



## Prevalence and Microbial Resistance of Uropathogenic Bacteria Isolated from Neonatal and Pediatric Patients in Western of Iran

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors MM, DA, MHH, AD and AF designed the study, author AH performed the statistical analysis, authors DA and AF wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors MM, DA, AD and AF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Backgrounds:** Urinary tract infections (UTIs) are the most common bacterial infections disease among children, pediatric and neonatal patients. These infections are found frequently in children. The global evolution of antibiotic resistance among urinary tract isolates has recently been reported.

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The aim of the present study was to investigate the prevalence and antibiotic resistance pattern of uropathogenic bacteria isolated from patients with urinary tract infections.

**Materials and Methods:** A total of 1754 urine specimens were obtained from children with UTI who referred to Emam Hossien hospital in Kermanshah city, Iran. The urine samples were cultured on the appropriate bacteriological media and identified by conventional bacteriological tests. Antibiotic susceptibility testing was then performed by disk diffusion method.

**Results:** Of 1754 samples, 193 (11%) urine samples were positive based on do the urine cultures method. Out of 193 urine specimens, were positive for: *E. coli*, *Kelebsiella* spp., *Enterococcus* spp., coagulase-negative staphylococci, *Pseudomonas* spp., *Staphylococcus aureus*, *Citrobacter* spp., *Serratia* spp., *Streptococcus* (viridans group) and *Proteus* spp. A sample was positive for *Acinetobacter* spp. Furthermore, *E. coli* infections showed high resistance to ampicillin (82%), trimethoperim-sulfamethoxazole, nalidixic acid (69%), and nitrofurantoin (61%).

**Conclusion:** Our results revealed that urinary tract pathogens isolated from Iranian children are particularly resistant to some commonly used antimicrobial agents. Regarding to antimicrobial susceptibility pattern in urinary tract pathogens showed that high levels of resistance to different antibiotics and treatment options are limited, and infection control measures remain of high importance. Routine surveillance and monitoring studies should be performed to provide knowledge to physicians on the updated and most effective empirical prescribing practice in the treatment of UTIs.

**Keywords:** Urinary tract infections; antibiotic resistance; gram-negative bacteria; gram-positive bacteria.

## 1. INTRODUCTION

Urinary tract infection (UTI) is a critical health problem and it is an infection that can happen anywhere along the urinary tract and is continued as one of the most frequently diagnosed cases [1,2]. In fact, UTIs are the leading cause of gram-negative bacteremia in patients of all ages and are associated with a high risk of morbidity and mortality, especially in the elderly, and account for significant health care costs [3]. These infections are found frequently in children, which can be asymptomatic or symptomatic and also characterized by a wide spectrum of symptoms ranging from mild irritative voiding to bacteremia, sepsis or death. In recent years, the incidence of antibiotic resistance has been increasing among urinary pathogens as a result of wide spread use of antibiotics [4,5]. Several studies have demonstrated that the geographical variability of pathogens occurrence in cases of UTI between inpatients and outpatients is limited by the predominance of gram-negative species, particularly *E. coli* and *Entrobacter* spp. [6,7]. The global evolution of antibiotic resistance among urinary tract isolates has recently been reported [8-10]. In developing countries, more than 80% of bacterial strains causing UTIs are now resistant to trimethoprim-sulphamethoxazole. Antibiotics are commonly used for the treatment of UTIs particularly uncomplicated cystitis [11]. Various studies have reported an increase in the incidence of UTIs in Iran, indicating that treatment has become more

complicated due to the emergence of pathogens with higher resistance to antimicrobial agents. The aim of present study was to determine the prevalence and antibiotic resistance pattern of uropathogenic bacteria isolated from urinary tract of suspected children with age range of newborn to 8 years olds during time period of 2010 to 2012 who were admitted to the Microbiology Laboratory of Emam Hossein Hospital in Kermanshah, Iran.

