ABSTRACT

Background: Urinary tract infection is of grave concern in children and is an important cause of morbidity. Urinary tract infection at young age commonly can lead to renal injury and scarring and ultimately to end stage renal disease in childhood.

Objectives: The purpose of the study was to identify different species of microorganisms, along with their antimicrobial susceptibility pattern, causing urinary tract infection in paediatric patients up to 13yrs at Jorhat Medical College and Hospital, Jorhat, Assam.

Methods: This was a cross sectional study of 806 culture positive cases of UTI who attended paediatric outpatient department or indoor over a period of 11months from August 2014 to June 2015. Urine samples were collected before antimicrobial therapy. Periurethral cleaning was done. About (5-10ml) urine was collected in sterile wide mouthed leak proof container and transported to the laboratory within 2hrs.Semiquantitative method of culture was done in CLED media. A urine sample was included in our dataset if it demonstrated pure growth of a single organism and accompanying antimicrobial susceptibility and subject demographic data were available.

Results: UTI was more common in females (52.65%) than in males (47.34%).Bacterial isolates were (51.61%) and Candida species(48.38%).Among the uropathogens isolated Escherichia coli(34.43%) was found to be the predominant organism followed by Klebsiella species(21.77%), Pseudomonas species(3.29%), Citrobacter(2.53%), Proteus(1.77%), Providentia (1.01%). Amongst them gram positive bacteria Staphylococcus aureus(21.013%) was commonest followed by Enterococcus (19.49%).Sensitivity pattern of Escherichia coli species showed maximum sensitivity to Nitrofurantoin (26.83%), followed by Gentamicin (18.98%), Norfloxacin(13.41%), Nalidixic Acid(10.88%), Cefazolin(10.63%), Cefpodoxime (9.37%) and most resistant to Nalidixic Acid(24.557%) followed by Cotrimoxazole (18.22%), Norfloxacin (16.96%), Cefpodoxime(16.45%).

Conclusion: Though various microorganisms are responsible for UTI in paediatric age group Escherichia coli species is the most common organism. Antimicrobial resistance has already emerged against many antibiotics, making empiric treatment of these infections challenging.

Keywords: Urinary tract infection, Bacterial isolates, Antimicrobial susceptibility pattern, Urine culture.

INTRODUCTION

Urinary tract infections are the most common bacterial diseases worldwide and the commonest genitourinary disease in children.[1,2] UTI can be asymptomatic or symptomatic characterized by a wide spectrum of symptoms ranging from mild irritative voiding to bacteremia, sepsis or even death.[3] Its prevalence varies with age and sex and clearly it involves women more than men because of anatomical differences. [4] Infection may occur at any part of the
genitourinary tract, including urethra, bladder, ureter, renal pelvis or renal parenchyma. Most infections are caused by retrograde ascent of bacteria from fecal flora to bladder and kidney via urethra, especially in females whom the urethra is shorter and wider. The diagnosis of UTI in young children is important as it may be the marker of urinary tract abnormalities. Early diagnosis is important to preserve renal function of the growing kidney. UTI is one of the most important risk factor in development of renal insufficiency or end stage renal disease. UTI is one of the most important risk factor in development of renal insufficiency or end stage renal disease. UTI is frequently termed acute cystitis. Although, UTI is mainly due to ascending infection from the urethra, microorganism may reach the urinary tract by hematogenous or lymphatic routes as well. Ascending routes accounts for almost 95% cases of UTI.

Aims and objectives:

- Isolation and identification of microbial agents causing urinary tract infection in the paediatric age group up to 13 yrs.
- To study the antimicrobial susceptibility pattern of the agents isolated.

