12.9%)	32 (6.3%)	261 (51.8%)	121 (24.0%)	469 (93.1%)	-	-	-
Klebsiella spp	79	1 (1.3)	15 (19.0%)	1 (1.3%)	36 (45.6%)	11 (13.9%)	32 (40.5%)	27 (34.2%)	72 (91.1%)	-	-	-
Enterobacter spp	8	0 (0%)	2 (25%)	0 (0%)	3 (37.5%)	0 (0%)	3 (37.5%)	3 (37.5%)	8 (100%)	-	-	-
Proteus spp	8	0 (0%)	0 (0%)	0 (0%)	5 (62.5%)	0 (0%)	3 (37.5%)	2 (25%)	8 (100%)	-	-	-
Pseudomonas aeruginosa	15	2 (13.3%)	4 (26.7%)	2 (13.3%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	-	-	-
Staphylococcus aureus	11	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)	-	2 (18.2%)	2 (18.2%)	8 (72.7%)	0 (0%)	0 (0%)	4 (36.4%)
Staphylococcus saprophyticus	43	6 (14.0%)	9 (20.9%)	0 (0%)	2 (4.7%)	-	27 (62.8%)	5 (11.6%)	25 (58.1%)	6 (14.0%)	0 (0%)	20 (46.5%)
Enterococci	8	3 (37.5%)	3 (37.5%)	0 (0%)	1 (12.5%)	-	7 (87.5%)	4 (50%)	8 (100%)	4 (50%)	0 (0%)	7 (87.5%)
Total	676	23	48	33	129	58	350	179	613	10	0	31

N, number of isolates; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; NIT, nitrofurantoin; NAL, nalidixic acid; SXT, trimethoprim–sulfamethoxazole; CEP, cephalexin; AMP, ampicillin; CAR, carbenicillin; VAN, vancomycin; OXA, oxacillin.

Antimicrobial susceptibility

The rates of resistance of isolates to a panel of antibiotics, including penicillins, cephalosporins, quinolones, aminoglycosides, and trimethoprim–sulfamethoxazole, which are routinely used to treat UTI infections, are shown in Table 2. *E. coli* as the predominant cause of UTI, showed the highest percentage of resistance to ampicillin (93.1%) and the lowest resistance to amikacin (2.2%). *Klebsiella spp* as the second most prevalent pathogen of UTI displayed a similar resistance pattern and were resistant to ampicillin in 91.1% of cases and susceptible to amikacin in 98.7% of cases. *P. aeruginosa* showed the highest antibiotic resistance rate and was significantly resistant to most of the antibiotics (Table 1). In this study, staphylococci were responsible for about 8% of UTI cases; among these, *S. saprophyticus* was the most common species isolated. *S. saprophyticus* and *Staphylococcus aureus* were resistant to ampicillin in 58.1% and 72.7% of cases, respectively, whereas, 46.5% of *S. saprophyticus* and 36.4% of *S. aureus* were resistant to oxacillin.

Discussion

Microbial infection of the urinary tract is one of the most common infectious diseases worldwide. In this study, of 5136 patients from who urine samples were taken, only 13.2% had a urinary tract infection. This is possibly because UTI symptoms are not a reliable indicator of infection and in children younger than 2 years of age are non-specific. In our investigation, most of the urine samples were collected from patients who did not have a combination of UTI symptoms, and most of the subjects had been referred by general practitioners not specialist physicians. These results indicate that urine culture is necessary for a definitive diagnosis of UTI, and that empirical therapy should only be done by specialist physicians in cases where it is necessary. Our results show a lower urinary tract infection rate of 7.2% in males. The reason for this may be due to the higher number of women than men in this study, and because males are less prone to UTIs, possibly because of their longer urethra and the presence of antimicrobial substances in prostatic fluid.

Although the prevalence of pathogens in different parts of the world is somewhat similar, antimicrobial resistance patterns reported from different regions are significantly different and antimicrobial resistance is increasing. The results of our study show that among the heterogeneous causative organisms of UTI, *Enterobacteriaceae* are the predominant pathogens, followed by Gram-positive cocci. These findings are consistent with reports published from other countries.[11–13]

The highest percentages of resistance were found for ampicillin (90.7%), trimethoprim–sulfamethoxazole (51.8%), and cephalexin (26.5%), whereas the highest percentages of susceptibility were seen for amikacin (96.6%), ciprofloxacin (95.1%), and gentamicin (92.9%); these results are basically in agreement with other studies carried out around the world.

