An observational study on emerging antimicrobial resistance pattern in urinary tract Infection in Nepalese children

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ABSTRACT

Aims

Evidence-based management and local antibiotic susceptibility pattern provides evidence to guide the development of urinary tract infection (UTI) treatments protocols decreasing the economic burden significantly. We aimed to investigate the aetiology and resistance pattern of bacterial uropathogens to commonly prescribed oral antibiotics (beta lactamase and fluoroquinolones) causing UTI and to recommend the most appropriate antibiotics.

Materials and Methods

This study is cross sectional, retrospective study. We evaluated causative agents and antimicrobial resistance in urine, culture positive samples collected from July 2019 to June 2021 in a single hospital in Kathmandu, Nepal. To obtain urine samples, a midstream clean-catch method used in children who were toilet trained and transurethral catheterization performed in non-toilet-trained children. Urine samples were sent to the laboratory where they were inoculated using a 4 mm calibre loop on CLED (Cysteine Lactose Electrolyte Deficient) agar plate, and incubated at 37 °C for 18–24 h. Conventional methods (colony morphology, Gram stain) were opted. Different biochemical tests – catalase test, coagulase test and inoculation in Bile Esculin Agar was done for Gram-positive organisms, while Triple Sugar Indole (TSI) agar, Sulphide indole motility (SIM) agar, Simon's citrate agar and Christensen's urea agar were used for identification of Gram-negative bacilli. Significant growth was evaluated as $\geq 10^5$ colony forming units (CFU)/ml of urine. Kirby-Bauer disc diffusion method was used to perform in vitro antimicrobial susceptibility tests in Mueller-Hinton agar plates. Total 13 drugs were tested for sensitivity pattern. To analyse resistance to antibiotics for different ages, subjects were divided into four age groups: Group I, 2 months - 1 year; Group II, 1 year - 5 year; Group III, 5 year - 10 year; Group IV, 10 year - 15 year.

Results: Among 970 samples sent, a total of 230 positive urine cultures were identified, of which 116 (50.4%) were from girls and 114 (49.6%) were from boys. The most common age group was 2 months to 1 year (49.1%). The most common causative agent was Escherichia coli (49.1%) followed by Enterococcus faecalis (14.3%) and Klebsiella pneumoniae (11.3%). The overall resistance to Nalidixic acid (66%), Ceftriaxone (54.8%) Cefotaxime (48.3%) Ciprofloxacin (47.9%) and Co-trimoxazole (46.9%) was significant. The least resistance was for Chloramphenicol, Nitrofurantoin, and Norfloxacin was 9.5 %, 31.5 %, and 38.3 % respectively. Chloramphenicol (90.5%) was the most active agent against E. coli and Klebsiella, whereas Linezolid (92.7%) and cloxacillin (64.9%) was most active against Enterococcus and Staphylococcus species.

Conclusion: Escherichia coli was the most common causative agent of urinary tract infection in children. Nalidixic acid, Ceftriaxone, Cefotaxime, Ciprofloxacin and Co-trimoxazole had the highest resistance rates against urinary tract pathogens in our centre. For oral empirical antibiotic therapy, Chloramphenicol is the most appropriate choice for Escherichia coli and Klebsiella strains and Linezolid for Enterococcus species.

Keywords: Urinary tract infection, Uropathogens, antimicrobial resistance, culture positive, causative agent

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INTRODUCTION

Urinary tract infection (UTI) is a common cause of fever and one of the most common community-acquired infections in children[1]. Delayed or inadequate treatment can lead to recurrent UTIs, which in particular can lead to permanent damage to the kidney like renal parenchymal scarring, poor kidney function, hypertension and even chronic kidney disease[2].Besides, UTI constitutes a serious economic burden, which can be large in regard to frequency of acutely unwell children visiting to hospital, additional diagnostic tests for structural abnormality and serious complications, and the wider impact of resistance antibiotic prescription. School abstinence for children and loss of parental workforce loss are indirect costs[3,4].Therefore, it is a major concern for children, parents and clinicians alike. So, prompt diagnosis and appropriate treatment are very important to reduce the morbidity associated with it[5].

