



## Antimicrobial Susceptibility Pattern and ESBL Production among Uropathogenic *Escherichia coli* Isolated from UTI Children in Pediatric Unit of a Hospital in Kerman, Iran

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### Authors' contributions

This work was carried out in collaboration between all authors. Author MRS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SA and MHS managed the analyses of the study. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** Emergence of antibiotic resistance and extended spectrum  $\beta$ - lactamase (ESBL) among uropathogens in the pediatric unit of hospitals created serious health care concern. This study deals with antimicrobial susceptibility and ESBL analysis of uropathogenic *Escherichia coli* isolated from children hospitalized in pediatric unit of a university hospital in Kerman, Iran.

**Methodology:** Fifty-five uropathogens positive samples were recovered from one hundred thirty five samples collected from urine of the children hospitalized with sign of UTI in pediatric unit of a hospital, in Kerman, Iran from April 2011 to November 2012.

Preliminary antimicrobial susceptibility testing was carried out using agar disk-diffusion breakpoint assay and minimum inhibitory concentrations (MICs) of different antibiotics were determined by agar dilution method. ESBL production was detected by a double disk synergy test and confirmed by a phenotypic confirmatory test.

**Results:** Of fifty-five positive samples isolated, *Escherichia coli* (69%) was the leading uropathogen followed by *Klebsiella* spp. (18.8%), *Proteus* (7.27%), *Staphylococcus aureus* (3.63%), *Citrobacter* (1.8%), *Enterobacter* spp. (1.81%) and *Enterococcus* (1.8%). Antimicrobial susceptibility tests revealed that almost all uropathogenic *E. coli* were

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sensitive to carbapenems (100%) and amikacin (94.4%), while, 100% of the strains were resistant to ampicillin (MIC range  $\pm 32 \mu\text{g/mL}$ ), 63.8% were resistant to amoxicillin/clavulanic acid (MIC range  $\pm 32 \mu\text{g/mL}$ ), 33% were resistant to trimethoprim- sulfamethoxazole (MIC range  $\pm 64.2 \mu\text{g/mL}$ ) and 61.1% of the strains were resistant to third generation of cephalosporins (MIC range  $\pm 8.0 \mu\text{g/mL}$ ) ( $P=0.05$ ). The ESBL confirmatory test for uropathogenic *E. coli* isolates resistant to third generation of cephalosporins revealed that only 20% were produced detectable ESBL enzymes.

**Conclusion:** From above results it can be concluded that *E. coli* was the most common nosocomial pathogen associated with UTI among hospitalized children in our hospital and amikacin, carbapenems were very effective drugs for treatment of UTI in these age group, while, care must be taken when third generation of cephalosporins and trimethoprim-sulfamethoxazole are administered.

**Keywords:** *Escherichia coli*; UTI; antimicrobial susceptibility; ESBL; pediatric unit.

## 5. INTRODUCTION

Urinary tract infection (UTI) is defined by infection of any part of urinary tract by the Microorganisms. UTI is a problem that is frequently encountered by pediatric healthcare system and is a very common infection observed in children. Although most patients have a good prognosis, UTI can cause significant morbidity, prolong hospital stay and complications (Bacteremia and sepsis) [1,2]. Clinically important ascending urinary tract infections in children usually occur due to bacteria and *Candida* especially in female. This is probably because they have shorter urethras, so easily contaminated by uropathogens. Common bacterial pathogens usually associated with UTI include gram-negative species such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Serratia* spp. And gram-positive organisms, including group B streptococci, *Enterococcus* spp. And coagulase negative *Staphylococcus* [3]. UTIs are often treated with different antibiotics like third generation of cephalosporins, trimethoprim- sulfamethoxazole, nitrofurantoin, aminoglycosides and nalidixic acid [4].

Information of resistance to antimicrobial agents is especially important in treatment of pyelonephritis in young children and fewer treatment options to treat hospitalized children compared with adults. Urinary infectious processes represent the second or third most common type of nosocomial infection [3].

