

**TRENDS IN ANTIMICROBIAL RESISTANCE AMONG UROPATHOGENS WITH SPECIAL REFERENCE TO ESCHERICHIA COLI IN COMMUNITY ACQUIRED PEDIATRIC URINARY TRACT INFECTIONS FROM KERALA**Nisha K.V<sup>1</sup>, Rathika D. Shenoy<sup>2</sup>, A. Veena Shetty<sup>3</sup>, Vijaya M. Shenoy<sup>4</sup>, Avinash K. Shetty<sup>5</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: CONTEXT:** Resistance of uropathogens to commonly used antibiotics is increasingly reported from India in adults. There is little data on resistance patterns in childhood community acquired urinary tract infections (UTI). **AIMS:** To study antibiotic resistance trends of uropathogens isolated in community acquired UTI in children from a geographic area. **SETTINGS AND DESIGN:** Prospective study conducted in a northern Kerala tertiary pediatric centre between November 2012 and October 2014. **METHODS:** Urine samples were obtained by clean catch midstream, bladder catheterization or supra-pubic aspiration. Bacterial growth, when significant were identified by standard biochemical reactions with antibiogram by Kirby Bauers disc diffusion method. **STATISTICAL ANALYSIS:** Proportions were calculated and statistical significance obtained by Chi-square. **RESULTS:** Of the 1387 cultures with suspected community acquired UTI, 274 (19.75%) were positive. Mean age of study group was 28.52 months and 64.6% were boys. Escherichia coli (E.coli) was the predominant pathogen [189(69%)] followed by Klebsiella 50(18.2%). Citrobacter, Enterococcus, Proteus and Pseudomonas constituted less than 6%. All urinary isolates showed high combined resistance to most commonly used antimicrobials including beta-lactam antibiotics (84.3% to cefotaxime, 83.2% to cefixime, 74.5% to ampicillin), quinolones (54.2% to ciprofloxacin, 46.4% to norfloxacin, 32.8% to levofloxacin), 52.9% to co-trimoxazole, 30.2% to gentamicin, 24.1% to nitrofurantoin and 14.2% to netilmicin. E.coli was highly resistant to cephalosporins and ampicillin (>80%). **CONCLUSIONS:** There is increasing resistance amongst E.coli coli and Klebsiella to third generation cephalosporins in pediatric age group. It is important to generate regional data on antibiogram pattern to guide therapy.

**KEYWORDS:** Community acquired Urinary tract infection, Escherichia coli, Ampicillin, Cephalosporins, Quinolones.

**KEYMESSAGES:** E.coli is a major uropathogen in community acquired urinary tract infection in children. Resistance to penicillins, cephalosporins, quinolones and aminoglycosides were statistically significant compared to antibiogram trends of other isolates. As initial therapy is empiric, regional resistance patterns can guide clinicians on antimicrobial choice.

**INTRODUCTION:** Urinary Tract Infection (UTI) is one of the most common childhood bacterial infections. They result in acute as well as long-term morbidity including hypertension and renal scarring with substantial financial burden to society.<sup>[1]</sup> Thus the current goal of management of UTI is early detection and appropriate antimicrobial treatment. Initial empirical antimicrobial treatment should be based on the knowledge of most likely pathogen and its local antibiotic susceptibility data. Enterobacteriaceae are the most common pathogens causing UTI in children and among these, Escherichia coli (E. coli) is the most frequent, accounting for more than 65% of cases followed by non-

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E.coli uropathogens which include Klebsiella spp, Proteus spp, Pseudomonas aeruginosa, Citrobacter spp and Enterococcus spp.<sup>[2]</sup>

