ORIGINAL ARTICLE

A Study on Microbiological Profile and Antibiogram in Suspected Cases of Pediatric Urinary Tract Infection: A Single Center Study from Rural India

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most prevalent bacterial infections in children. Fever with or without focus is one type of clinical manifestation. Urinary tract anomalies are linked to high morbidity and death rates, so it's critical to diagnose and treat UTIs promptly. Isolating microbiological agents simplifies treatment. Purpose of this study was to identify the microbiological profile and antimicrobial susceptibility pattern that cause paediatric UTIs. **Material and Method:** 938 urine samples were taken from the suspected UTI cases. The semi-quantitative method known as the calibrated loop method was employed to isolate bacterial pathogens from urine specimens. The modified Kirby Bauer disc diffusion technique was used to conduct the antibiotic sensitivity test. Extended Spectrum Beta-Lactamase (ESBL) in GNB and Methicillin resistance in *Staphylococcus* was detected according to CLSI guidelines. **Result:** This study included 938 urine samples from clinically suspected UTI cases, with 38.2% showing substantial growth. The majority of 354 instances were reported in the 6-12 age-group. E. coli (29%) and *Enterococcus* (13.3%) were the most frequently identified Gram-negative and Gram-positive uropathogens. Nitrofurantoin was the most effective against Gram-negative organisms and Gram-positive organisms, followed by Vancomycin. 29.7% were ESBL producers. 28.1% were Methicillin resistant *Staphylococcus*. **Conclusion:** To optimize empirical therapy, it's important to assess the antibiotic sensitivity of UTI pathogens to commonly used antimicrobial drugs in specific location on a regular basis. Antibiotic resistance among paediatric urine culture isolates is on rise. We recommend empirical antibiotic selection based on local bacterial prevalence and sensitivities, rather than universal guidelines.

KEY WORDS: UTI, Pediatric population, ESBL, GNB, CLSI.

Introduction

A urinary tract infection (UTI) is described as a microbial invasion of the genitourinary tract that has a high risk of morbidity and mortality if left untreated. It is estimated that between 150 and 250 million instances of UTIs occur annually worldwide, making it one of the most prevalent forms of infectious disease.[1]

Worldwide, there is a 30% reported incidence of UTI among children.[2] The age and sex of children affect the incidence of UTIs. From 2.8 to 5.4:1, the male-to-female ratio indicates that males outnumber females in infants. With a male-to-female ratio of 1:10, there is a high female preponderance beyond infancy (>1 year).[3]

Low socioeconomic level, cultural practices including cleaning the perineum, and the use of diapers are risk factors for UTIs in children.[4] Particularly in newborns, post-urethral valves, neurogenic bladder,

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stricture urethra, and pelvic-ureteric junction obstruction are associated with pediatric UTIs. The most frequent pathogens causing UTIs are gram-negative bacteria, particularly Escherichia coli and Klebseilla spp., however, in recent years, yeasts, *Enterococcus* species, and *Staphylococcus aureus* have become well-known pathogenic agents.[5]

The variety of non-specific clinical signs makes it challenging to make a diagnosis based solely on these features. To protect the developing kidney's renal function, early detection is crucial.[6] The natural history of paediatric UTIs has changed over the last 30 to 50 years due to advancements in antibiotic treatment.[7]

Multidrug resistant bacteria have been mostly caused by over-use, insufficient course of antibiotics, and empirical antibiotic therapy. Treatment should therefore be based on the regional patterns of antibiotic susceptibility. This study is among the few that examine patterns of antibiotic susceptibility in rural population. More comprehensive AMR surveillance data is the need of the hour.

