Original Research Article

Antibiotic resistance pattern of uropathogens in urinary tract infections in children at State Referral Hospital, Falkawn, Mizoram, India

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ABSTRACT

Background: Analysing antibiotic susceptibility pattern of uropathogens help to overcome the therapeutic difficulties created by the rising anti-microbial resistant bacteria and guides in choosing appropriate antibiotics. Hence, we aimed at evaluating the uropathogens causing urinary tract infections and study their antibiograms.

Methods: Midstream urine samples were collected, cultured and appropriate biochemical tests were performed for proper identification of urinary tract pathogens in State Referral Hospital Falkawn, Mizoram, India from 1st January to 30th June 2018.

Results: The most common isolated gram-negative uropathogens were Escherichia coli (40.9%), followed by Klebsiella spp. (17.6%), Proteus spp. (10.7%) and Pseudomonas spp. (3.1%). Among gram-positive organisms Enterococcus spp. (20.1%) and Staphylococcus spp. (7.6%) were grown. Urinary tract infections were more prevalent in girls (64.8%) than boys (35.2%). High level of resistance was found to amoxycillin/ampicillin by all the organisms isolated.

Conclusions: Almost all the test organisms exhibited multiple antibiotic resistance. The study identified multiple antibiotics resistance by uropathogens which were earlier used as first line of treatment of UTI in children.

Keywords: Antibiotic, Resistance, Susceptibility, Urinary Tract Infection, Uropathogens

INTRODUCTION

Urinary tract infection (UTI) is a common site of infection in the pediatric population. Unlike the generally benign course of UTI in the adult population, UTI in the pediatric population is well recognized as a cause of acute morbidity and chronic medical conditions, such as hypertension, renal insufficiency and morbidity among pediatric population due to inconspicuous clinical manifestations.¹ The diagnosis of urinary tract infection must be based on a positive urine culture.² Approximately 8% of girls and 2% of boys have a UTI by 11 years of age. The lifetime incidence of UTI in females is about 30% compared to only 1% in males.

Approximately 75% of infants younger than 3 months with bacteriuria are male compared with only 10% between 3 and 8 months of age. After 12 months of age, UTI in healthy children is usually seen in girls.³ UTI is the most common bacterial infections ranging from

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asymptomatic to severe sepsis. Bacteria are the primary organisms that cause UTI. Significant bacteriuria is defined as colony count of >10^5/ml of a single species in a midstream clean catch sample. Effective antimicrobial therapy for UTI is important and can reduce adverse effects in patients with UTI. Therefore, there is growing concern regarding the resistance of urinary pathogens to antimicrobial agents because of the increasing number of therapeutic failure after empiric treatment. In acute infection, E. coli is the most frequent infecting organisms. But the prevalence of other antibiotic resistance organisms such as Klebsiella spp., Proteus spp., Serratia spp., Enterobacter spp. and Pseudomonas spp. are increasing in complicated UTI. Among gram - positives, S. sprophyticus, E. faecalis, S. agalactiae, S. pyogenes and S. aureus are usually prevalent which are resistant to a variety of different antibiotics .There are many types of antibiotics available for UTIs and the choice depends upon many factors including severity of infection and acute or recurrent infection. The rate of resistance is very high among uropathogens and the frequency of resistance to antibiotics directly linked with the consumption of antibiotics. Often treatment of UTI is started empirically and therapy is based on information determined from the antimicrobial sensitivity pattern of the uropathogens of a given community. Due to aberrant use of antibiotics in practice the prevalence of antimicrobial resistance among uropathogens has been increasing worldwide. Distribution of uropathogens and their susceptibility to antibiotics varies regionally. Therefore, it becomes necessary to have knowledge of distribution of these pathogens and their susceptibility to antibiotics in a particular setting.

Unfortunately, little has been published regarding Indian scenario of the range and antimicrobial susceptibility patterns in uropathogens particularly among children. Awareness of antimicrobial resistance patterns of common uropathogens in children, according to local epidemiology, it is essential for providing clinically appropriate, cost effective therapies for UTIs. To the best of our knowledge, there is a lack of available information on bacterial agents and their antibiotic susceptibility pattern regarding pediatric UTIs in State Referral Hospital, Falkawn, Mizoram.