MATERIALS AND METHODS

A cross sectional study was done from August 2014 to June 2015 in children upto 13 yrs of age attending JMCH on OPD or as inpatient. Total of 832 urine samples were collected during this period. As the collection of urine samples from the children was difficult the consent from the parents were taken on collection of midstream urine samples. Urine samples from the infants were collected by bladder aspiration. Older children were asked to collect urine samples after proper cleaning of the external urethra and perineum. For collection of urine samples patients allowed to collect a clean catch midstream urine in a sterile, wide mouthed leak proof disposable containers (4-5ml) supplied by the laboratory. Samples were processed within two hours of collection for microscopy examination and culture. Quantitative estimation of urine by means of bacterial counts has been accepted as a routine procedure. Quantization is done by culture of urine by using a calibrated loop. Can be examined under microscopy, semiquantitative culture, and quantitative culture method. Microscopical examination of a wet film of uncentrifuged urine is done to determine whether polymorphs (pus cells) are present in numbers indicative of infection in UTI.A wet film is made from uncentrifuged urine. Urine sample is mixed carefully and 0.05ml of urine is transferred on to the middle of microscopic slide and

renal failure. Although, UTI is mainly due to ascending infection from the urethra, microorganism may reach the urinary tract by hematogenous or lymphatic routes as well. Ascending routes accounts for almost 95% cases of UTI. [15,16]
cover slip is applied on it. For all cases of suspected UTI, urine culture was done by semiquantitative technique on cysteine lactose electrolyte deficient medium (CLED agar - (Hi-Media, Mumbai) One μl urine was cultured using a calibrated bacteriological loop on CLED agar, and colonies were counted after overnight incubation at 370C. Number of colonies obtained was multiplied by 1000 to form colony forming units (cfu)/ml. For positive cultures, culture media is examined for the quantity and morphological type of organisms present. With a 0.001-ml loop, one colony equals 1,000 CFU/ml. With a 0.01-ml loop, one colony equals 100 CFU/ml. When the colonies are too numerous to count, the maximum readable using the 0.001-ml loop is $10^5$ CFU/ml. The maximum readable on the 0.01-ml loop is $10^4$ CFU/ml. CLED is more specific for detection and differentiation of urinary pathogens, it distinguishes LF and NLF. Isolates were identified by Gram stain, motility test and routine biochemical reactions. After final identification, to perform drug susceptibility test, the standard method of disk diffusion (Kirby Bauer) was used on Mueller - Hinton agar plates and following the National Committee of Clinical Laboratory Standards (NCCLS) criteria, appearing of inhibition zone and its diameters were examined. 14 antibiotics, frequently used in urinary tract infection treatment, were tested. All Enterobacteriaceae and Gram positive bacteria were tested against first line agents: gentamicin (10mcg), amikacin (30mcg), cefoperazone (75mcg), cefpodoxime (10mcg), Cefazolin (30mcg), cefaclor (30mcg), nitrofurantoin (300mcg), cotrimoxazole (1.25mcg), nalidixic acid (30mcg), norfloxacin (10mcg) and ceftazidime (30mcg) nalidixic acid (30mcg), amoxicillin and clavulanic acid (20mcg), pipercillin (100mcg) and cefoperazone + sulbactam (27mcg). Data were entered and analysed in Microsoft Excel. Chi-Square test is used and P value less than 0.05 were considered as statistically significant.

Ethical clearance was obtained from Institutional Ethics Committee of Jorhat Medical College, Jorhat.

**RESULTS**

In this study 806(96.87%) out of 832 patients showed positive urine cultures of which there were 390(48.38%) males and 416(51.61%) females and there was a statistically insignificant relation between gender and urinary tract infection (p = 0.2907). (TABLE-1)

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>PERCENTAGE</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>390</td>
<td>48.38%</td>
<td>0.839</td>
</tr>
<tr>
<td>Female</td>
<td>416</td>
<td>51.61%</td>
<td>(Insignificant)</td>
</tr>
</tbody>
</table>

Among the isolates from urinary tract, 256 cases of the isolated bacteria were Gram negative bacilli while 160 cases were Gram positive cocci. Bacterial isolates were 416(51.61%) and Candida species 390 (48.38%). Amongst the bacterial isolates *Escherichia coli* (n = 136 ; 34.43%) was found to be the predominant organism followed by *Klebsiella species* (n= 86; 21.77%), *Pseudomonas* species (n=13;3.29%), *Citrobacter*(n= 10; 2.532%), *Proteus*(n= 7; 1.772%), *Providentia*(n= 4; 1.013%). Amongst the Gram positive bacteria *Staphylococcus Aureus* (n=83; 21.03%) was commonest followed by *Enterococcus* (n= 77; 19.49%). (TABLE-2)