Material and Methods

- **Study setting:** In a clinical environment, a cross-sectional approach was employed to perform the research. The investigation was carried out between January and December of 2023. The study was carried out with the institute's ethical committee's clearance.
- **Inclusion criteria:** The samples were taken from patients who were suspected of having UTI aged one month to 12 years presenting to OPD, IPD, and the emergency department of Paediatrics.
- **Methodology:** The first mid-stream urine sample was collected with aseptic precautions, in infants; either supra-pubic aspiration or transurethral bladder catheterization was used to collect a urine sample. The samples that could not be examined right away were kept at 4°C in a refrigerator for a maximum of 12 to 24 hours. In contaminated cases, a second urine culture was performed.

The semi-quantitative method and quantitative method i.e. unspun wet mount microscopy on well-mixed, uncentrifuged urine samples were used to detect pyuria (> 1pus cell/7 high power fields).[8] The semi-quantitative method known as the calibrated loop method was employed to isolate bacterial pathogens from urine specimens. Cysteine lactose electrolyte deficient (CLED) agar (Hi Media Laboratories, Mumbai, India) was inoculated with a loopful of urine. Plates were incubated for 24 hours at 35–37°C in an aerobic environment in order to precisely determine the number of colonies. A colony count of more than 100,000/mL of organisms from one bacterial species in a midstream urine sample is considered significant.

Culture isolates were tested by using routine and conventional laboratory procedures utilizing biochemical procedures such as triple sugar iron agar, IMViC test, oxidase test, coagulase test, catalase test, and Gram stain to determine their identities. In compliance with Central Laboratory Standard Institute (CLSI) recommendations, the antibiotic sensitivity test was carried out using the Kirby Bauer disc diffusion technique on Mueller Hinton agar plates using commercially available Hi-Media antibiotic discs.

In accordance with CLSI, the development of Extended Spectrum Beta-Lactamase (ESBL) in GNB and Methicillin resistance in *Staphylococcus* was detected. Using cephalosporin, ceftazidime (30 μg), and cefotaxime (30 μg) discs alone or combined with clavulanate, an ESBL production test was conducted on Muller-Hinton agar using the disc diffusion method. If the zone diameter of the organism for either antibiotic tested when combined with clavulanate was greater than 5 mm compared to its zone diameter when tested alone, it was determined to be producing ESBLs.[9]

Result

In this study, 938 cases were enrolled based on the clinical features. There was a dominance of those with females (59%), age group between 6-12 years (37.7%), and presenting with fever and dysuria (Table 1).

In this study, out of 938, 359 (38.2%) showed significant growth. Wet mount microscopy was used to detect infections in 292 patients. Its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 80.3, 60.3, 41.4, and 91.3 respectively, in identifying the presence of bacteria, yeast, or pus cells in a significant amount per field. 156 individuals had significant pyuria alone, with corresponding sensitivity, specificity, PPV, and NPV values of 53.6, 62.6, 36.1, and 77.4%.

In this study, *E. coli* followed by *Klebsiella* spp. was the most predominant Gram-negative uropathogen to be isolated (Table 3).

Table 1: General profile of the study population

S. No	Characteristics	Number
1.	**Age**	
	< 1 year	84 (8.9%)
	1-5 years	199 (21.2%)
	6-12 years	354 (37.7%)
	12-18 years	301 (32%)
2.	**Gender**	
	Male	384 (40.9%)
	Female	554 (59%)
3.	**Clinical features**	
	Fever	154 (43.5%)
	Dysuria	89 (25.1%)
	Pain abdomen	63 (17.8%)
	Hematuria	37 (10.4%)
	Poor feeding	11 (3.1%)

Table 2: Total number of samples tested

Urine culture	Number of samples	Percentage
Significant growth	359	38.2%
Insignificant growth	87	9.2%
Sterile	492	52.4%

In the present study, 244 Gram negative uropathogens were isolated, they showed, highest sensitivity to Nitrofurantoin (73.1%), Amikacin (77.4%), Gentamicin (70%) followed by Imipenem, Amoxyclav, Cefoperazone-sulbactum. They showed least sensitivity to norfloxacin, nalidixic acid and cefixime (Table 4). ESBL production was seen a combined percentage of 29.7% in *E. coli* and *Klebsiella* species.

