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Microbiological Profile in Urinary Tract Infections among Children in a Tertiary Care Center in Kumaun region, India

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A B S T R A C T

Urinary tract infection is one of the commonest bacterial infections encountered in daily clinical practice and a major problem that is frequently encountered by pediatrics healthcare providers. This study aimed to find bacterial profile of urinary tract infection (UTI) in children between less than 1 year to 15 years of age. This is a hospital based retrospective study conducted at Government Medical College, Haldwani over a period of January 2012 to March 2013. Clean catch mid-stream urine samples from 458 clinically suspected cases of urinary tract infections were processed as per standard procedures. Antimicrobial susceptibility test was performed for the isolated pathogens using Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Among the 458 children, 75 (16.37%) were found to be culture positive. Most common age group having culture positivity was 6-10 years (29.33%). Present study showed that UTI is more common in boys (54.66%) than in girls (45.33%). The most common organism causing the urinary tract infection in this study was *Escherichia coli (*n=48, 64%*)*. Other were *Enterococcus spp.* (n=8, 12%), *Klebsiella spp. (n=4, 5.33%), Proteus spp. (n=4, 5.33%), Pseudomonas spp. (n= 3, 4%), Coagulase negative staphylococcus (n=2, 2.66%), Citrobacter spp. (n=2, 2.66%), Acinetobacter spp. (n=1, 1.33%), MRCONS (n=1, 1.33%),* and *Staphylococcus aureus (n=1, 1.33%). E. coli* showed 87.5% and 79.2% resistance to Ampicillin/sulbactum and Ciprofloxacin & Levofloxacin respectively; while it showed less resistance (18.7%) to Nitorfuratoin. *Enterococcus spp.* showed 88.88% resistance to Norfloxacin and Ciprofloxacin.

Introduction

Urinary Tract Infection (UTI) implies the presence of actively multiplying organisms in the urinary tract (Singh and Madhup, 2013; Lambert and Coulthard, 2003) and it occurs in 3-5% of girls and 1% of boys during childhood, while it is more common in boys during the first year of life (Owa J.A., 2003). Every year about 250 million people worldwide are affected by UTIs. UTI is the third most common cause of

admission to hospitals in India. It has been estimated that about 6 million patients per year are visited worldwide for UTI, out of which around 30,000 are treated in the wards (Bano *et al*., 2012). Urinary Tract Infection (UTI) is among the most common serious bacterial infections in infants and children, and is frequently missed, probably because of its non-specific presentation (Akash and Adarsh, 2013). It distresses the child and concerns the parents. It has a wide variety of clinical presentation, ranging from the asymptomatic presence of bacteria in the urine to potentially life-threatening infection of the kidney (Chang *et al*., 2006). Although, UTI is mainly due to ascending infection from the urethra, (Sharma *et al*., 2011) micro-organisms may reach the urinary tract by hematogenous or lymphatic routes as well. But ascending route accounts for almost 95% of cases of UTI (Goldman, 2000).

Common nonbacterial causes of UTI include hemorrhagic cystitis from adenovirus and Candida infection in immunocompromised individuals. Common bacterial pathogens include gram-negative species such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Serratia* spp. and gram-positive organisms, including group B streptococci, *Enterococcus* sp., and *Staphylococcus aureus* (Zorc JJ, et al, 2005). *Escherichia coli* is the most frequent bacteria to cause UTI in infants and children. The diagnosis of UTI remains complicated by nonspecific symptoms and the difficulty in obtaining an uncontaminated urine specimen. The most recent guidelines issued by the American Academy of Pediatrics (1999) suggest that a UTI should be considered in infants and children who present with symptoms and they should also be evaluated with a urinalysis and urine culture (Johnson, 2003).

In recent years, widespread use of antibiotics has been resulted in an increasing incidence of antibiotic resistance among the urinary tract pathogens all over the world. Worldwide, emerging of antibiotic resistance is increasing among the urinary pathogens (Kahlmeter, 2003; Runehagen *et al*., 2002). The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogens (Ashkenizi *et al*., Marray, 1990; Alon *et al*., 1987). The knowledge of etiology and antibiotic sensitivity and resistance pattern of the organisms causing urinary tract infection is essential (Akash *et al*., 2013). The aim of this study was to isolation and identification of uropathogens causing urinary tract infection and to obtain data on resistant pattern of these pathogens.

