

Antimicrobial Resistance and Genotyping of Bacteria Isolated from Urinary Tract Infection in Children in an Iranian Referral Hospital

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Introduction: Urinary tract infection (UTI) is one of the most common bacterial infections in childhood, and the increasing rate of antibiotic resistance to the commonly prescribed antimicrobial agents against it has become a major concern. The aim of this study was to determine the antibiotic resistance and genotyping of bacteria isolated from urine cultures in patients referred to the Children's Medical Center, Tehran, Iran.

Methods: During the 1-year period, antimicrobial susceptibility profiles of strains isolated from patients with UTI were determined. Typing of the isolates causing nosocomial infections was performed by random amplified polymorphic DNA (RAPD) analysis, and the results were analyzed by Gelcompar II software.

Results: In this study, 203 children (130 girls and 73 boys) were included. The patients' age ranged from 1 day to 16 years (IQR average=4 months to 4 years). The most frequent isolated organisms were *Escherichia coli* (118 isolates, 58%), followed by *Klebsiella pneumoniae* (30 isolates, 15%). Sixty-two strains (18 strains of *E. coli*, 13 strains of *K. pneumoniae*, 11 strains of *Enterococcus faecium*, and five strains of *Burkholderia cepacia* complex) had criteria of nosocomial infection. A high resistance rate to trimethoprim-sulfamethoxazole (69%) and cefotaxime (60%) was reported in *E. coli* and *K. pneumoniae* strains, respectively. *Pseudomonas aeruginosa* strains showed high sensitivity to amikacin (100%). All *E. faecium* strains were susceptible to trimethoprim-sulfamethoxazole (100%), and 23% of the strains were resistant to vancomycin. The analysis of RAPD-typing revealed the presence of three clusters in *E. coli*, two clusters in *E. faecium*, and one clone in *K. pneumoniae*. Besides, four out of five isolates of *B. cepacia* complex had more than 90% genetic similarity.

Conclusion: The most frequent isolated pathogen was *E. coli*, and an increasing rate of antibiotic resistance to the commonly prescribed antimicrobial agents such as trimethoprim/sulfamethoxazole and cephalosporins was observed. Moreover, the results of this study showed the presence of clones with $\geq 80\%$ similarity in *E. coli*, *K. pneumoniae*, *E. faecium*, and *B. cepacia* complex isolates; therefore, the transmission of nosocomial infections from one patient to another or one ward to another is probable.

Keywords: urinary tract infection, antibiotic resistance pattern, genotyping

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Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in children aged <2 years¹ and considered as a main cause of hospitalization in childhood.² The incidence rate of UTIs is up to 7% among febrile infants with no other recognizable causes.³ *Escherichia coli* has been known as the most common isolated organism in pediatric patients with UTI with a prevalence of 80–90%,^{4,5}

followed by others such as *Enterococcus species* (spp.), *Enterobacter spp.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus spp.*^{5,6} In the preantibiotic era, UTI caused a mortality rate as high as 20%.⁷ The early treatment of UTI decreases the rate of morbidity;² however, the increasing rate of antibiotic resistance to the commonly prescribed antimicrobial agents such as trimethoprim/sulfamethoxazole, cephalosporins, and fluoroquinolones⁸ has become a major concern.⁹ Hence, it is crucial to know the frequency of uropathogens and antimicrobial resistance in pediatrics.¹⁰ Prompt and efficient antimicrobial therapies for UTIs are vital for an appropriate outcome and prevention of long-term morbidity associated with renal scarring such as hypertension, development of chronic kidney disease, and ultimately the need of renal transplantation.¹¹ The aim of this study was to determine the antibiotic resistance pattern and genotyping of strains isolated from pediatric patients with UTI.

Materials and Methods

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.CHMCREC.1397.47).