Globally, prevalence of urinary tract infection in children below 19 years of age is 7.8%[6]. However, the prevalence varies according to age, race, sex and circumcision status, commonest being in uncircumcised boys less than 3 months (20.1%) of age followed by girls in the same age group (7.5%)[6]. Similarly, prevalence of UTI in Nepalese children is 12%[7]. Other risks factors includes toilet training in toddlers which can lead to volitional holding and bladder stasis[8]. Prevalence peaks in adolescent females when sexual activity disrupts bacteria near the urethral orifice[9]. Structural voiding disorder like urogenital anomalies and functional disorder like neurogenic bladder, constipation and behavioural withholding. Altered immune function and malnutrition increases the risk of uncommon viral and fungal causes of UTI [10].

Most pediatric UTIs are caused by Gram negative coliform bacteria arising from faecal flora and Escherichia coli (E. coli) being the commonest of all, responsible for approximately 80% of pediatric UTIs. Other organisms include Klebsiella, Proteus, Enterobacter, and Enterococcus species[9,10]. Globally, antibiotic resistant to common antibiotics in UTI is on the rise [10,11]. Particularly high in resource-limited settings and developing countries[11] where oral antibiotics such as ampicillin and cotrimoxazole resistance can be as high as 97%–100% [12].

Different studies are showing that resistance and treatment of UTIs is becoming a global economic burden[13]. However, bacterial patterns and resistance properties depends upon local antimicrobial usage and geographic variations [14]. Because of the emerging and continuing antibiotic resistance pattern, regular monitoring of resistance patterns is necessary. Thus, use of evidence-based management and local antibiotic susceptibility pattern provides evidence to guide the development of UTI treatments protocols decreasing the economic burden significantly [15].So the rationale of the study is to improve and generate local guidelines for empirical antibiotic therapy.

MATERIAL AND METHODS

Data collection Tools and Technique:

This is a retrospective study and followed convenient sampling technique. All urine cultures of children younger than 15 years of age from July 2019 to June 2021 were reviewed. Data collected by reviewing of case notes and medical records from laboratory for sensitivity pattern. Demographic data were received from outpatient and inpatient records from the Department of Pediatrics of Nepal Mediciti Hospital, Nepal. Children less than 2 months of age and children with malnutrition and altered immune function were excluded from the study.

Sample collection technique: A clean-catch midstream urine was collected in children who were toilet trained and transurethral catheterization was performed in non-toilet-trained children. Urine samples were collected in a sterile, dry, wide-necked, leak-proof container.

Collected urine samples were sent immediately to the laboratory for processing.

Procedure

A. Sample processing

Semi-quantitative method was opted for urine culture. With the help of the calibrated loop of 4mm size, well-mixed midstream urine sample was inoculated into CLED (Cysteine Lactose Electrolyte Deficient) agar plate. A loopful of the urine was touched to the center of the plate from which the inoculum is spread in diameter across the plate. Without flaming or reentering the urine, a loop is drawn across the entire plate, crossing the first inoculum streak numerous times.

The plates were then incubated at 37 °C for 24 hours. The following day the plates were observed for the growth of the organism, their characteristics on the plates, or any possible contamination.Conventional methods (colony morphology and Gram stain) were opted. Significant growth was evaluated as ≥10^5 colony forming units (CFU)/ml of urine.

Different biochemical tests – catalase test, coagulase test and inoculation in Bile Esculin Agar was done for Gram-positive organisms, while media such as Triple Sugar Indole (TSI) agar, Sulphide indole motility (SIM) agar, Simon's citrate agar and Christensen's urea agar were used for identification of Gram-negative bacilli.

B. Antimicrobial susceptibility testing

Mueller-Hinton agar was used for in vitro antimicrobial susceptibility test. Modified Kirby-Bauer method was used. The inoculum was prepared by picking 3-5 colonies of similar appearance from CLED agar plate and emulsified in normal saline. The turbidity was adjusted with 0.5 McFarland standard using Wickerham card.

Disk of cefotaxime, ceftriaxone, ciprofloxacin, norfloxacin, amikacin, cotrimoxazole, gentamicin, nalidixic acid, chloramphenicol, linezolid and nitrofurantoin were placed on each MHA plate using sterile forceps. The plates were incubated at 35^0C for 24 hours. The antimicrobial susceptibility testing was done based on CLSI (clinical and laboratory standard institute) 2019 guidelines. Only presumptive identification of Enterococcus species was done (catalase test, screening with bacitracin and BEA). So the species differentiation could not be obtained. Screening and confirmatory identification of Extended spectrum of beta lactamase (ESBL) isolates requires different methods which was not opted in this study. Coagulase-negative Staphylocacci (CoNS) were identified based on catalase and coagulase test. Coagulase was used as a sole test to differentiate cons from staphylococcus aureus and disk diffusion with novobiocin for species identification was not done due to lack of the same.