Materials and Methods

The study population was drawn from patients attending or admitted to pediatric department of Government Medical College, Haldwani from January 2012 to March 2013. Early morning mid-stream urine samples were collected by clean-catch method by using sterile wide mouthed glass bottles with screw cap tops. The specimens were transported to the bacteriology laboratory immediately or if there was a delay, refrigerated for 4 hours before processing. Isolation of uropathogens was performed by a surface streak procedure on Cysteine Lactose Electrolyte Deficient (C.L.E.D) medium with 4 mm sterilized inoculation loop, which can take about 0.01 ml of urine (one colony of a particular bacteria will be equal to 100 colony per ml of urine (Cheesbrough, 1984) in zigzag motion. All were then incubated at 37^0C aerobically for 24 hours. Colonies were counted and multiplied by 1000 which results in $>10^5$ colony forming unit (CFU) / ml of urine sample. Bacterial count less than this were considered insignificant. Growth

of 3 or more organisms was considered contamination. Subculture of the colonies was done on blood agar and MacConkey agar to characterize the isolate. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques (Collee *et al.*, 1996). For susceptibility testing Muller-Hinton agar plate was swabbed with the suspension using sterile cotton swab and the antibiotic discs were placed over the agar and left for 30 minutes for diffusion of the antibiotics in the disc. The zones of inhibition were then read as resistant and sensitive using calibrated ruler and compared with the standard chart (Baurer *et al.*, 1966).

Antibiotics agents employed for susceptibility testing were Ampicillin/sulbactum, Ceftriaxone, cotrimoxazole, Gentamicin, Levofloxacin, Linezolid, Nitrofurantoin, Norfloxacin and Ciprofloxacin. All isolates were tested for antimicrobial susceptibility against different antibiotics using the disc diffusion method (Cheesbrough, 2000). Standard control strains of *E. coli* ATCC 11774, *S.aureus* ATCC43300, *Enterococcus faecalis* ATCC 51299, *Klebsiella* ATCC 13883, *Pseudomonas aeruginosa* ATCC 15442 and *Proteus mirabilis* ATCC 7490 were used during culturing and antibiotics susceptibility testing as a control throughout the study.

Results and Discussion

A total of 458 urine samples were collected from children with clinically suspected urinary tract infections, 255 (55.67%) samples were from male patients and 203 (44.32%) samples were from female patients. Out of which 75 (16.37%) urine samples were identified positive as shown in Table 1.

Present study shows that UTI is more common in boys (54.66%) than in girls (45.33%). Majority of the cases were of less than 10 years of age. Maximum number of cases was seen in age group 6-10 years of age, where the percentage of male were 11(50%) and female were 11(50%) respectively. The age and gender wise distribution of children from whom the urine samples were collected is shown in Table 2.

The most common organism causing the urinary tract infection in this study was *Escherichia coli (*n=48, 64%*)*. Other were *Enterococcus spp.* (n=8, 12%), *Klebsiella spp. (n=4, 5.33%), Proteus spp. (n=4, 5.33%), Pseudomonas spp. (n= 3, 4%), Coagulase negative staphylococcus (n=2, 2.66%), Citrobacter spp. (n=2, 2.66%), Acinetobacter spp. (n=1, 1.33%), MRCONS (n=1, 1.33%),* and *Staphylococcus aureus (n=1, 1.33%). E. coli* (25 from male and 23 from female) and *Enterococcus spp*. (5 from male and 4 from female) were the most common microorganisms isolated from both male as well as female patients. Similarly *E. coli and Enterococcus spp.* were the most common isolates in all age group. Distribution of organisms isolated from urine samples were shown in figure 1.

Among the 62 gram-negative bacteria, *E. coli* showed 87.5% and 79.2% resistance to Ampicillin/sulbactum and Ciprofloxacin & Levofloxacin respectively while it showed less resistance 18.75% to Nitorfuratoin. *Pseudomonas spp*., showed 100% resistance to Ampicillin /sulbactum, Cotrimoxazole, Ceftriaxone, Ceftriaxone/ sulbactum, Nitrofurantoin while less resistance to Levofloxacin. *Citrobacter spp.* showed 100% resistance to all drugs, and *Acinetobacter spp.* was fully sensitive to all drugs except to Ceftriaxone and Cotrimoxazole.