## 2. MATERIALS AND METHODS

### 2.1 Specimen Collection and Isolation

The specimens were obtained from all suspected pediatric and neonatal children with UTI who were admitted to Emam Hossein Hospital, in Kermanshah, Iran during a time course of November 2010 to March 2012. At first, physical examination and complete history were taken for each patient and all urine samples (1745 urine specimens: 933 females and 821 males) were then obtained. Following obliteration for another source of infection from the urogenital superficial region by povidone-iodine, the urine specimens were collected by midstream clean-catch method. After inoculation on EMB (Eosin Methylene Blue Agar), Blood agar and MacConkey agar followed by overnight incubation at 37°C, a general urine analysis including white blood cell (WBC) count and the amount of bacteria using a direct microscopy was performed.

## 2.2 Isolation and Identification Procedures for *E. coli* and Other Bacterial Pathogens

The number of bacterial colonies as colony formation unit (CFU) was determined after overnight incubation at 37°C. Bacterial colony number of  $>10^5$  was considered as pathologic count for *E. coli*. It was also portrayed as significant bacteriuria and the isolates were further subjected to antibiogram analysis [12,13]. Coagulase-negative and coagulase-positive *Staphylococcus spp* were identified by standard biochemical tests such as production of coagulase, colonial morphology, DNase and the API 20 staph test (Bio Merieux). The isolated bacterial strains were further differentiated microbiologically according to standard laboratory methods. Classification of positive urinary specimens was carried out based on the guidelines of Infectious Disease Society of America (IDSA) [14]. Isolated strains were differentiated by Gram staining and conventional biochemical methods [15].

## 2.3 Antimicrobial Susceptibility Testing

Antibacterial disc diffusion tests were performed according to the Clinical and Laboratory Standard Institute (CLSI) standards with antibacterial tablets manufactured by ROSCO Diagnostica. The antibiotics selected for the panel were following: trimethoprim-sulfamethoxazole (SXT, 30 µg), ampicillin (AM, 10 µg), nalidixic acid (NA, 30 µg), cefixim (CFM, 30 µg), ciprofloxacin (CP, 5 µg), ceftriaxone (CRO, 30 µg), cefotaxim (CTX, 30 µg), ofloxacin (OFX, 5 µg), gentamicin (GM, 10 µg), amikacin (AN, 30 µg), nitrofurantoin (FM, 300 µg), imipenem (IPM, 10 µg), penicillin (P, 10 µg), vancomycin (V, 30 µg), oxacillin (OX, 5 µg), cloxacillin (CX, 5 µg), cephalothin (CF, 30 µg), and erythromycin (E, 15 µg).