The aim of this study was to investigate common uropathogens and their antibiotic resistance patterns in patients admitted in the pediatric ward of State Referral Hospital, Falkawn, Mizoram, India.

**METHODS**

This cross-sectional descriptive study was conducted from 1st January to 30th June 2018 on 220 children admitted with signs and symptoms of UTI in the pediatric ward of State Referral Hospital, Falkawn. Patient’s information, including age, gender, clinical status and the type and number of isolated bacteria, were recorded in a designed form. The study subjects were divided to four age groups: 0-1year, 2-5years, 6-10 years and 11-15 years. The study included cases, who had not received antibiotic treatment before hospitalisation. This study was approved by the ethical committee of State Referral Hospital Falkawn, Mizoram, India. The study did not harm any patients and all the cases were kept confidential.

**Microbiological methods and antibiotic susceptibility testing**

Samples were collected in a sterile container and sent to the laboratory by midstream, urine bag, catheter and suprapubic methods based on age and physical status of the subjects. 5% blood agar, eosin methylene blue and Mac Conkey agar (Himedia, India) were used for culture. Bacterial identification was done using conventional methods. Positive results were considered if the number of colonies of a single organism were >105 CFU. Antimicrobial susceptibility testing was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI) on the most prevalent gram-negative and gram- positive isolated bacteria. The following antibiotic disks (Mast, UK) were used: amikacin (30 µg), gentamycin HLG (120 µg), gentamycin (30 µg), amoxyclav (30 µg), ampicillin (10 µg), erythromycin (15 µg), ceftazidime (30µg), imipenem (10 µg), co-trimoxazole (1.25/23.75 µg), cefotaxime (30 µg), norfloxacin (10 µg), linezolid (30 µg), nitrofurantoin (300 µg), vancomycin (30 µg), meropenem (10 µg), piperacillin + tazobactam (100/10 µg), colistin (10 µg). Standard strains used for the susceptibility tents were Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, and Pseudomonas aeruginoa ATCC 27853. The isolates were classified as sensitive or resistant according to CLSI guidelines. Microsoft Excel was used for data compilation, analysis and preparation of chart.

**RESULTS**

Of the 220 urine culture of children under study, 159 (72.27%) were culture positive, of which 103 (64.8%) belonged to girls and 56 (35.2%) were boys (Figure 1).

![Figure 1: Distribution of uropathogens in patients with culture positive.](image_url)
The maximum age group with culture positive were between 2 to 5 years (36.5%) followed by 0 to 1 year (33.3%), 6 to 10 years (19.5%) and 11 to 15 years (10.7%) (Table 2). The commonest uropathogens were *E. coli* (40.9%), followed by *Enterococcus spp.* (20.1%), *Klebsiella spp.* (17.6%), *Proteus spp.* (10.7), *Staphylococcus spp.* (7.6%) and *Pseudomonas spp.* (3.1%) as shown in Table 1. *E. coli* and *Klebsiella spp.* were the two most common gram-negative bacteria isolated among group 2 to 5 years whereas *Enterococcus spp.* were the maximum gram-positive organism isolated and is the commonest cause of UTI in the age group 0 to 1 year shown in Table 2.

### Table 1: Distribution of Uropathogens isolated (N=159).

| Type of isolated bacteria | No. of bacteria isolated (n=159) | % |
|---------------------------|----------------------------------|---|
| *E. coli*                 | 65                               | 40.9 |
| *Enterococcus spp.*       | 32                               | 20.1 |
| *Klebsiella spp.*         | 28                               | 17.6 |
| *Proteus spp.*            | 17                               | 10.7 |
| *Staphylococcus spp.*     | 12                               | 7.6  |
| *Pseudomonas spp.*        | 5                                | 3.1  |

### Table 2: Age wise distribution of Uropathogens (N=159).

| Age (year) | E. coli | Enterococcus spp. | Klebsiella spp. | Proteus spp. | Staphylococcus spp. | Pseudomonas spp. | Total |
|------------|---------|-------------------|-----------------|--------------|---------------------|-----------------|-------|
| 0-1        | 18      | 14                | 8               | 6            | 6                   | 1               | 53 (33.3%) |
| 2-5        | 24      | 12                | 11              | 4            | 4                   | 3               | 58 (36.5%) |
| 6-10       | 14      | 4                 | 7               | 4            | 1                   | 1               | 31 (19.5%) |
| 11-15      | 9       | 2                 | 2               | 3            | 1                   | 0               | 17 (10.7%) |
| Total      | 65      | 32                | 28              | 17           | 12                  | 5               | 159 (100%) |