Unfortunately because of excessive application of antibiotics in pediatric unit of different hospitals especially in developing countries, the antimicrobial resistance among uropathogenic bacteria across the globe are emerging [3,4], for e.g. in recent years, spread of the plasmid mediated ESBL production in gram negative bacteria has been resulted in an increasing incidence of resistance to third generation of cephalosporins among the urinary pathogens in pediatric unit of many hospitals. The antibiotic prescribing patterns for pediatric UTI from 1998 to 2007 revealed that third generation cephalosporins constituted the majority of parenterally administered antibiotics (77%) and among these, ceftriaxone in 95% of the visits while, trimethoprim-sulfamethoxazole was prescribed in 49% of UTI [5].

Little information is available regarding antimicrobial susceptibility and ESBL among *E.coli* strains in Iran [6,7].

This study deals with antimicrobial susceptibility pattern and ESBL production among uropathogenic *E. coli* isolated from children hospitalized in pediatric unit of a university hospital in Kerman, Iran.

## 2. MATERIALS AND METHODS

### 2.1 Sampling and Bacterial Identification

A total of fifty- five bacterial strains were recovered from one hundred thirty five samples obtained from urinary tract infected children hospitalized in pediatric unit of a university hospital in Kerman, Iran during April 2011 to November 2012. Midstream urine were collected in clean sterile 10mL urinary containers, centrifuged at 5000 rpm for 5 minutes and 0.1mL of the lower portion of the pellet was then inoculated into 5mL sterile transport medium. The samples were then transferred to the Department of Microbiology, Kerman University of Medical Sciences for further analysis within 1 to 2 hours of the sampling. Information about the patient's age and sex were collected from medical records. Several criteria were included in this study like patient clinical condition (pain in time of urination, chill and fever), direct microscopic count and presence of leukocytes in the urine.

A loopful of the bacterial culture was suitably diluted ( $10^{-2}$ ) with 5mL sterile 0.1N normal saline and streaked onto MacConkey and sheep blood agar medium (E-Merck, Germany), The plates were incubated for 24 hours at 37°C. Bacterial identification were performed by routine biochemical tests such as gram reaction, motility, ability to ferment lactose, H<sub>2</sub>S, urease production, triple sugar iron agar (TSI), phenyl alanine deaminase (PAD), lysine decarboxylase (LDC), MR, VP and indole as described previously [8,9]. The identified isolates were mixed with 2mL sterile trypticase soy broth (TSB) containing 40% glycerol in True North™ Cryogenic Vials (TNC) and preserved at -70°C for further investigation.

### 2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of all fifty- five bacterial uropathogens was tested by the Kirby-Bauer (KB) agar disk diffusion breakpoint method according to Clinical Laboratory Standards Institute (CLSI 2011) guide lines [10] using commercially available paper disks (Padtan-Teb, Iran). Antibiotics were used in the following concentrations (in  $\mu\text{g mL}^{-1}$ ): ceftazidime (CAZ) (30 $\mu\text{g}$ ), trimethoprim-sulfametoxazole (SXT) (10 $\mu\text{g}$ ), cefotaxime (CTX) [30 $\mu\text{g}$ ], amoxicillin / clavulanic acid (AMC) (30 $\mu\text{g}$  + 10 $\mu\text{g}$ ), gentamicin (Gm) (10 $\mu\text{g}$ ), amikacin (AN) (30 $\mu\text{g}$ ), cefepime (FEP) (30 $\mu\text{g}$ ), imipenem (IMP) (10 $\mu\text{g}$ ), meropenem (MEN) (10 $\mu\text{g}$ ), cefixime (CFM) (5 $\mu\text{g}$ ) and nalidixic acid (NA) (30 $\mu\text{g}$ ). A standard 264neumonia adjusted to 0.5 McFarland was swabbed on to Muller-Hinton agar (Hi-media, India) and antibiotic disks were dispensed after drying the plate for 3 to 5 min and incubated at 37°C for 24 hours. Zone of inhibition surrounding each disk was measured and labeled as resistance, intermediate, sensitive according to CLSI procedure. Similarly, MIC of following antibiotics CAZ, AMP, AMC, SXT and AN against resistant isolates were determined by agar dilution method [9]. The isolates were considered susceptible if the MIC was  $\leq 2 \mu\text{g/mL}$  and resistant if the MIC was  $\geq 8 \mu\text{g/mL}$ . In order to guarantee the quality of antimicrobial susceptibility testing, a laboratory reference strain of *E. coli* with known susceptibility was run along to the routine isolates.