Changes in antibiotic susceptibility patterns and emergence of antibiotic resistance in community and hospital acquired pediatric uropathogens affect empiric therapy of resultant infections.<sup>[3-5]</sup> There are very few reports on resistance pattern of community acquired UTI from India especially in children.<sup>[6-8]</sup> E.coli is the most common organism isolated in all these studies. Comparison of the antibiogram trends in these studies suggests an increasing resistance trend to ampicillin and cephalosporins over the last few years. In the study by Taneja et al consisting of 558 isolates, 32.7% and 75.5% were resistant to ampicillin and cephalosporin respectively.<sup>[6]</sup> Ghadage et al have documented 98% and 87% resistance to ampicillin and cephalosporin in their 390 culture positive samples.<sup>[7]</sup> In the study by Sharan et al consisting of 64 samples, 98% and >60% were resistant to ampicillin and cephalosporins respectively.<sup>[8]</sup> Similarly, Rao et al in their 857 culture positive urine samples show 89% resistance to cephalosporins.<sup>[9]</sup> There is no regional data from Kerala state till date. It is well known that there may be marked differences in antibiotic sensitivity pattern between various geographic areas within the same country. Thus this study aims to assess microbiological profile and antibiotic resistance trends of uropathogens isolated in community acquired UTI in children from Kerala.

**SUBJECTS AND METHODS: Setting:** The present study was carried out in the central clinical microbiology laboratory of a tertiary care hospital with a large out- and in-patient pediatric facility in Northern Kerala, India. The study was conducted after due approval from the institutional ethics committee and informed consent was obtained from either parent.

**Study Design:** It is a prospective microbiological study of all significant isolates from urine specimens of pediatric patients with suspected community acquired UTI who attended outpatient department between November 2012 and October 2014. Isolates included were those of children aged  $\leq 18$  years with culture-proven UTI (significant bacteriuria) and fulfilling definition of community acquired infection. Urinary tract infection was defined according to the American Academy of Pediatrics (AAP) guidelines.<sup>[1]</sup> Cultures were considered positive when there was a growth of a single pathogen in a urine specimen of  $>10^5$  CFU/ml collected by midstream catch,  $>10^4$  CFU/ml for catheterized sample, or any growth obtained by a suprapubic aspiration. Isolates excluded were those with health care associated UTI defined as follows: i. UTI during hospitalization ( $>48$ hrs) or within 48 hours of hospital discharge, ii. Hospitalization during 90 days preceding culture, iii. Children with chronic systemic illnesses, iv. On intravenous therapy or specialized wound care, v. on hemodialysis or antineoplastic chemotherapy within 30 days and vi. Residing in a nursing home or transferred from another hospital.<sup>[10]</sup> Bag collected samples and mixed cultures were excluded from the study.

**Sample Collection and Processing:** Cultures were obtained by midstream catch, bladder catheterization or suprapubic aspiration. Urine microscopy was performed. Semi quantitative urine culture was done using calibrated loop method. A loopful (0.001 mL) of well mixed un-centrifuged urine was inoculated onto the surface of MacConkey and blood agar. The culture plates were incubated aerobically at 37°C for 18-24 hours and bacterial count were expressed as CFU/ml. The culture isolates were identified by standard microbiological methods with the recommended media

and standard control strains.<sup>[11]</sup> Antimicrobial susceptibility testing was done by the standard Kirby-Bauer disc diffusion method.<sup>[12]</sup> After inoculation with organisms and placement of discs, Mueller-Hinton agar plates were incubated at 37°C for 18-24 hours and the inhibition zones were measured. The following standard antibiotic discs (mcg) were used: ampicillin (10mcg), cefixime (30), cefotaxime (30), gentamicin (10), netilmicin (30), co-trimoxazole (25), ciprofloxacin (5), norfloxacin (10), levofloxacin (5), doxycycline (30), nitrofurantoin (300) and imipenem (10). Imipenem resistance was confirmed by E strip method (Imipenem with & without EDTA Ezy MIC™ Strips EM078). All culture media and antibiotic discs were procured from HiMedia Laboratories, Mumbai, India. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI) 2010.<sup>[13]</sup> The quality control strains used were *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212.

**Statistical analysis:** Data was analyzed using SPSS (Statistical Package for Social Sciences) software version 16. Student's t test was used to compare continuous variables, and the chi-square test was used to compare categorical variables. A p value <0.05 was considered as significant.