In other studies conducted on 311 urinary and fecal isolates of *E. coli* from the southeast and 76 clinical isolates of *E. coli* from the capital city of Iran, similar susceptibility patterns were observed.[14,15]

Our study, as with previous studies, shows that *E. coli* is the predominant etiology of UTI,[16,17] and also reveals a very high microbial resistance rate to antibiotics. This was especially the case for *P. aeruginosa*, which was totally resistant to ampicillin, cephalexin, nitrofurantoin, nalidixic acid, and trimethoprim–sulfamethoxazole; this resistance is higher than that found in other reports.[17,18] Most cases of urinary infection with *P. aeruginosa* were in patients over 60 years of age and under 9 years of age. This indicates that this bacterium is an opportunistic pathogen, causing infection in those in a weak immunological condition.

Other surveys in Iran have shown a high resistance to antibiotics. Khotaii et al. reported resistance rates of 87.5% to ampicillin, 39.5% to gentamicin, 67.5% to trimethoprim–sulfamethoxazole, and 57.7% to cephalothin.[19] This significantly higher bacterial resistance to antibiotics in our region in comparison with other countries seems to be due to a higher rate of antibiotic usage by families, even in the absence of a prescription, and to the high percentage of younger population, since UTIs are more common in the early years of life.

Resistance to β-lactamase sensitive penicillins is highly prevalent among these bacteria.[20] In this study the resistance rate to ampicillin was 58.1% for *S. saprophyticus* and 72.7% for *S. aureus*. Resistance to oxacillin as a representative of β-lactamase resistant penicillins has developed in UTIs with Gram-positive cocci isolated, where resistance rates to oxacillin were 46.5% for *S. saprophyticus* and 36.4% for *S. aureus*. There were no significant differences observed in antimicrobial resistance by age or gender of patients.

S. saprophyticus is a prevalent pathogen during the period of sexual activity in women. Although sexual transmission has not been defined as the main transmission route of this pathogen, frequent or recent sexual activity is a major risk factor. The division time for this pathogen is longer than that for other UTI pathogens, therefore a lower colony count (100–1000) is of clinical worth.[21] In this study *S. saprophyticus* was responsible for 7% of UTIs in women. The rate of UTIs gradually increases with age to 30 years in women (average 20–40 years). *S. aureus* is an important uropathogen and was responsible for 1.6% of UTI cases in our study. It has been emphasized that any amount of this bacterium should be subjected to antibiogram test.[20]

Over the last decade there has been a substantial increase in resistance of uropathogens to antibiotics. Resistance rates among *S. aureus* strains are increasing, and a major part of this species has become resistant to β-lactamase resistant penicillins.[22,23] For such resistant species, vancomycin is the effective choice of drug. Resistance to vancomycin is reported among enterococci,[24,25] but this resistance has also begun to develop among staphylococci.[26] In this study we concentrated on resistance to vancomycin and fortunately resistant strains to vancomycin were not observed, but 72.7% of cases were resistant to ampicillin and 36.4% to oxacillin.

The regional variations of resistance to antibiotics may be explained in part by different local antibiotic practices.[27,28] The influence of excessive and/or inappropriate antibiotic use on the development of antibiotic-resistant strains, particularly broad-spectrum agents prescribed empirically, has been demonstrated. Reducing the number of prescriptions of a particular antibiotic can lead to a decrease in resistance rates.[29,30] Transmission of resistant isolates between people and/or by consumption of foods originated from animals that

have received antibiotics and greater mobility of individuals worldwide has also contributed to the expansion of antibiotic resistance.[31,32]

In conclusion, because the pattern of sensitivity of bacteria to antibiotics varies over time and in different geographical regions, antibiotic treatment of infections should be based on local experience of sensitivity and resistance patterns. In this study, nitrofurantoin and nalidixic acid were found to be the most appropriate oral antibiotics, and amikacin and third-generation cephalosporins were the most appropriate parenteral antibiotics, for the empirical therapy of UTIs.

Conflict of interest: No conflict of interest to declare.

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