In the present study, 47 non fermenters were isolated, and their antibiotic sensitivity pattern is shown in Table 5.

In the present study, 80 (22.2%) Gram- positive pathogens were isolated out of which *Enterococcus* species (13.3%) was the most common organism followed by *Staphylococcus aureus* (7.2%). They showed highest sensitivity towards Linezolid, Amikacin and Nitrofurantoin, Vancomycin and Teicoplanin. 9 (28.1%) out of 32 *Staphylococci* isolates showed resistance to Cefoxitin disk (Table 6).

Table 3: Organism isolated

Organism isolated	Number	Percentage
E. coli	103	29%
Klebsiella pneumoniae	66	18.3%
Klebsiella oxytoca	6	1.6%
Proteus mirabilis	6	1.6%
Proteus vulgaris	3	0.8%
Morganella morganii	1	0.2%
Citrobacter koseri	3	0.8%
Citrobacter freundii	5	1.4%
Entrerobacter aerogenes	4	1.1%
Pseudomonas aeroginosa	42	11.6%
Acinetobacter spp.	5	1.4%
Staphylococcus aureus	26	7.2%
CONS	6	1.6%
Enterococcus fecalis	48	13.3%
Candida albicans	28	7.8%
Candida glabrata	7	1.9%

Table 4: Antibiotic sensitivity pattern of Gram negative pathogens

Antibiotics	Sensitivity percentage
Norfloxacin	23.6%
Nitrofurantoin	73.1%
Nalidixic Acid	6%
Amoxyclav	42.3%
Cefoperazone sulbactum	43.1%
Ceftriaxone	36.2%
Amikacin	77.4%
Cefixime	19.4%
Gentamycin	70%
Imipenem	45.4%
Co-trimoxazole	42.8%

Discussion

One of the most prevalent illnesses in kids is urinary tract infection. They also contribute significantly to morbidity, which can have long-term effects including hypertension and renal failure. Therefore, in order to save patients needless financial hardship and stop the emergence of long-term problems, early diagnosis, the identification of antibiotic sensitivity patterns, and appropriate treatment of UTIs are crucial.

Table 5: Antibiotic sensitivity pattern of Non-fermenters

Antibiotics	Sensitivity percentages
Norfloxacin	55%
Nitrofurantoin	75%
Nalidixic acid	22.8%
Ceftazidime	35.5
Cefepime	40.2
Piperacillin-tazobactum	58%
Amikacin	76
Gentamycin	66.6
Aztreonam	63.3%
Ciprofloxacin	54%
Imipenem	52%

Table 6: Antibiotic sensitivity pattern of Gram-positive pathogens

Antibiotics	Percentage sensitivity
Norfloxacin	23.9%
Nitrofurantoin	72%
Amoxyclav	40%
Amikacin	73.8%
Ciprofloxacin	39.2%
Cotrimoxazole	45.6%
Gentamycin	63.6%
Linezolid	87.5%
Vancomycin	100%
Teicoplanin	100%

In the present study, 938 patients were enrolled according to signs and symptoms. Of the total, 59% were female and 40.9% were male. Our results are consistent with prior research that also found a preponderance of women, with male-to-female ratios of 1:221 & 1:1.220, correspondingly.[10,11] Females have UTIs more frequently, most likely as a result of unique physiological and anatomical alterations such as their shorter urethra and closer closeness to the anus.

Of the 938 cases of UTI, children aged 6 to 12 accounted for a significant prevalence of the infections. It is consistent with investigations by Gautam G. et al. that indicated that the age range of 6 to 10 years old had the highest number of cases.[12] This could be brought on by a lack of knowledge about appropriate toilet training and an increase in faecal flora infection in this age range.