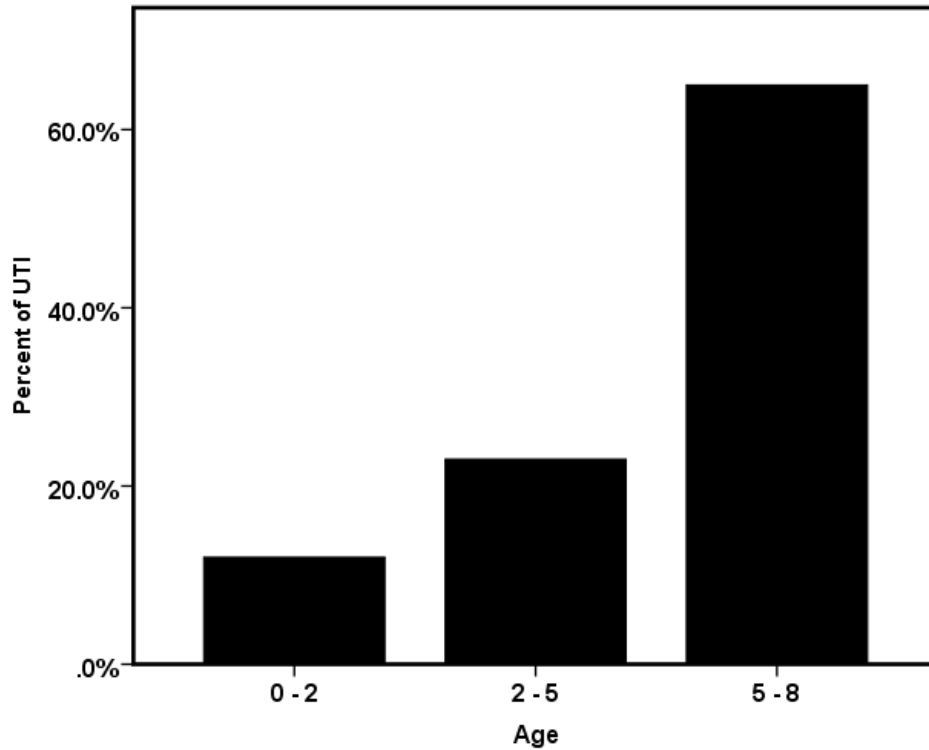
## 2.4 Statistical Data Analysis

The number of patients and antibiotic resistance pattern of uropathogens were compared for each antibiogram test using Chi-square and Fisher exact tests by SPSS statistics software version 11.0. Statistical significance difference were considered at value of  $p < 0.05$ .

## 3. RESULTS

A total of 1754 urine specimens were collected from pediatric patients in both gender (female

933 cases and male 821 cases) with proportion of 53.47%. The age range of the patients was between 1 week-8 years (Fig. 1). Of these, 193 (11%) were showed significant positive growth for uropathogenic bacteria either gram-positive or gram-negative. Among isolated gram-negative uropathogens, one specimen was positive for *Acinetobacter spp*. while the most common isolates were positive for other uropathogenic bacteria with significant proportion of 136 (70.5%) for *E. coli*. The most common isolated bacterial species were as following: *Klebsiella spp.* (7.3%), *Enterobacter spp.* (5.7%), coagulase-negative staphylococci (5.2%), *Pseudomonas spp.* (2.6%), *Staphylococcus aureus* (2.6%), *Citrobacter spp.* (2.1%), *Serratia spp.* (1.5%), *Streptococcus (viridans group)* (1.5%), *Proteus spp.* (1%), *Acinetobacter spp.* (0.5%). In respect to the antimicrobial susceptibility test (AST), *E. coli* isolates were exhibited highest susceptibility (93%) to imipenem and low susceptibility (18%) to ampicillin. The highest susceptibility of *Klebsiella spp.* was observed (86%) to imipenem and low susceptibility(14%) to ampicillin. The highest susceptibility of *Enterobacter spp.* was observed to (82%) imipenem and, similar to two latter species, highest resistance was demonstrated (91%) to ampicillin. The most level resistance of *Pseudomonas spp.* (80%) was to trimethoprim-sulfomethoxazol, cefixim, ampicillin and nitrofurantoin. Also the low level resistance was (20%) to imipenem, amikacin and ciprofloxacin. Isolated *Citrobacter spp.* showed the highest resistance (75%) to trimethoprim-sulfomethoxazol and ampicillin. *Serratia spp.* strains were the most sensitive (100%) to ceftriaxone, amikacin and imipenem. *Proteus spp.* isolates showed 100% resistance to trimethoprim-sulfomethoxazol, ampicillin and nalidixic acid and no resistance to ciprofloxacin, amikacin and imipenem. The *Acinetobacter spp.* exhibited 100% of resistance to trimethoprim-sulfamethoxazole, ampicillin, nalidixic acid, cefixim, ciprofloxacin, cefotaxim and nitrofurantoin. Suseptibility level of gram-positive isolates was portrayed as following: Coagulase-negative Staphylococci exhibited susceptibility of 10% to penicillin, nalidixic acid and vancomycin and showed 20% resistance to imipenem. *Staphylococcus aureus* isolates were 100% resistant to nalidixic acid, whereas their resistance to oxacillin and nitrofurantoin was low (20%). *Streptococcus (viridans group)* isolates was 100% resistant to ampicillin, nalidixic acid and cloxacillin while 33% of *Streptococcus* strains were resistant to ceftriaxone, imipenem, but 0% of *Streptococcus* strains were resistant to