## 2.3 ESBL Detection Tests

The extended spectrum  $\beta$ -lactamase (ESBL) phenotypic confirmatory test with cefotaxime, cefotaxime + clavulanic acid and amoxicillin + clavulanic acid, were performed for all isolates that were resistant to third generation of cephalosporins. In this test, an overnight culture suspension of the test isolates which were adjusted to 0.5 McFarland's standard were inoculated by using sterile cotton swab on the surface of a Mueller Hinton Agar plates. The cefotaxime (30 $\mu$ g) and cefotaxime- clavulanic acid (30 $\mu$ g/10 $\mu$ g) disks were placed 20 mm apart on the agar. Similarly, the amoxicillin + clavulanic acid (30 $\mu$ g/10 $\mu$ g) disk was placed 20mm apart. After incubating overnight at 37°C for 24 hours, a  $\geq$  5mm or more increase in the zone diameter around cefotaxime/clavulanic acid (CTX/Clav) was considered positive for ESBL production [11]. *E.coli* ATCC25922 was used as negative ESBL control.

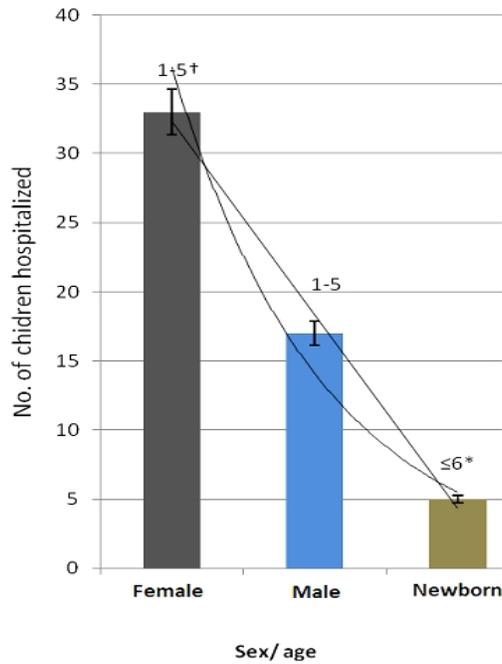
## 2.4 Statistical Analysis

The difference in susceptibility patterns was analyzed by the Pearson Chi-square or two-tailed Fisher exact test. A  $P=0.05$  was considered as statistically significant for two-tailed tests. SPSS v17.0 software (SPSS Inc., Chicago, IL) was used for the statistical analysis.