Table.1 Distribution of Culture Positive and Culture Negative Samples

Culture results	Sample	% (n=458)
Positive	75	16.37%
Negative	383	83.63%
Total	458	100%

Table.2 Age and Gender Wise Distribution of Urinary Tract Infection

Age (years)	Male	Female	Total
<1	7(46.66%)	8(53.33)	15(20%)
1-5	15(71.42)	6(28.57%)	21(28%)
6-10	11(50%)	11(50%)	22(29.33%)
11-15	8(47.05%)	9(52.94%)	17(22.66%)
Total	41(54.66%)	34(45.33%)	75(100%)

Table.3 Antibiotic Susceptibility of Gram-Negative Organisms

Antibiotics	% of isolates resistant to antibiotic					
	***E. coli* (n=48)**	***Klebsiella* spp. (n=4)**	***Pr. mirabilis* (n=4)**	***Pseudomonas* spp. (n=3)**	***Citrobacter* spp. (n=2)**	***Acinetobacter* spp. (n=1)**
Ampicillin/sulbactum	42(87.5%)	3(75%)	2(50%)	3(100%)	2(100%)	0(0%)
Ceftriaxone	32(66.66%)	4(100%)	0(0%)	3(100%)	2(100%)	1(100%)
Ceftriaxone/sulbactum	17(35.41%)	2(50%)	0(0%)	3(100%)	2(100%)	0(0%)
Cotrimoxazole	29(60.41%)	2(50%)	4(100%)	3(100%)	2(100%)	1(100%)
Nitrofurantoin	9(18.75%)	3(75%)	4(100%)	3(100%)	2(100%)	0(0%)
Norfloxacin	39(81.25%)	3(75%)	1(25%)	1(33.33%)	2(100%)	0(0%)
Ciprofloxacin	38(79.16%)	3(75%)	2(50%)	2(66.66%)	2(100%)	0(0%)
Levofloxacin	38(79.16%)	3(75%)	0(0%)	1(33.33%)	2(100%)	0(0%)

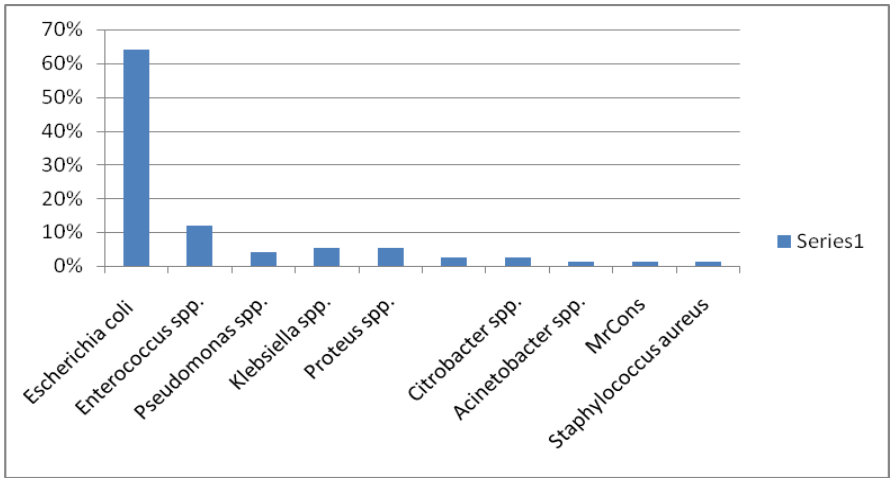
n= number of isolates, *E. coli* - *Escherichia coli*, *Pr. mirabilis*. - *Proteus mirabilis*

Table.4 Antibiotic Susceptibility of Gram-Positive Organisms

Antibiotics	**% of isolates resistant to antibiotic**			
	Enterococcus spp. (n=9)	***CONS spp. (n=2)***	**MRCONS *(n=1)***	***S.aureus (n=1)***
Ampicillin/sulbactum	7(77.77%)	0(0%)	1(100%)	1(100%)
Cotrimoxazole	5(55.55%)	0(0%)	1(100%)	1(100%)
Norfloxacin	8(88.88%)	0(0%)	1(100%)	1(100%)
Nitrofurantoin	5(55.55%)	0(0%)	1(100%)	1(100%)
Ciprofloxacin	8(88.88%)	0(0%)	1(100%)	1(100%)
Levofloxacin	7(77.77%)	0(0%)	1(100%)	1(100%)
Cephoxitin	5(55.55%)	0(0%)	1(100%)	1(100%)
Linezolid	3(33.33%)	0(0%)	1(100%)	1(100%)
Cephalexin	7(77.77%)	1(50%)	1(100%)	1(100%)