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Isolates number (n=256)</th>
<th>Percentage (%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>136</td>
<td>53.12%</td>
<td>0.001</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>86</td>
<td>33.59%</td>
<td>(Significant)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>13</td>
<td>5.078%</td>
<td></td>
</tr>
<tr>
<td>Citrobacter</td>
<td>10</td>
<td>3.90%</td>
<td></td>
</tr>
<tr>
<td>Proteus</td>
<td>7</td>
<td>2.73%</td>
<td></td>
</tr>
<tr>
<td>Providentia</td>
<td>4</td>
<td>1.56%</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Distribution of Gram positive organisms isolated from urine samples

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Isolates number (n= 160)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>83</td>
<td>51.87%</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>77</td>
<td>48.12%</td>
</tr>
</tbody>
</table>

*Escherichia coli* is the predominant uropathogens in this study.

Sensitivity pattern of *Escherichia coli* species showed maximum sensitivity to Nitrofurantoin 106(77.94%) followed by Gentamicin 75(55.47%), Norfloxacin 53(38.97%), Nalidixic acid 43(31.618%), Cefazolin 42(30.88%), Cefpodoxime 37(27.2%) and most resistant to Nalidixic acid 97(71.324%), Cefazolin 72(52.94%), Cotrimoxazole 71(52.20%), Norfloxacin 67(49.26%), Cefpodoxime 65(47.77%), Ampicillin 62(45.58%), Cefaclor 54(39.70%), Nitrofurantoin 37(27.20%), Gentamicin 28(20.58%) and Piperacillin 24(17.64%). Klebsiella strains showed highest susceptibility to Gentamicin 54(62.79 %), Norfloxacin 53 (61.62%), Nitrofurantoin 46 (53.48%) and higher resistance to Cefpodoxime 58 (67.44%), Cefazolin 54 (62.79%) and Ampicillin 52(60.46%). On Proteus and Pseudomonas strains highest sensitivity was seen to Gentamicin and Cotrimoxazole and resistance to Cotrimoxazole, Cefazolin, Ampicillin and Cefpodoxime. The rates of sensitivity and graphical representation of 14 selected antimicrobial agents against Gram negative bacilli and Gram positive cocci are summarised below.

![Antibiotic Sensitivity of Escherichia Coli](image1.png)

![Antibiotic Sensitivity of Klebsiella Species](image2.png)

![Antibiotic Sensitivity of Staphylococcus Aureus](image3.png)

![Antibiotic Sensitivity of Enterococcus Species](image4.png)

Figure 1: Antimicrobial sensitivity patterns of bacterial pathogens isolated from positive culture.
DISCUSSION

This study shows the distribution of microbial species isolated from patients with urinary tract infection at Jorhat Medical College and Hospital and their susceptibility pattern to antimicrobial agents. Furthermore, we described the relationship between gender and isolated bacterial agents of urinary tract infection. The gender distribution of patients in our study is consistent with those in other studies [16, 17] showing female predominance. Study done by Sharma et al and Malla et al also showed female preponderance. It is assumed that the short urethra in females predisposes them to ascending infection. Study done by K Gautam et al. showed that UTI is more common in girls than in boys, girls (52%), boys (48%) since women who are prone to urinary tract infection possess epithelial cells with significantly more receptors for uropathogenic bacteria than healthy controls. But this result is not consistent with the study done at Kanti children’s hospital by G Rai and et al; male children (53.3%), female children (46.7%). Another study done by K K Malla et al revealed that there were male children (32.7%) and female children (67.2%) with the male female ratio 1:2. 21 this result is consistent with the present study.