**Fig. 1. Prevalence of UTI among pediatric patients according to the age**

nitrofurantoin, vancomycin and oxacillin (Tables 1 and 2). However, there was no significant difference between antimicrobial resistance and gender and age of pediatric patients ( $\chi^2 = 1.442$ ,  $P = 0.23$ ).

#### 4. DISCUSSION

Urinary tract infection (UTI) is one of the most common occurred in female and male and important cause of mortality and morbidity in pediatrics patient [16]. The most common causative organisms are intestine flora, typically gram-negative bacteria. Previous studies revealed that *E. coli* is still the principal etiological agent of UTI and consist of 77-80 percent of cases. To treat pediatric patients with UTIs and to prevent the recurrence, a course of antibiotics therapy is routinely prescribed [4, 17]. Empirical clinical practise antibiotics should be prescribed for the coverage of several UTI causing bacteria including *Enterococcus* spp., *Proteus* spp., *Klebsiella* spp., *Citrobacter* spp., *Serratia* spp., *Acinetobacter* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus* spp. [18,19]. There are a great deal of study reporting that

these pathogens are the most common cause of UTI worldwide [20]. The patients with pyelonephritis are sensitive for infected by Uropathogenic Bacteria. For suspected pyelonephritis, parenteral antibiotics are recommended [21-25].

Earlier studies suggested to up accession of *E. coli* strains separated in most group and ages in UTIs women in Canada and 16 European countries revealed [26]. A study by Hansson et al. [19] indicated that *E. coli* is the most common pathogen in males (79%) and females (89%) with UTI [27,28]. As demonstrated in Tables 1 and 2, *E. coli* strains are the most common uropathogen bacteria (70.5%), followed by *Klebsiella* spp. (7.25%) and *Enterococcus* spp. (5.7%). Only one *Acinetobacter* spp. was isolated from UTIs. Among gram-positive cocci, the most dominant pathogens causing UTI were coagulase-negative *Staphylococcus* spp. (5.2%). These results showed that these pathogens are still important causes of UTI worldwide and antibiotic resistance in uropathogens bacteria is remained as a critical health issue and such resistance pattern is increasing in all around the world [29,30].

**Table 1. Prevalence and antimicrobial resistance of gram-negative uropathogens**

Uropathogens	No.	SXT%	AM%	NA%	CFM%	CP%	CRO%	CTX%	OFX%	GM%	AN%	FM%	IPM%
<i>E. coli</i>	136	73	82	69	58	37	40	53	10	37	26	58	7
<i>Klebsiella</i> spp.	14	43	86	21	64	36	43	57	36	43	50	50	14
<i>Enterobacter</i> spp.	11	73	91	64	64	36	45	45	36	36	55	55	18
<i>Pseudomonas</i> spp.	5	80	80	60	80	20	60	60	40	40	20	80	20
<i>Citrobacter</i> spp.	4	75	75	50	25	25	50	50	50	25	25	50	25
<i>Serratia</i> spp.	3	100	100	66	66	66	0	66	33	33	0	66	0
<i>Proteus</i> spp.	2	100	100	100	50	0	50	50	50	50	0	50	0
<i>Acinetobacter</i> spp.	1	100	100	100	100	100	0	100	0	0	0	100	0

Trimethoprim-Sulfamethoxazole (SXT), ampicillin (AM), nalidixic acid (NA), cefixim (CFM), ciprofloxacin (CP), ceftriaxone (CRO), cefotaxim (CTX), ofloxacin (OFX), gentamicin (GM), amikacin (AN), nitrofurantoin (FM), imipenem (IPM)