There was a 38.2% cultural positive rate noted. Gram-negative bacilli constituted most of the organisms in this study. The same kind of uniformity between culture isolates has been demonstrated by many studies.[13,14] The study by Taneja et al. showed that *Acinetobacter* spp. 6.6% and *Pseudomonas* in 10.9% of children were significant infections in PICU settings, which is in contradiction to our findings.[5] A distinct geographic distribution of the pathogen may be the cause of this. Another reason may be the difference in age group or the presence of congenital urinary tract abnormalities.[15]

Antibiotics have completely changed healthcare since the discovery of penicillin. It has undoubtedly had a major impact on bringing down the death rate from a variety of infections. Unfortunately, due to its indiscriminate and extensive usage, antibiotic resistance has developed, particularly in nations where the health sector is not well regulated.[16]

The majority of Gram-negative uropathogens in our study were most sensitive to gentamicin, imipenem, amoxyclav, and cefoperazone-sulbactum, following these three antibiotics. They showed the least sensitivity to Norfloxacin, Nalidixic acid, and Cefixime. Our findings are consistent with Gupta P et al., A Sharma et al**.,** who found pathogens to be sensitive to Nitrofurantoin, followed by Amikacin, Imipenem, and the least sensitive was Cefotaxime.[17,18]

The high prevalence of ESBL-producing organisms causing UTI in our hospital has reached an alarming proportion. In our study, 52 out of 175 (29.7%) isolates of *E. coli* and *Klebsiella* species were ESBL producers. According to studies done in Rohtak and Nagpur, 18.5% and 40% of *E. coli*, respectively, produce ESBLs.[19,20] Over the course of a 2.5-year study period, an increasing trend in the isolation of *E. coli* that produces ESBL was seen in Jaipur, with numbers rising from 9.52% to 30.08%.[21]

Notably, the majority of our *K. pneumoniae* and *E. coli* bacteria that produced ESBLs were multi-drug resistant. Most of them specifically exhibited resistance to the amoxicillin-clavulanic acid combination. Nitrofurantoin can be taken into consideration as an alternate drug for treating lower UTIs according to Tasbakan et al. and Chen et al.[22,23]

Gram-positive organisms have drawn increased attention recently as potential causes of urinary tract infections and bacteuria. Coagulase-negative *Staphylococcus*, *S.aureus*, *Streptococci*, and *Enterococci* have been reported by various authors as an important cause of UTI.[24,25] In the current study, *Enterococcus* was the most common gram-positive to be isolated followed by *Staphylococci*. They exhibited 100% susceptibility to vancomycin and teicoplanin but were resistant to amoxyclav, norfloxacin, and ciprofloxacin. There were 2 *Enterococci* which were only sensitive to vancomycin and teicoplanin. Amongst *Staphylococci*, 28.1% showed resistance to cefoxitin. These findings were consistent with other studies.[26,27]

Due to varying hospital-based antibiotic policies and the widespread availability of over-the-counter medications, the pattern of antimicrobial resistance varies between studies. One of the challenges that can prevent an effective course of treatment is the uropathogens' resistance to antibiotics. The incidence of ESBL producers among Enterobacteriaceae was <10% in North America, notably lower than in Asia and the Middle East countries (>40%), as per the "Study for Monitoring Antimicrobial Resistance Trends (SMART)".[28]

Limitation

The study has several limitations despite its best efforts. Firstly, the urine culture and sensitivity findings from one institution, which had a smaller sample size, cannot be generalized to all UTI patients. Secondly, as the majority of the data is centered on urine culture results, epidemiologic, demographic, and comorbid circumstances of patients are not fully reported.

Conclusion

In conclusion, a policy for appropriate use of antibiotics should be developed, along with routine monitoring to find reliable data regarding the resistance pattern of urine pathogens and provide the best empirical therapy. There should be a greater emphasis on expanding the worldwide AMR surveillance studies. There should be research into vaccines, next-generation antibiotics, and sophisticated 'point-of-need' diagnostics to distinguish between bacterial and viral infections.

Disclosure

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