Significant growth was evaluated as $\geq 10^5$ colony forming units (CFU)/ml of urine. All antibiotics included in this study cover the appropriate organisms and are used in the treatment of UTIs with varying degrees of success. Only the first positive urine culture obtained per patient on admission was included in the analysis to eliminate any possibility of recurrence. Polymicrobial cultures and cultures with multidrug-resistant uropathogens were not included in the analysis for not including nosocomial infections and contaminations. Cultures with Candida growth were excluded.

Statistical Analysis

Data entry was done in Microsoft excel. Data was imported to SPSS 25th version and analysed for sensitivity and resistance pattern of uropathogen to different drugs. Descriptive statistics including frequency and percentage were calculated for age and gender.

RESULTS

A total of 970 urine samples were reviewed and 230 urine cultures were eligible. Mean patient age was 38.5 ± 55.5 months. One hundred sixteen pathogens (69.2%) were isolated from girls and One hundred fourteen (30.8%) were from boys. According to age group, the most common age group was Group I (49.1%), followed by Group II(28.7%), Group III (11.3 %) and Group IV (10.9%) shown in table 1.

Table 1. Age and sex distribution of children

Variables	Frequency	Percentage
Age		
0-1 year	113	49.1%
1.1-5 years	66	28.7%
5.1-10 years	26	11.3%
10.1-15 years	25	10.9%
Gender		
Male	114	49.6%
Female	116	50.4%

The most common causative agent was Escherichia coli (49.1% of cases) followed by Enterococcus faecalis (14.3%), Klebsiella pneumoniae (11.3%), Citrobacter koseri (6.5%), Staphylococcus aureus (4.8%), Citrobacter ferundii (2.2%), Klebsiella oxytoca (2.2%), Enterobacter aeruginosa (1.3%), Streptococcus species (1.3%) and others (7%) given in table 2.

Table 2. List of causative uropathogens in children aged 2 months to 15 years

Organism	Frequency	Percentage
Escherichia coli	113	49.1%
Enterobacter cloacae	1	0.4%
Enterobacter aerogenes	3	1.3%
Streptococcus species	3	1.3%
CoNS*	2	0.9%
MRSA*	1	0.4%
Proteus vulgaris	1	0.4%
Acinetobacter baumannii	1	0.4%
Klebsiella pneumoniae	26	11.3%
Citrobacter koseri	15	6.5%
Staphylococcus aureus	11	4.8%
Proteus mirabilis	1	0.4%
Pseudomonas aeruginosa	9	3.9%
Enterococcus faecalis	33	14.3%
Klebsiella oxytoca	5	2.2%
Citrobacter ferundii	5	2.2%

**MRSA-methicillin resistant staphylococcus aureus*
**CoNS-coagulase negative staphylococci*

The overall resistance to Nalidixic acid was highest (66%), followed by Ceftriaxone (54.8%) Cefotaxime (48.3%) Ciprofloxacin (47.9%) and Co-trimoxazole (46.9%).The least resistance was for Chloramphenicol, Nitrofurantoin, and Norfloxacin was 9.5 %, 31.5 %, and 38.3 % respectively. Chloramphenicol (90.5%) was the most active agent against E. coli and Klebsiella, whereas Linezolid (92.7%) and Cloxacillin (64.9%) was most active against Enterococcus and Staphylococcus species presented in table 3.