**Table 2. Prevalence and antimicrobial resistance of gram-positive uropathogens**

Uropathogens, antibiotics & number of isolates	Coagulase negative <i>Staphylococcus</i>		<i>Staphylococcus aureus</i>		<i>Streptococcus</i> (viridians group)	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Ampicillin (10 µg)	70%	30%	80%	20%	100%	0
Amikacin (30 µg)	40%	60%	40%	60%	66%	34%
Ceftriaxone (30 µg)	80%	20%	80%	20%	33%	67%
Cephalothin (30 µg)	50%	50%	60%	40%	66%	34%
Cloxacillin (5 µg)	80%	20%	80%	20%	100%	0
Erythromycin (15 µg)	70%	30%	60%	40%	66%	34%
Gentamicin (10 µg)	50%	50%	60%	40%	66%	34%
Imipenem (10 µg)	20%	80%	40%	60%	33%	67%
Nalidixic acid (30 µg)	90%	10%	100%	0	100%	0
Nitrofurantoin (300 µg)	30%	70%	20%	80%	0	100%
Oxacillin (5 µg)	70%	30%	20%	80%	0	100%
Penicillin (10 µg)	90%	10%	80%	20%	66%	34%
Trimethoprim-sulfamethoxazole (30 µg)	70%	30%	60%	40%	66%	34%
Vancomycin (30 µg)	90%	10%	20%	80%	0	100%
No.	10		5		3	

Overall, our study demonstrated that trimethoprim-sulfamethoxazole and ampicillin are not effective against gram-negative uropathogenic bacteria, however, these bacteria were susceptible to other studied drugs. The wide spectrum activity of imipenem (10.5%), ofloxacin (32%), gentamicin (33%) and amikacin (22%) have made them as one of the best therapeutic options for UTIs. Ampicillin resistance in UTI isolates was 89.2%, which is comparable to obtained results from Canada, European and African studies [4–6,12,31].

Although, UTI caused by gram-negative bacteria effectively treated with ceftriaxone (36%) and ciprofloxacin (40%) but these antibiotics are not suitable therapeutic options for gram-positive (*Staphylococcus aureus* and Coagulase-negative *Staphylococci*) urinary tract infections. In respect to antimicrobial susceptibility test, the highest susceptibility level of isolated gram-positive bacteria was observed to nitrofurantoin (83.3%) and imipenem (79%) and these antibiotics thereby would be prescribed as an empirical treatment regimen for UTIs. Gram-negative bacteria that were less common in UTI had maximum sensitivity to cephalosporins antibiotics, nalidixic acid and imipenem. In present study, *Staphylococcus aureus* showed high sensitivity (about 80%) to nitrofurantoin and oxacillin. None of *Staphylococcus aureus* strains were completely resistant to vancomycin; however, 20% of strains were diagnosed as hetero-VRSA (hVISA) strains by according to the protocols previously described by Mohajeri et al. [32].

In urinary tract infections by *Streptococcus viridians*, 100% susceptibility were observed to nitrofurantoin and vancomycin and also 67% susceptibility to ceftriaxon and imipenem.

## 5. CONCLUSION

In conclusion, our results also revealed that urinary tract pathogens isolated from Iranian children are particularly resistant to commonly used antimicrobial agents and antibiotic resistance pattern. Susptibility pattern in urinary tract pathogens and its impact on empirical treatment, it is suggested that *in vitro* resistance pattern, routine surveillance and monitoring studies should be performed to provide knowledge to physicians on the updated and most effective empirical prescribing practice in the treatment of UTIs. We suggest that empirical antibiotic prescriptions should be based

on the local prevalence of bacteria and also their suseptibility pattern to commonly used antibiotics. Briefly, prevalence of urinary tract pathogens were observed in Neonatal and Pediatric Patients and high levels of resistance to different antibiotics show that, treatment options are limited, and infection control measures remain of high importance.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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