### Table 3: Distribution of the pathogenic organism according to sex: male, N= 56 and female, N= 103.

| Sex          | E. coli | Enterococcus spp. | Klebsiella spp. | Proteus spp. | Staphylococcus spp. | Pseudomonas spp. | Total |
|--------------|---------|-------------------|-----------------|--------------|---------------------|-----------------|-------|
| Female       | 42 (40.8%) | 20 (19.4%)       | 18 (17.5%)     | 12 (11.7%)  | 8 (7.8%)            | 3 (2.9%)        | 103 (100%) |
| Male         | 23 (41.1%)  | 12 (21.4%)       | 10 (17.9%)     | 5 (8.9%)    | 4 (7.1%)            | 2 (3.6%)        | 56 (100%) |

### Table 4: Antibiotic resistance pattern of gram-negative isolates.

| Antibiotics                  | *E. coli* (N = 65) | *Klebsiella spp.* (N = 38) | *Proteus spp.* (N = 17) | *Pseudomonas spp.* (N = 5) |
|------------------------------|--------------------|-----------------------------|-------------------------|-----------------------------|
| Ampicillin/Amoxycillin       | 60 (92.3%)         | 26 (68.4%)                  | 16 (94.1%)              | 3 (60%)                     |
| Piperacillin                 | 6 (9.2%)           | 1 (2%)                      | 2 (11.8%)               | 2 (40%)                     |
| Cefotaxime                   | 21 (32.3%)         | 2 (5.3%)                    | 8 (47.1%)               | 2 (40%)                     |
| Cephalexin                   | 7 (10.8%)          | 1 (2.6%)                    | 2 (11.8%)               | 2 (40%)                     |
| Ceftazidime                  | 21 (32.3%)         | 2 (5.3%)                    | 2 (11.8%)               | 2 (40%)                     |
| Ceftriazone                  | 6 (9.2%)           | 1 (2.6%)                    | 4 (23.5%)               | 1 (20%)                     |
| Meropenem                    | 2 (3%)             | 1 (2.6%)                    | 1 (5.9%)                | 1 (20%)                     |
| Cefoperazone+ Sulbactum      | 2 (3%)             | 1 (2.6%)                    | 2 (11.8)                | 1 (20%)                     |
| Piperacillin+ Trazobactum    | 11 (16.9%)         | 2 (5.3%)                    | 1 (5.9%)                | 1 (20%)                     |
| Gentamycin                   | 10 (15.4%)         | 2 (5.3%)                    | 4 (23.5%)               | 0 (0%)                      |
| Amikacin                     | 8 (12.3%)          | 2 (5.3%)                    | 2 (11.8%)               | 1 (20%)                     |
| Co-trimoxazole               | 24 (36.9%)         | 6 (15.8%)                   | 9 (52.9%)               | 2 (40%)                     |
| Nitrofurantion               | 20 (30.8%)         | 6 (15.8%)                   | 2 (11.8%)               | 3 (60%)                     |
| Norfloxacin                  | 23 (35.4%)         | 1 (2.6%)                    | 5 (29.4%)               | 1 (20%)                     |
| Colistin                     | -                  | -                           | -                       | 0 (0%)                      |

The sex ratio is 1.83:1 (female: male), of which *E. coli* was the maximum isolated in both the sex (40.8%, N= 103 in female and 41.1%, N= 56 in male) as depicted in Table 3. Antibiotic resistant pattern of the uropathogens
are shown in Table 4 and 5. All the gram-negative isolated bacteria showed maximum resistance to ampicillin/amoxicillin (E. coli 92.3%, Klebsiella spp. 8.4%, Proteus spp. 94.1% and Pseudomonas spp. 60%) followed by co-trimoxazole (E. coli 36.9%, Klebsiella spp. 15.8%, Proteus spp. 52.9% and Pseudomonas spp. 40%), norfloxacin (E. coli 35.4%, Klebsiella spp. 2.6%, Proteus spp. 29.4% and Pseudomonas spp. 20%), cefotaxime (E. coli 32.3%, Klebsiella spp. 5.3%, Proteus spp. 47.1% and Pseudomonas spp. 40%), cefazidime (E. coli 32.3%, Klebsiella spp. 5.3%, Proteus spp. 11.8% and Pseudomonas spp. 40%), nitrofurantoin (E. coli 30.8%, Klebsiella spp. 15.8%, Proteus spp. 11.8% and Pseudomonas 20%) and piperacillin + tazobactum (E. coli 16.9%, Klebsiella spp. 5.3%, Proteus spp. 5.9% and Pseudomonas spp. 20%). Pseudomonas spp. was not resistant to colistin.