Table 3. Sensitivity and resistance status of drugs

Sensitivity of drugs	Frequency	Percentage
Amikacin		
Sensitive	95	55.2%
Resistant	77	44.8%
Gentamycin		
Sensitive	63	63%
Resistant	37	37%
Ciprofloxacin		
Sensitive	73	52.1%
Resistant	67	47.9%
Cotrimoxazole		
Sensitive	91	53.5%
Resistant	79	46.5%
Ceftriaxone		
Sensitive	33	45.2%
Resistant	40	54.8%
Linezolid		
Sensitive	38	92.7%
Resistant	3	7.3%
Nitrofurantoin		
Sensitive	148	68.5%
Resistant	68	31.5%
Norfloxacin		
Sensitive	127	61.7%
Resistant	79	38.3%
Cloxacilin		
Sensitive	24	64.9%
Resistant	13	35.1%
Chloramphenicol		
Sensitive	38	90.5%
Resistant	4	9.5%
Ofloxacin		
Sensitive	30	66.7%
Resistant	15	33.3%
Nalidixic acid		
Sensitive	72	34%
Resistant	140	66%
Cefotaxime		
Sensitive	45	51.7%
Resistant	42	48.3%

DISCUSSION

In our study E. coli was the most common causative organism for UTI among children below 15 years, similar results were found in studies carried out in other countries[1][2].However regular evidence of Klebsiella bacterial strain isolated from urine culture was found in studies[3][4]. Enterococcus faecalis (14.3%) and Klebsiella pneumoniae (11.3%) were the second and third most common organisms in the present study.

We found significantly higher resistance to Nalidixic acid as we did not include ampicillin as it was not choice of drug for children in our setting for this age group.[1][2][4][5] In our study, Ceftriaxone, Ciprofloxacin, and Co-trimoxazole were also found resistant with more than 45%. A similar result was shown in other studies for Co-trimoxazole, however, the least resistance was found for Ceftriaxone, Ciprofloxacin[1][2].

The least resistance was found for Chloramphenicol, Nitrofurantoin, and Norfloxacin in our study. Most of the other studies in the past showed least or no resistance to Nitrofurantoin[1][3][4].In this study, Chloramphenicol was the most potent agent against E. coli and Klebsiella whereas Linezolid and cloxacillin were most efficient against Enterococcus and Staphylococcus species.

Paediatrician, Physician, and other medical professional must be mindful of the resistance patterns of uropathogens in their practice area and prescribe empirical antibiotics. Appropriate treatment requires information regarding the susceptibility patterns of the current bacteria to give effective antibiotics promptly. According to the international practice guideline, an antibiotic with less than 20% resistance to probable infecting variant is considered as a first-line empirical treatment for urinary tract infection[6].

Resistance rates were detected higher against Nalidixic acid, Ceftriaxone, Ciprofloxacin in our centre, among which only Ciprofloxacin is a choice of drugs for UTI treatment among children in Nepal. These higher resistance rates suggest that these antibiotics should not be selected for empiric treatment in children of our Lalitpur municipality. The reason behind this resistance might be attributed to changes with time.

According to our study, Chloramphenicol was the most effective oral antibiotic for UTI caused by E. coli and Klebsiella. Linezolid and Cloxacillin were most effective against Enterococcus and Staphylococcus in our study. The study identifies Chloramphenicol as an appropriate choice for empirical treatment of E. coli initiated UTI in our centre.

Globally, drug resistance is a recognized threat, which can be more dangerous towards children. In recent decades, all kinds of microbes including pathogens in the urinary tract are developing resistance to antimicrobials. Rational use of antibiotics is vital in prescription practice by doctors. We should consider the surging resistance of specific uropathogens to Nalidixic acid and other resistant antibiotics while treating children with UTI in the future in Nepal.

There were some limitations in this study. Only laboratory-based data was taken to avoid subjective bias caused by retrospective study. Another limitation was we could not follow up with the patients, so we did not have information about any other diagnostic tests or the outcomes of the patients.

CONCLUSION

E. coli was the most common causative agent of urinary tract infection in children. Nalidixic acid, Ceftriaxone, Cefotaxime, Ciprofloxacin and Co-trimoxazole had the highest resistance rates against urinary tract pathogens in our centre. For oral empirical antibiotic therapy, Chloramphenicol is the most appropriate choice for Escherichia coli and Klebsiella strains and Linezolid for Enterococcus species. This study will be useful for paediatrician in Nepal to improve appropriate empirical treatment for UTI. Though there are universal guidelines, we suggest that empirical antibiotic selection should be done based on the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal guidelines.

Authors' Contribution

Dr. Sujeeta Bhandari designed and supervised the study from proposal writing till report writing. Dr. Anakita Guragain helped with acquisition of data, Dr. Anna Sharma and Dr Shobha Sapkota helped with interpretation of data and drafting of manuscript.

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