This study was performed over a period of 1 year, from March 2018 to February 2019 in the Children's Medical Center, an Iranian referral pediatrics hospital in Tehran, Iran. The inclusion criteria were patients having a history of fever >38°C or symptoms of UTIs including dysuria, frequency, urgency, hesitancy, small volume voids, or lower abdominal pain.⁹ UTI was defined in infants and children as growth of a single pathogen of >10⁵ colony forming units/mL by properly collected urine specimen (suprapubic aspiration, transurethral catheterization, or mid-stream urine) in febrile children or the ones who had other urinary symptoms.¹² Bacteria identification was performed using Gram staining (for bacterial morphology) and differential biochemical tests. Identification of organisms was carried out using conventional biochemical methods such as catalase, oxidase, sugar fermentation, methyl red/Voges-Proskauer (MR/VP) test, growth on Kligler's Iron Agar (KIA), growth on Bile-Esculin Agar and 6.5% NaCl media, and motility test.¹³

All isolated bacteria were checked for their susceptibility towards different antibiotics by disk diffusion method based on Clinical Laboratory Standard Institute (CLSI) guidelines, 2019.¹⁴ The list of antibiotic discs

used for gram-negative bacteria were: nitrofurantoin (300 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), cefepime (30 µg), cefotaxime (30 µg), imipenem (10 µg), amikacin (30 µg), piperacillin/tazobactam (100–10 g), ceftazidime (30 µg), and gentamicin (10 µg). Disks used for Gram-positive bacteria were gentamicin (10 µg), penicillin (30 µg), vancomycin (30 µg), nitrofurantoin (300 µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), piperacillin/tazobactam (100–10 g), imipenem (10 µg), meticillin (30 µg), and ampicillin (10 µg). High-level resistance to gentamicin was also determined using a 120 µg gentamicin disk. The minimal inhibitory concentration of vancomycin was determined by E-test methods. All disks were prepared by the Mast Group Ltd., Merseyside, UK. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used as control strains. All recovered strains which had caused nosocomial infections were typed by Random Amplified Polymorphic DNA PCR (RAPD-PCR) in a mixture consisting of 2.5 µL of the PCR buffer (10-times concentrated), 0.75 µL of MgCl₂ (final concentration 250 µM), 0.6 µL of dNTPs (Fermentas, Vilnius, Lithuania, final concentration 3 mM), 1 µL of primer (5'- AGCGGGCCAA-3'), final concentration of 0.4 mM, 0.3 µL (1.5 U) of the DFS Taq DNA polymerase (BIORON, Germany), 1 µL of the boiled DNA (final concentration 2 ng/µL), and DNase-, RNase-free deionised water (Biomedicals) to a final volume of 25 µL.¹⁵ Amplification was carried out in a thermal cycler (Applied Biosystems, USA) as follows: the primary denaturation for 5 minutes at 95°C; followed by 30 cycles of 60 seconds for 94°C, 60 seconds at annealing temperature, and 5 minutes at 72°C; and the final extension phase for 15 minutes at 72°C. The analysis of the amplified products was performed in 1% agarose (Sigma), and DNA bands were visualized by staining with gel red (Biotium), analyzed under UV light, and photographed using the GEL Doc 2000 documentation system (Bio-Rad). Comparison of RAPD-PCR banding patterns was performed using Gelcompar II, version 6.5 (Applied Maths, Sint-MatensLatem, Belgium), and the unweighed pair group method with arithmetic mean (UPGMA) and dendrograms were constructed.

Statistical Analysis

Descriptive statistics were reported by total numbers, percentages, means, and medians as appropriate.

Table 1 The Distribution of Antimicrobial Resistance Pattern in Gram Negative Bacteria Isolated from UTIs

Gram Negative Bacteria	Total	Trimethoprim-sulfamethoxazole	Nitrofurantoin	Piperacillin/Tazobactam	Amikacin	Gentamycin	Cefepime	Cefotaxime	Imipenem	Cefazidime
	N	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<i>E. coli</i>	118	81 (69)	9 (8)	20 (17)	3 (2/5)	21 (18)	67 (57)	74 (63)	-	-
<i>K. pneumoniae</i>	30	11 (37)	15 (50)	14 (47)	10 (33)	7 (23)	15 (50)	18 (60)	-	-
<i>P. aeruginosa</i>	13	-	-	1 (8)	0	1 (8)	1 (8)	-	2 (15)	1 (8)
<i>E. aerogenes</i>	3	0	3 (100)	-	0	1 (33)	1 (33)	-	-	-
<i>B. cepacia</i> complex	6	0	-	2 (33)	6 (100)	6 (100)	4 (67)	-	6 (100)	0
<i>K. oxytoca</i>	3	1 (33)	0	1 (33)	1 (33)	1 (33)	1 (33)	-	-	-

Results

A total of 203 children with positive urine cultures, collected in the tertiary referral Children's Medical Hospital in Tehran, Iran, were included in our study. Among these patients, 130 (64%) subjects were female. Median age was 1 year (IQR=4 months to 4 years), and the age range was 1 day to 16 years.