**RESULTS:** During the study period, 1387 pediatric urine samples with suspected community acquired UTI were processed of which 274 (19.75%) had significant bacteriuria. Mean age was 28.52±1.77 months and 64.6% (177) were boys. About 243 (88.6%) were under five of which 119 (43.4%) were infants. Figure 1 shows the distribution of uropathogens in the isolates. *E. coli* was the predominant pathogen with 189 isolates (69%) followed by *Klebsiella* 50(18.2%), *Citrobacter* 15(5.5%), *Enterococcus* 9(3.3%), *Proteus* 8(2.9%) and *Pseudomonas* 3(1.1%). Table 1 shows the overall resistance pattern of these pathogens to various antibiotics. Overall resistance trends of all isolates to antimicrobial agents were 74.5% to ampicillin, 84.3 and 83.2% to cefotaxime and cefixime, 52.9% to co-trimoxazole, 35.4% to doxycycline, 54.2, 46.4 and 32.8% respectively to ciprofloxacin, norfloxacin and levofloxacin, 30.2% and 14.2% to gentamicin and netilmicin and 24.1% to nitrofurantoin.

More than 80% of *E. coli* were resistant to ampicillin, cefixime and cefotaxime; more than 50% to co-trimoxazole and quinolones except levofloxacin. Least resistance was observed for netilmicin and nitrofurantoin and none for imipenem. Figure 2 depicts the antibiotic resistance percentage of community acquired *E. coli* to various antimicrobials tested. Table 2 allows the comparison of the resistance of each uropathogen. *Klebsiella* and *Pseudomonas* also were resistant to cephalosporins (>80%) while *Proteus* and *Citrobacter* showed relatively lower resistance. Six strains of *Klebsiella* were resistant to imipenem. *Enterococcus* showed resistance to ampicillin, cephalosporins, co-trimoxazole (>55%), quinolones (>75%) except levofloxacin and were more sensitive to gentamicin (3% resistance). Antimicrobial resistance of *E. coli* were statistically significant for beta lactams (p0.000), co-trimoxazole (p0.017), ciprofloxacin (p0.005), levofloxacin (p0.037), norfloxacin (p0.000), gentamicin (p0.022), netilmicin (p0.004), imipenem (p0.006), and nitrofurantoin (p0.000) in comparison with other isolates.

**DISCUSSION:** The present study provides data on microbial prevalence and their antibiotic resistance pattern in community acquired UTI from Northern Kerala, India and Table 3 allows comparison with other studies. The yield rate of a positive culture of 19.75% was comparable to 17.9% by Sood et al.<sup>[14]</sup> However studies by Taneja et al, Ghadage et al and Dash et al have shown

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higher yield rate of 28.2, 24.7, and 39.7 percentages respectively.<sup>[6,7,15]</sup> The present study shows that boys (64.6%) had higher prevalence of UTI similar to the study by Taneja et al who showed 77.8% male prevalence.<sup>[6]</sup> There is almost equal prevalence of UTI in boys (51%) and girls (49%) in the study by Ghadage et al.<sup>[7]</sup> As per our study majority were under five specifically infants which is comparable with Taneja et al with 64.25% in under five of which 25.6% were infants and Ghadage et al with 71% in under five of which 37.7% were infants.<sup>[6,7]</sup> E. coli is the most common etiologic agent (69%) of community-acquired UTI in our study. Kothari et al and Dash et al report similar prevalence of E. coli in their studies.<sup>[15,16]</sup> Sharan et al have shown 76.5% E.coli in childhood UTI whereas Tanja et al and Ghadage et al have recorded a lower prevalence 47.1% and 45.12% respectively.<sup>[6-8]</sup> Worldwide data also show that E. coli and Klebsiella spp. are the commonest uropathogens isolated in community acquired UTI patients.<sup>[16,17]</sup>

This study shows higher antibiotic resistance in Gram-negative bacteria when compared to Gram-positive isolates. Among Gram-negative bacteria, E.coli showed high level of resistance to commonly used antibiotics like ampicillin, cephalosporins (Cefixime, cefotaxime), fluoroquinolones (Ciprofloxacin and norfloxacin) and co-trimoxazole. This is similar to other studies on community acquired UTI from elsewhere.<sup>[14-20]</sup> Six strains of Klebsiella spp showed imipenem resistance, which is a reserve drug for multidrug resistant uropathogens. AAP guidelines recommend third generation cephalosporins, tobramycin, gentamicin and piperacillin as empirical parenteral therapy for UTI where as co-amoxiclav, co-trimoxazole and second generation cephalosporins are recommended for oral therapy for uncomplicated UTI in children.<sup>[1]</sup> However, the guidelines add that local antimicrobial susceptibility patterns must be considered before choosing an agent. The alarmingly increasing prevalence of multidrug resistant uropathogens in the community setting calls for prudent use and right choice of antibiotics both for treatment and prophylaxis in children.