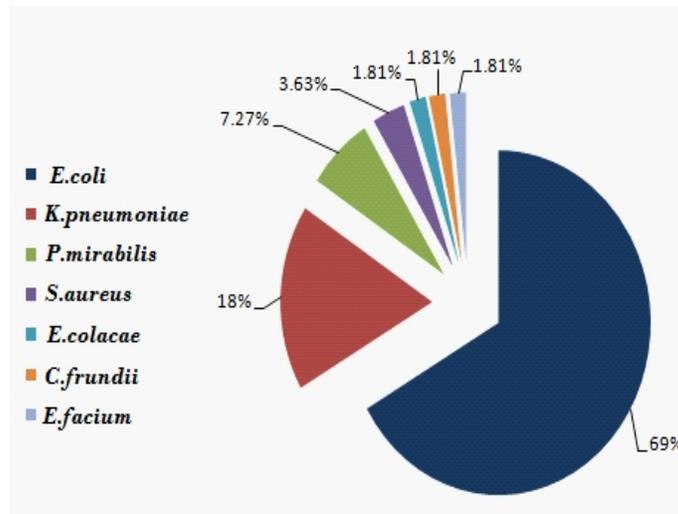
## 3. RESULTS

### 3.1 Distribution Uropathogenic *E. coli* and Antimicrobial Susceptibility

Fig.1 shows distribution of children hospitalized in our hospital with sign of UTI according to sex and age. Only inpatients were included in this investigation. 33 (60%) were female, 18 (30%) were male and 4 (7.2%) were newborn. We included all patients between  $\leq$ 5 months and 5 years of age. Majority of children exhibited mild to severe fever, malaises and chill. Some of them were also demonstrated sign of pain when urinated. Among gram negative bacteria, *E.coli* was leading uropathogen follow by *Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter*, while, among gram positive bacteria *S. aureus*, *Enterococci* were predominant (Fig.2). Antimicrobial susceptibility of the *E.coli* isolated during this study (Table1) revealed that 100% were sensitive to carbapenems and 94.4% to amikacin, while, all the uropathogenic *E.coli* isolates were resistant to ampicillin (100%). Most of them (33%) were resistant to trimethoprim- sulfamethoxazole, while, 63.8% of the isolates were resistant to amoxicillin/clavulanic acid as well as third generation of cephalosporins (68.8%), gentamicin (22.2%) and cefepime (13.8%).



**Fig. 1. Distribution of children hospitalized with sign of UTI in pediatric unit of a university hospital according to sex and age. A description for the signs shown on bars**



**Fig. 2. Percentage and types of bacteria isolated from UTI patients in pediatric unit of a university hospital during April 2011 to November 2012**

**Table 1. Antimicrobial susceptibility of uropathogenic *E. coli* isolated from UTI\* of children hospitalized in our pediatric unit to routinely used antibiotics.**

Antibiotic	<i>E. coli</i> (n=36)		
	R	S	I
AMC	23(63.8)	3(8.3)	10(25)
AMP	36(100)	0(0)	0(0)
Gm	8(22.2)	25(69.4)	3(8.3)
SXT	12(33)	8(22.2)	16(44)
AN	0(0)	34(94.4)	2(5.5)
CAZ	9(25)	22(61.1)	5(13.8)
CTX	14(38.8)	18(50)	4(11.1)
NA	17(47.2)	15(40.9)	4(11.1)
CFM	16(44.4)	19(52.7)	1(2.7)
IPM	6(16.6)	30(83.3)	0(0)
MEN	0(0)	36(100)	0(0)
FEP	5(13.8)	28(77.5)	3(8.3)

\* UTI, urinary tract infection. † P=0.05

Muller-Hinton agar was used for susceptibility testing. Inoculums diluted to obtain  $1 \times 10^8$  CFU/mL.

AMC= amoxicillin + clavulanic, AMP= ampicillin, Gm=gentamicin, SXT= trimethoprim + sulfamethoxazole, AN= amikacin, CAZ=ceftazidime, CTX= cefotaxime, NA= nalidixic acid, CFM= cefixime, IMP= imipenem, MEN= meropenem, FEP=cefepime. Figures in bracket represent the percentage of bacterial isolates. R=resistant. S=sensitive. I=intermediate.

### 3.2 Minimum Inhibitory Concentrations (MIC)

The MICs of the five routinely used antibiotics in our pediatric unit hospital are shown in Table 2. 66.6% [n=24] of uropathogenic *E. coli* isolates exhibited MIC 32µg/mL and 22.2% [n=8] showed MIC ≥64µg/mL to ampicillin. It was found that 72.2% [n=26] demonstrated MIC ≥64µg/mL to trimethoprim- sulfamethoxazole and 80.3% exhibited MIC 32µg/mL to amoxicillin + clavulanic acid. The results further supported disk diffusion method.