E. coli (118 samples, 58%) was the most prevalent microorganism isolated from urine cultures, followed by *K. pneumoniae* (30 samples, 15%) and *P. aeruginosa* (13 samples, 6%). Among the isolated gram positive microorganisms, the most frequent isolated bacteria were *E. faecium* (13 samples, 6%) and *S. epidermidis* (5 samples, 2.5%), respectively. The other strains were identified as: *B. cepacia* complex, *E. aerogenes*, *K. oxytoca*, *E. cloacae* complex, *P. mirabilis*, *Morganella morganii*, *Acinetobacter baumannii-baumannii*, *P. vulgaris*, *Enterobacter spp.*, *E. faecalis*, *S. haemolyticus*, and *Alpha Hemolytic Streptococcus* (viridans group).

A high percentage of *E. coli* strains were isolated from female children (81%), while 70% of *K. pneumoniae* strains were isolated from male children. Most of the bacteria were isolated from the emergency ward (N=51, 25%), followed by the urology (N=33, 16%) and nephrology (N=32, 16%) wards.

The results of the antibiotic resistance pattern to the mentioned antibiotic disks for each microorganism are shown in Tables 1 and 2. Sixty percent of *K. pneumoniae* and 69% of *E. coli* strains were resistant to cefotaxime and trimethoprim-sulfamethoxazole, respectively. Twenty-three percent of *E. faecium* strains (n=3) showed resistance to vancomycin, which was lower than our previous reports.^{15,16}

Out of 203 strains isolated, 62 strains had nosocomial infection criteria which were: 18 *E. coli*, 13 *K. pneumoniae*, 11 *E. faecium*, and five *B. cepacia* complex. Molecular typing and phylogenetic analysis of these 62 isolates causing hospital-acquired infection revealed that *E. coli* strains were divided into three clusters with 70% genetic similarity (Figure 1A). Also, the presence of all *K. pneumoniae* strains in one cluster with high genetic similarity was reported (Figure 1B), most of which were isolated from the Nephrology ward and Neonatal Intensive Care Unit (NICU). Among five *B. cepacia* complex isolates, four strains had 90% genetic similarity (Figure 1C).

The dendrogram, based on analysis of *E. faecium* strains, indicated the presence of two clusters with more

Table 2 The Distribution of Antimicrobial Resistance Pattern in Gram Positive Bacteria Isolated from UTIs

Gram Positive Bacteria	Total	Trimethoprim-Sulfamethoxazole	Nitrofurantoin	Piperacillin/Tazobactam	Ampicillin	Gentamycin	Vancomycin	Penicillin	Imipenem	Meticillin
	N	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<i>E. faecium</i>	13	–	3 (23)	5 of 12 (42)	5 of 12 (42)	3 of 3 (100)	3 (23)	8 (61/5)	3 (23)	–
<i>S. epidermidis</i>	5	2 (40)	0	–	–	–	0	4 (80)	–	4 (80)

than 70% genetic similarity (Figure 1D). Two strains were completely similar to each other, isolated from different wards.

Discussion

In this study, we report the antimicrobial resistance and genotyping of bacteria isolated from UTIs in children in an Iranian referral hospital. The current concern of UTIs is the prevalence of multi drug resistance and also a main problem in the treatment of UTIs.^{17,18}

In our study among 203 positive cultures, the most frequent pathogen was *E. coli* (58%), which is generally the leading uropathogen and was isolated from 56.6–84.6% of Iranian children with febrile UTI.^{19,20} Similarly, it is reported as frequent as 62% in Raman et al's²¹ study. However, in Heidary et al study *P. aeruginosa* was reported as the most common pathogen (49.65%).