**Limitation:** Extended spectrum beta lactamase (ESBL) confirmation test was not done for the isolates which is a limitation of our study.

**CONCLUSION:** The study allows comparison of resistance patterns to antimicrobials in community acquired childhood UTI from a geographic region of Southern India. Urine culture is essential in pediatric febrile illness and the yield rate can be as high as one-fifth with an increasing resistance amongst E.coli and Klebsiella to cephalosporins, penicillins and quinolones. Traditional first line drug used for treating childhood UTI such as ampicillin, cephalosporins and co-trimoxazole might no longer be adequate. There is a need for continued monitoring of antimicrobial susceptibility of uropathogens isolated from community acquired UTI and the generated regional data on antibiogram pattern should guide therapy.

Antibiotic	Number of isolates with resistance (Percent)
Ampicillin	204(74.5)
Cefixime	228(83.2)
Cefotaxime	231(84.3)
Co-trimoxazole	105(52.9)
Ciprofloxacin	150(54.7)
Doxycycline	97(35.4)

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Gentamicin	84(30.7)
Imipenem	3(1.1)
Levofloxacin	90(32.8)
Netilmicin	39(14.2)
Nitrofurantoin	66(24.1)
Norfloxacin	127(46.4)

Table 1

**Table 1: Overall resistance percentage of community acquired uropathogens to various antimicrobials.**

Organism (n = 274)	Percentage of Resistance										
	Amp	Cfm	Ctx	Cot	Cip	Gen	Ipm	Le	Net	Nit	Nx
E coli (189)	82	88	92	53	60	29	0	37	10	15	55
Klebsiella (50)	70	80	76	62	46	34	6	18	26	56	22
Citrobacter (15)	26	46	40	26	26	13	0	13	6	6	20
Proteus (8)	37	50	50	12	12	12	0	25	0	62	0
Pseudomons (3)	66	100	66	55	77	77	0	33	33	0	33
p value	0.00	0.00	0.00	0.017	0.005	0.022	0.006	0.037	0.004	0.00	0.00

Table 2

**Table 2: Comparison of resistance patterns in percentage to Enterobacteriaceae family for various antimicrobials in community acquired pediatric urinary tract infection**

Amp-Ampicillin, Cfm-Cefixime, Ctx-Cefotaxime, Cot-Co-trimoxazole, Cip-Ciprofloxacin, Gen-Gentamicin, Ipm-Imipenem, Le-Levofloxacin, Net-Netilmicin, Nit-Nitrofurantoin, Nx-Norfloxacin

Study (Year)	Sample size	Antibiotic resistance (%)						Remarks
		Amp	Ctx	Cot	Nit	Cip	Gen	
Taneja N <sup>6</sup> (2006)	558	32.7	75.5	89	26.8	79.6	74.2	Pediatric
Ghadage <sup>7</sup> (2010)	390	98	87.5	82.5	28.5	-	17	Pediatric
Sharan <sup>8</sup> (2012)	64	98	61.3	-	0	63.3	16.4	Pediatric
Rao <sup>9</sup> (2014)	614	-	89.1	-	-	-	-	Adults,ESBL done
Selvakumar <sup>19</sup> (2007)	936	96	92	71	62	92	73	Adults,ESBLdone
Kothari <sup>16</sup> (2008)	531	82	-	74	24	72	-	Adults, ESBLdone
Sood <sup>14</sup> (2011)	346	81.2	69	67.8	5.8	74.7	26	Adults, ESBLdone
Shaifali <sup>20</sup> (2012)	139	52.2	-	39.2	13.1	60.9	54.4	Adults, ESBLdone
Dash <sup>15</sup> (2012)	577	94.7	58.2	51.9	9.8	53.4	38.3	Adults,ESBLdone
Present study	274	82	92	53.4	15.9	60.3	29.6	Pediatric