CONS: Coagulase negative Staphylococci, MRCONS: Methcillin resistant Coagulase negative Staphylococci, *S. aureus*: *Staphylococcus aureus*, n = number of isolates

Figure.1 Distribution of Organisms Isolated from Urine Samples



Among the gram-positive bacteria *Enterococcus* showed 88.88% resistance to Norfloxacin and Ciprofloxacin. MRCONS and *S.aureus* was found resistant to all drugs. Only CONS showed 100% sensitivity to all drugs. Table 3 and table 4 shows the Antibiotic resistance pattern of gram-positive and gram-negative organisms respectively.

Urinary tract infection (UTI) is one of the commonest bacterial infections seen by Pediatricians in children. However, diagnosis remains a difficult task probably because its presentation is non-specific and similar to other common illnesses. Therefore, it is often missed diagnosis in children (Adedoyin *et al.,* 2003; Owa *et al.,* 1999).

The urinary culture positive rate was 16.37% in this study which was similar to rates of 16.2% Ranjbar *et al.,* 19.3% Shreshtha *et al.*, 18.49% Raghubanshi *et al.,* in previous studies. However another study from Nepal showed 28% of positivity rate

out of 1878 study subjects (Runehagen, 2002). Another study done at KCH by G.K. Rai *et al.,* found 28.6% 28% culture positive from 205 urine samples. In our present study culture positivity rate is less (16.37%) than that of study done by GK. Rai *et al.,* and K. Gautam *et al.,* and may be attribute to sample size.

UTI is a common problem in children but the prevalence varies with in the age and sex of children it occurs in about one percent of boys and three to five percent of girls (Wald, 2004; Sherestha *et al.,* 2005; Elder *et al.,* 2007). However, in contrast to this, present study shows marginally higher positive rate among male children compared with female children (Male 56% vs. Female 44%). This could be due to the relatively more number of male children coming to the hospital and might have been attributed to the preference given to the male children in the Indian society (Pooja *et al.,* 2014). Similarly, GK Rai *et al.,* 2007 also observed higher positive rate among male children compared with female children (Male 51.7%% and Female 48.3%).

In this study *Escherichia coli* (64%) was found to be the most common organism causing UTI in females as well as males this finding is very similar to Muhammad *et al,* 2009, Bosch *et al.,* 2011; Nadir, 2010; Eshwarappa *et al.,* 2011; Mumke *et al.,* 2013; Ines *et al.,* 2013. On the other hand present finding was lesser than the finding of GK *et al* (93.3%), and higher than the findings of Godwin *et al*. (36.4%), Ranjbar *et al. (*40%), Taneja *et al.* (47.1%), and Jha B K *et al.* (1.39%). This variation can be due to age groups, congenital anomalies of urinary tract (Roberts, 1996).

Out of 62 gram-negative isolates, the four highest observed resistances were for Ampicillin /sulbactum 87.5%, Norfloxacin 81.25%, Ciprofloxacin 79.16% and Levofloxacin 79.16% indicating over prescription of these drugs. The commonly used antibiotics such as Nitrofurantoin, Ampicillin/sulbactum and Co-trimoxazole were poorly effective against majority of the organisms isolated in this study. High percentage of isolates showed resistance to sulfa drugs such as Cotrimoxazole that is line with previous finds (Tambekar *et al*., 2005). Antibiotic sensitivity and resistance pattern vary over time and places. The study showed a high resistance to antimicrobials like Ampicillin, Co-trimoxazole, Ciprofloxacin, Norfloxacin; could be these antibiotics were in general use for a long period. This study provides a glimpse of emerging antimicrobial resistance pattern. This type of study should be done periodically to assess the pattern of microorganisms causing UTI and their antimicrobial susceptibility, which will guide in selection of antibiotics for the empiric treatment.

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