In this study, *E. coli* isolates revealed a high resistance rate to trimethoprim-sulfamethoxazole (69%) which has increased compared with the percentage of this antibiotic in previous studies.²¹ According to a report by Bryce et al,²² pooled prevalence of resistance to trimethoprim-sulfamethoxazole was as high as 30% in OECD (Organization for Economic Cooperation and Development) countries and resistance to trimethoprim-sulfamethoxazole was more than twice as high in non-OECD compared with OECD countries. On the other hand, the highest percentages of susceptibility of *E. coli* in our current study were reported for amikacin (97.5%) and nitrofurantoin (91%), which was similar to our previous study.²³

A high *P. aeruginosa* susceptibility rate to amikacin (100%), ceftazidime, cefepime, gentamycin, piperacillin/tazobactam (92%), and imipenem (85%) was reported in the current study which was higher than the frequency reported by Zhang et al.²⁴ Genotyping of 18 *E. coli* strains causing nosocomial infections depicted the presence of three clusters with more than 70% genetic similarity, 12 of 18 strains were isolated from urology or surgical units. These results suggested the possibility of dissemination of these clones, especially in these two units.

More than half of the *K. pneumoniae* isolates were resistant to cefepime and cefotaxime. In addition, all *K. pneumoniae* isolates belonged to one cluster and most of the strains were isolated from the nephrology ward or NICU, suggesting a nosocomial infection between these two units. Similar to the current result, our previous study reported the presence of 93% of *K. pneumoniae* isolates causing nosocomial infections in one cluster.²⁵