Table 5: Antibiotic resistance pattern of gram-positive isolates.

| Antibiotics       | Enterococcus spp (N=32) | Staphylococcus spp (N=12) |
|-------------------|-------------------------|---------------------------|
| Penicillin        | 22 (68.8%)              | 2 (16.7%)                 |
| Ampicillin/amoxicillin | 19 (59.4%)           | 4 (33.3%)                 |
| Vancomycin        | 0 (0%)                  | 0 (0%)                    |
| Cefoxitine        | 4 (6.2%)                | 5 (41.7%)                 |
| Gentamycin (HLG)  | 19 (59.4)               | 6 (50%)                   |
| Amikacin          | 14 (43.8%)              | 8 (66.7%)                 |
| Nitrofurantion    | 14 (43.8%)              | 1 (8.3%)                  |
| Norfloxacin       | 28 (87.5%)              | 10 (83.3%)                |
| Linezolidone      | 1 (3.1%)                | 0 (0%)                    |
| Erythromycin      | 15 (46.9%)              | 5 (41.7%)                 |

The gram - negative uropathogens are less resistance to cefoperazon + sulbactum followed by meropenen. As seen in Table 4, the gram positives are most resistance to norfloxacin (E. coli 87.5% and Staphylococcus spp. 83.3%) followed by ampicillin/amoxicillin (Enterococcus spp. 59.4% and Staphylococcus 33.3%), penicillin (E. coli 68.8% and Staphylococcus spp. 16.7%) gentamycin HLG (E. coli 59.4% and Staphylococcus spp. 50%), erythromycin (E. coli 46.9% and Staphylococcus 41.7%), Amikacin (E. coli 43.8% and Staphylococcus 66.7%) and cefoxitine (E. coli 6.2% and Staphylococcus spp. 41.7%).

DISCUSSION

Present study showed that UTI in children was more prevalent in females than male patients. The ratio of female: male in present study was 1.8:1 (64.8% females and 35.2% males) which is nearly similar to other study done in Coimbatore Tamil Nadu by Kavitha J et al in (59.8% females and 40.2% males) and in Bangladesh by Sanjida KS et al (61.68% females and 38.32% males). The reason for low percentage of UTI in males is due to longer course of urethra and bacteriostatic secretions by the prostate gland which supported present study. Majority of growth positive cases were in the age group of ≤6 years which is higher than study done by Sumit G et al in north India.

Serious study on uropathogens resistance pattern in children is broadly lacking in the state of Mizoram. The present study being an important step towards this direction in the wake of increasing reported cases of antibiotic resistance among uropathogens across the globe. E. coli (40.9%) was the commonest organism isolated in both the sex from in present study which was well correlating to other studies in India. The second most common uropathogens isolated was Enterococcus spp. (20.1%). Enterococcus was the most frequently isolated pathogen in the year 1996 to 2000, during a study on catheter associated UTIs in UK hospital. The researchers found that Enterococcus was the second most frequent microbe. Prevalence of E. coli (40.1%) and Enterococcus spp. (19.4%) high in girls which was similar to study done by Rekha T et al. Though E. coli and Enterococcus spp. were commonest uropathogens we studied other organisms as our interest is their resistance pattern like Klebsiella spp., Proteus spp., Staphylococcus spp. and Pseudomonas spp., there resistance pattern were as high as E. coli and Enterococcus spp.

The least isolated were Pseudomonas spp. (3.1%) which may be due to nosocomial infection. The percentage of resistance of uropathogens to different antimicrobial agents range from 0 to 94.1% in present study. Higher resistance to antimicrobials like ampicillin, co-trimoxazole, norfloxacin, nitrofurantion, cephalosporins and aminoglycosides were seen.