Escherichia coli (53.12%) is the most common organism causing urinary tract infection in paediatric age group in this study followed by Klebsiella species. This corresponds with the data obtained by other investigators. [18,19] According to statistical calculation, there was significant association of urinary tract infection caused by Escherichia Coli (p<0.001) in our study which matched with one of the study done by S GK Rai et al at Kanti Children Hospital showed. This may be explained that the large intestine and the perineal area serve as a reservoir for pathogenic bacteria such as Escherichia Coli. Uropathogenic strains of Escherichia Coli have an adherence factor called P fimbriae, or pili, which binds to the P blood group antigen. These P fimbriae mediate the attachment of Escherichia Coli to uroepithelial cells. Thus, patients with intestinal carriage of Escherichia Coli that contains P fimbriae are at greater risk of developing UTI than the general population. Klebsiella was isolated in (33.59%) cases in our study. A study done in Aligarh, India by Akram et al showed similar data (32.0%). In one of the study, [20] Klebsiella was isolated in(5.45%) which does not match with our study. Proteus was isolated in only (2.73%) in this study whereas Proteus isolated ranged from 5.8% to 12.4% in different studies which is not consistent with our study. Pseudomonas was isolated to be (5.078%) in our study which is almost consistent to one of the study. [21] While studying the bacteriological profile of urinary tract infection in our study we also isolated increased number of Candida species which is not consistent to one of the study. [22] Another study done by Alka Nerurkar et al showed increased number of Candida species due to indiscriminate use of antibiotic in hospitalised patients, so fungal coinfection is more likely.

With regard to antibiotic sensitivity pattern of isolates Escherichia coli was found to be most sensitive to Nitrofurantoain (77.94%) followed by Gentamicin (55.47%) and Norfloxacin (38.97%) that corresponds with other studies. [23] In the same study at KCH by GK Rai et al showed, Escherichia Coli was sensitive to Nitrofurantoain (47%), Amikacin (62.2%), Ofloxacin (45.5%),
Gentamicin (30.4%), Cefotaxime and Ceftriaxone to (38.3%) and (36.3%) respectively and is not consistent to our present study. In our present study, Klebsiella was found to be sensitive to Gentamicin (62.79%) and Norfloxacin (61.62%) which matched to one of the study. [24] Sharma et al showed 100% sensitivity to amikacin and 83.3% to nitrofurantoin and Kumari et al [25] showed 96.0% of Klebsiella sensitive to amikacin. This variation in sensitivity pattern in Klebsiella may be either due to increasing resistance of organism or due to limited number of isolates. On Proteus strains norfloxacin, nitrofurantoin and amikacin showed the least (0%, 0%, 14.28%, respectively) and gentamicin, meropenem, cefotaxime showed the highest susceptibility (71.42%, 57.14%, 42.85%, respectively) that were almost similar to one of the study. [26] In a study obtained by other investigators, [27] Proteus was 100.0% sensitive to ofloxacin, norfloxacin, nitrofurantoin and amikacin and 100.0% resistant to nalidixic acid which do not matched to our present study. In this study Gram positive organism were responsible for about 40.52% of urinary tract infection cases; among these Staphylococcus isolates as most frequent Gram positive bacteria showed high sensitivity to cefazolin, gentamicin (72.28%, 71.08% respectively) which showed harmony to one study. [28] Cefixime and Ceftriaxone are least sensitive to all 3 common organisms isolated in this study. So, use of these drugs for the treatment of UTI is found to be of not much helpful.

CONCLUSION

Urinary tract infection is a common problem in paediatric age. Escherichia Coli is the most common microorganism causing UTI. Antimicrobial resistance has already emerged to all antimicrobials. None of the antimicrobials showed 100% sensitivity. Similarly antimicrobial susceptibility pattern vary in different regions and according to time. This makes empiric treatment of UTI difficult. Finally this type of study should be repeated periodically to assess the pattern of microorganisms causing UTI and their antimicrobial susceptibility which will guide in choosing antibiotics for the empiric treatment. Present study together with others are suggestive of need of periodic monitoring of antibiotic sensitivity pattern of the bacterial isolates, to monitor pathogen trends and also to avoid the indiscriminate use of ineffective antibiotics to decrease the menace of drug resistance and to provide effective treatment and thereby to make it more cost effective.

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