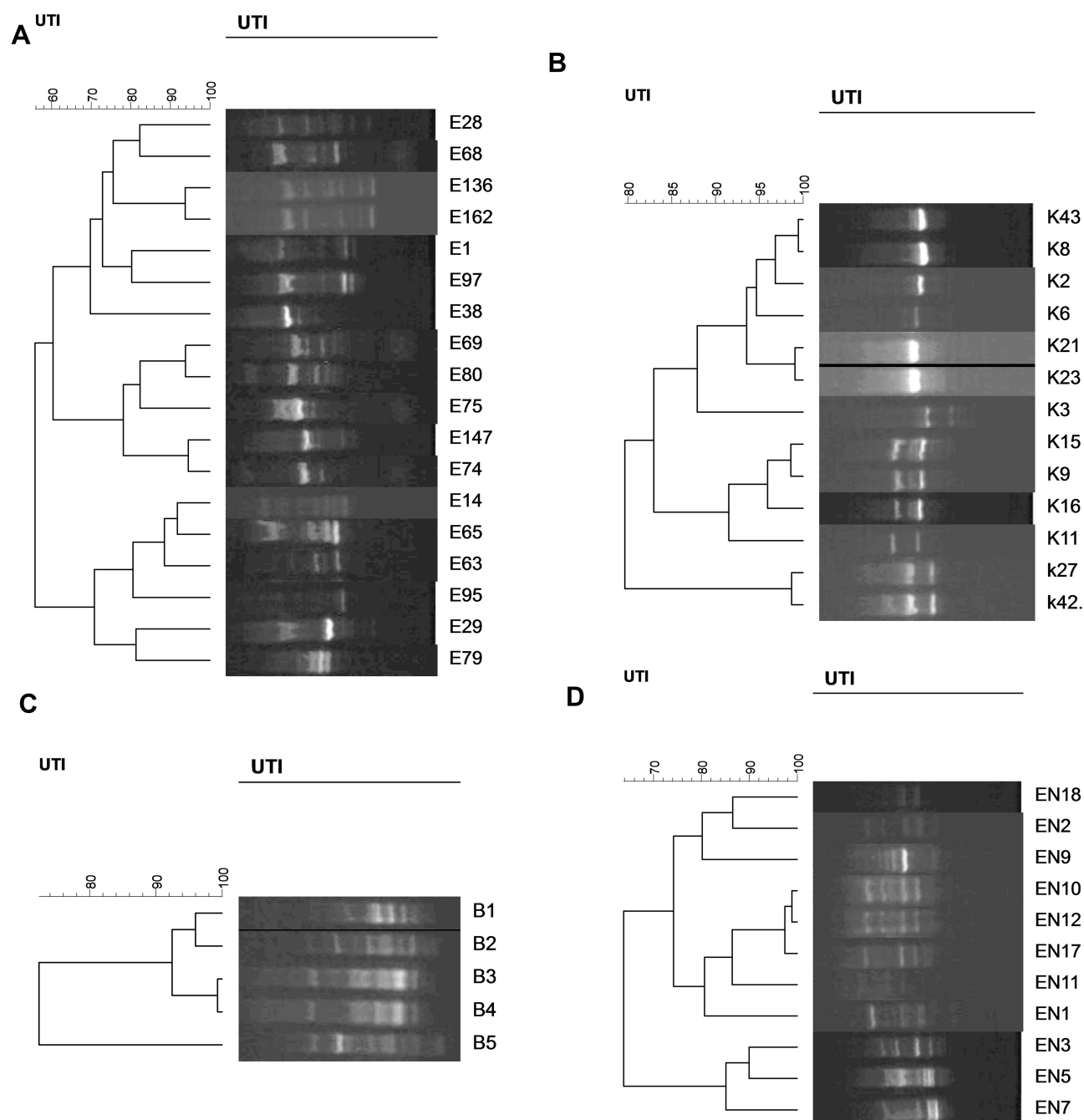


Figure 1 Genotyping of *E. coli* (A), *K. pneumoniae* (B), *B. cepacia* complex (C), and *E. faecium* strains (D) that met hospital-acquired infection criteria using RAPD-PCR.

Treatment of infections caused by *B. cepacia* complex, opportunistic nosocomial pathogens, relies on ceftazidime and other extended-spectrum cephalosporins, as intrinsic resistance prevents the action of many other classes of antimicrobials.²⁶ In our study, all *B. cepacia* complex isolates were susceptible to ceftazidime and 100% resistant to amikacin and gentamycin. In a study conducted in Taiwan, 24% of *B. cepacia* complex were resistant to ceftazidime and, similar to ours, had a high susceptibility

to trimethoprim-sulfamethoxazole.²⁷ The genotyping of *B. cepacia* complex depicted that 80% of isolates had high genetic similarity, isolated from urology, nephrology units, and NICU. Therefore, the spread of one strain between these three wards is probable.

The analysis of RAPD-typing of *E. faecium* strains showed the presence of two clusters with more than 70% genetic similarity and most of the strains were isolated from urology and nephrology units.

The RAPD-PCR is a simple, rapid, easy, inexpensive, and reproducible method that has been widely used in bacterial epidemiology and finding of bacterial genetic similarity that is crucial for cross-infection assessment.^{15,25,28,31} Cross-sectional transmission, especially from the urology unit to other wards, may lead to strains transmission causing nosocomial infection through the hospital. Therefore, much attention should be paid to the basic methods of preventing infection (standard precautions).^{32,33} The length of hospitalization and the use of antibiotics in patients, particularly during long-term hospitalization and unnecessary transfer of patients between different units might have impacts on the reduction of antibiotic resistance and the occurrence of nosocomial infection.

There are several limitations for this study. First, this is a single-center retrospective study using existing data collected over years with small samples size. It is possible that we could have missed cases such as those who were treated without any culture testing, or received antibiotics before urine collection for culture. Second, there was no access to some clinical information of the patients such as the results of treatment, so determining the rate of the outcome was impossible.

In conclusion, the most frequent isolated pathogen was *E. coli* and the increasing rate of antibiotic resistance to the commonly prescribed antimicrobial agents such as trimethoprim/sulfamethoxazole and cephalosporins was observed. Moreover, the results of this study showed the presence of clones with $\geq 80\%$ similarity in *E. coli*, *K. pneumoniae*, *E. faecium*, and *B. cepacia* complex isolates; therefore, the transmission of nosocomial infections from one patient to another or one ward to another is probable.

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Disclosure

The authors report no conflicts of interest in this work.

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