**Table 2. MIC\* of *E. coli* isolated from UTI patients in pediatric unit to five antibiotics routinely used in our hospital**

Antibiotic	MIC (µg/mL)									
	≤0.063	0.25	0.5	1.0	2	4	8	16	32	≥64
CAZ	0(0)	19(52)	0(0)	2(5.5)	1(2.7)	1(2.7)	8(22.2)	5(13.8)	ND	ND
AMP	0(0)	0(0)	0(0)	0(0)	1(2.7)	2(5.5)	1(2.7)	ND	24(66.6)	8(22.2)
SXT	ND	ND	0(0)	0(0)	5(13.8)	0(0)	2(5.5)	1(2.7)	2(5.5)	26(72.2)
AMC	ND	ND	ND	0(0)	1(2.7)	2(5.5)	3(8.3)	3(8.3)	16(44.4)	11(30.5)
AN	25(69.4)	8(22.2)	2(5.5)	1(2.7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

\*MIC= Minimum inhibitory concentration. CAZ= Cefazidime, AMP= Ampicillin, CIP=Ciprofloxacin, SXT= Trimethoprim/sulfamethoxazole, AMC= Amoxicillin/clavulanic acid, ND= Not determined. Figure in barracked indicate the percentage.

Amikacin was the most effective antibiotic along with imipenem and meropenem. 69.4% [n=25] exhibited MIC ≤0.063µg/mL, while, 22.2% [n=8] showed MIC 0.25µg/mL to amikacin.

### 3.3 ESBL Production

The ESBL production revealed that only 20% of isolated *E. coli* were produced detectable ESBL enzymes and remaining *E. coli* were negative for ESBL. The results were further supported by ESBL confirmatory test.

## 4. DISCUSSION

Periodic surveillance of antibiotic sensitivity in pediatric unit of hospitals is core stone in antimicrobial stewardship, since it prevent urosepsis, give idea regarding the most efficient antibiotic should be used in routine therapy and possible emergence of multiple antibiotic resistant (MDR) strain of bacteria. Antimicrobial susceptibility patterns of pediatric uropathogens to six of the most common antibiotics in use for urinary tract infections in children were showed that resistance rates for females and males children, respectively, were as follows: ampicillin 44.3% and 44.6%; trimethoprim –sulphamethoxazole 24.5% and 36.7%; amoxicillin/ clavulanic acid 12.4% and 27.5%; Cefazoline 10.9% and 27.1%; ciprofloxacin 0.9% and 2.4% [4].

McLoughlin and Matar [4] isolated 126 urine cultures; the majority of organisms were *E.coli* accounting for 89% of the patients. Other organisms identified were *Klebsiella* 3.7%, *Proteus* 1.2%, *Citrobacter* 1.2%, *Staphylococcus* 1.2%, and *Enterococcus* 3.7% (all in children/4 years old).

Similarly, It has been reported that *E. coli* was the most frequently occurring pathogen (54.8%), followed by *K. pneumoniae* (16.0%), coagulase negative *Staphylococci* (11.2%), *Enterobacter* spp. (9.6%), *Proteus* spp. 1.4% and *P. aeruginosa* (1.4%) [7]. Resistance rates of *E. coli* isolates were 85.9% to trimethoprim- sulfamethoxazole, 80% to nafcillin, 77% to ampicillin, 68% to chloramphenicol, 12.9% to ciprofloxacin, 12.9% to ceftriaxone, 12.9% to cephalotin, and 14% to amikacin. Results obtained from Iran [8], suggested that *E. coli* was extremely resistant to ampicillin but highly sensitive to amikacin in most of the hospitalized children.

A survey of antibiotic susceptibility in pediatric infections in a teaching hospital in Mato Grosso do Sul, Brazil revealed that imipenem was the most effective drugs, inhibiting all or almost all of the Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter calcoaceticus* [11]. In a recent study from United States, among the multidrug-resistant *E. coli* isolates, 97.8% were resistant to ampicillin, 92.8% to trimethoprim- sulfamethoxazole, 86.6% to cephalothin, 38.8% to ciprofloxacin, and 7.7% to nitrofurantoin.