The resistance rates to ampicillin were found to be as high as 45%, 50% and 100% in Canada, Europe and Africa respectively. In present study the frequency of resistance to ampicillin/amoxicillin were high in all the gram negative and positive bacteria isolated except for Staphylococcus spp. (E. coli 92.3%, Klebsiella spp. 68.4%, Proteus spp. 94.1%, Pseudomonas 60%, Enterococcus spp. 68.8% and Staphylococcus 16.7%). The use of ampicillin/amoxicillin or co-trimoxazole as an agent for empirical treatment would not cover the majority of uropathogens in Mizoram. The combination of ampicillin and an aminoglycoside are being used commonly for acute febrile illnesses of different causes in the hospital set up which may be the reason for increase resistance to ampicillin/amoxicillin by uropathogens in this part of the country. A study in Germany confirmed that initial empirical intravenous therapy of UTIs with the combination of ampicillin and aminoglycosides would be appropriate: resistance rates of causative agents to ampicillin and netilmicin were 51% and 7%, respectively. In Australia, gentamicin has been proposed as monotherapy for the effective and safe treatment of UTI requiring parenteral treatment in children aged 1 month to 12 years. In present study, however, resistance to gentamicin was 59.4% and 50% in Enterococcus spp. and Staphylococcus spp respectively though the resistance rate were low among the gram...
negative uropathogens in present study. In the area covered in this study the common uropathogens have less resistance rate to amikacin (E. coli 12.3%, Klebsiella spp. 5.3%, Proteus spp. 11.8% and Pseudomonas spp. 20%) compared to other antibiotics so it is quite safe to use as empirical treatment in the state for UTI before the culture reports are out provided that there are contraindications to use the drug in the hospitalised patients. Varying percentages of resistance to cephalosporins by both the gram negative and positive uropathogens isolated was observed in this study coinciding with the study done by Rekha T et al.18 The reason for high resistance to co-trimoxazole is due to the fact that it has been the commonest drug for treating any acute febrile illnesses in the villages and interior part of the state.

Most of the children with suspected lower UTI are initially treated with co-trimoxazole or oral cephalosporins as first line before culture or where urine culture sensitivity cannot be performed. Due to the reasons outlined above there is moderately high resistance to co-trimoxazole by the gram negative uropathogens in our state (E. coli 36.95%, Klebsiella spp. 15.8%, Proteus spp. 52.9% and Pseudomonas spp. 40%).

We therefore suggest that unless the uropathogen culture is positive for this drug it should not be used as first line of treatment for UTI to cut down the resistance rate of this drug in the state. Similar study in part of north India found that there were resistance to co-trimoxazole by gram-negative uropathogens in children (E. coli 25%, Klebsiella spp. 32%, Pseudomonas spp. 9% and Proteus spp. 14%), which is lower in comparison to present study.15

In present study Staphylococcus spp. showed zero resistance to linezolid which is similar to the study done by Rekha T et al.18 Enterococcus spp. has zero resistance to vancomycin and showed some resistance to linezolid (3.1 %) which coincides with the study done by Marwan O et al showing 100% sensitivity to these to drugs by Enterococcus spp.25

We found that there is zero resistance to colistin by Pseudomonas spp. conforming to a study done by Marwan O et al in 2017 among age group 1 year to 97 years,24 though there is high resistance to ampicillin / amoxycillin (60%) and nitrofurantoin (60%) which is more compared to study done by Sumit G et al.15 The resistance rate to norfloxacin by gram negative uropathogens is quite low compared to previous study done by Rekha T et al.18

The review of the patients’ data showed that the most common antibiotics, which was prescribed by physicians for hospitalised children with UTI before obtaining culture were aminoglycosides and ceftriaxone though most effective antibiotics found in the present study meropenem and vancomycin (gram negative - meropenem and gram positive- vancomycin).

CONCLUSION

Even though Mizoram is a small state of North East India, there are many resistant strains of uropathogens which shows the necessity for doing culture sensitivity before starting antimicrobials in UTI pediatric patients. Hence, it is necessary to prescribe antibiotics under an exact surveillance in teaching hospitals as it can prevent unnecessary expenses for using inappropriate antibiotics. This will further prevent the resistant strains in the study area.

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