Table 3

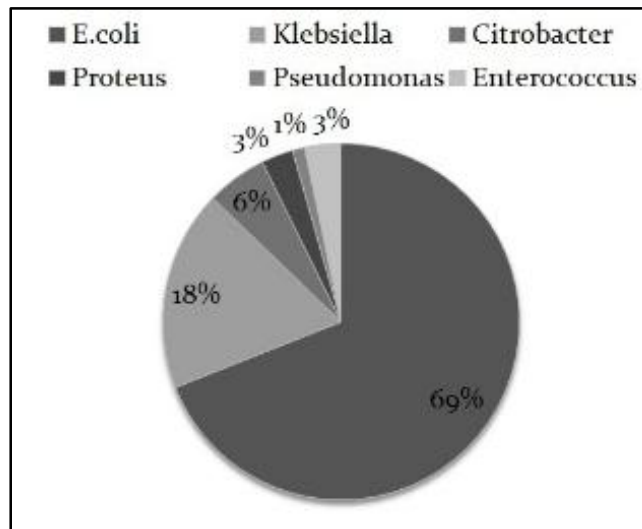
**Table 3: Regional antibiotic resistance pattern of community acquired Escherichia coli isolates.**

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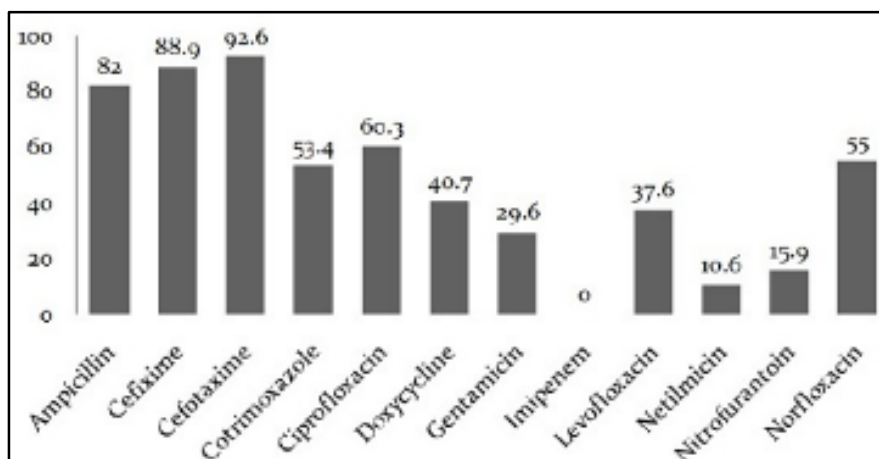
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**Fig. 1: Distribution of community acquired uropathogens from children with febrile illness**



**Fig. 2: Percentage of antibiotic resistance of community acquired Escherichia coli isolated from urinary tract infection**

**AUTHORS:**

1. Nisha K. V.
2. Rathika D. Shenoy
3. A. Veena Shetty
4. Vijaya M. Shenoy
5. Avinash K. Shetty

**PARTICULARS OF CONTRIBUTORS:**

1. Assistant Professor, Department of Microbiology, Century International Institute of Dental Sciences & Research Center, Kasargod, Kerla.
2. Professor, Department of Paediatrics, K. S. Hegde Medical Academy, Nitte University, Mangalore.
3. Associate Professor, Department of Microbiology, K. S. Hegde Medical Academy, Nitte University, Mangalore.

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**COMPETING INTERESTS:** None

4. Professor, Department of Paediatrics, K. S. Hegde Medical Academy, Nitte University, Mangalore.
5. Professor, Department of Paediatrics, School of Medicine, Wake Forest University, USA.

**NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. Nisha K. V,  
Assistant Professor,  
Department of Microbiology,  
Century International Institute of Dental Sciences  
and Research Centre,  
Kasaragod- 671541, Kerala.  
E-mail: nishabiju14@yahoo.in

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