*Khotayi* et al. [12] studied 90 pediatric patients with urinary tract infection. The most common pathogen in 77.7% of specimens was found to be *E. coli*. The most effective antibiotics were amikacin with sensitivity in 91.5% of cases and oral nalidixic acid in 76.9% of specimens collected. Ampicillin was the most ineffective antibiotic (resistance in 87.5% of specimens). In other investigation [13] in Mashhad city, it was found that *E.coli*, *Klebsiella* and *Proteus* were the causative organisms in 87.3% of UTI in hospitalized children. They were sensitive to cefotaxime, cefixime, cephalotin, amikacin, ciprofloxacin, nitrofurantoin and gentamicin in more than 96% while resistant to trimethoprim-sulfamethoxazole in about 75%.

In our study, *E. coli* was isolated in 69% of cases followed by *Klebsiella* and *Proteus*. This observation was in accordance with work carried out in Kuwait and Iran [14,15].

Since antimicrobial susceptibility patterns are and have always been the most important research topics in microbiology, therefore we decided to carry the antibiotic susceptibility test and ESBL production among the isolates. As our data indicated, it was found that the uropathogenic *E. coli* were still susceptible to valuable antibiotics such as imipenem, meropenem and amikacin, while, the susceptibility test revealed majority of the isolates were exhibited MIC  $\geq 64$   $\mu\text{g/mL}$  to SXT and MIC  $\geq 64$  to AMC. The low rate of resistance of uropathogenic *E. coli* to amikacin, and carbapenems in our study were probably due to a very restricted antibiotic prescribing policy in our hospital. The periodic susceptibility test also is important regarding emergence of carbapenemase type of ESBL in our hospital. It should be noted that we denote a possible bias in the epidemiological evaluation of our data (we cannot exclude that some contaminants were improperly enumerated as significant pathogens) due to the lack of the precise evaluation on microbial count in our urine specimens.

The antimicrobial susceptibility of all clinical isolates in the pediatric intensive care unit in India was found that among the gram negative organisms, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* were the 3 leading bacterial isolates. Carbapenem resistant *P. aeruginosa* accounted for 34% of *P. aeruginosa* isolates [16]. Haller et al. [17], were studied antibiotic resistance pattern of Gram negative bacteria associated with UTI infection in pediatric university hospital of Freiburg, Germany. They found that high resistance rates to alternative agents such as trimethoprim- sulfamethoxazole (almost 40%) and the oral cephalosporins (almost 30%), which is frequently used in Germany for community-acquired infection in children. Similarly, Bardsiri and Shakibaie [18] compared the antibiotic resistance pattern of biofilm producing and non producing *Proteus* spp. Recently in our laboratory, Jafari et al. [19], isolated a novel plasmid from ICU isolate of *P. aeruginosa* confer resistance to third generation of cephalosporins and aminoglycosides.

The ESBL confirmatory test for our uropathogenic *E. coli* isolates resistant to third generation of cephalosporins revealed that only 20% *E. coli* were able to produce a detectable ESBL enzyme, while for the other resistant isolates the ESBL test was negative. The ESBL producing isolates were sensitive to amikacin, imipenem and meropenem. The data indicate that further research is needed to study the mechanism of resistance to third generation of cephalosporins for these isolates. In our case the ampicillin administration must be discontinued due to overwhelming resistant to this antibiotic.

## 5. CONCLUSION

From above results it can be concluded that *E. coli* was leading pathogen associated with UTI among hospitalized children in our hospital in Iran and amikacin, carbapenems were very effective drug for treatment of UTI in these age group, while, care should be taken when third generation of cephalosporins, nalidixic acid and trimethoprim- sulfamethoxazole are administered. It should be emphasized that periodic antibiotic susceptibility test in this age group is a matter of healthcare priority and antimicrobial stewardship.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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