Antibiotic Sensitivity Pattern of Uropathogenic *Escherichia coli* in Pediatric Patients in a Tertiary Care Center in Kerala

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Abstract

Introduction: Urinary tract infections (UTI) are one of the most common but difficult to reliably diagnose clinically in children. The etiopathogenesis may range from urinary stasis due to improper toilet training or physical obstruction like phimosis to neural causes like neurogenic bladder or urogenital abnormalities. The most common uropathogen isolated is *Escherichia coli*. Prompt diagnosis and proper treatment as per the culture and sensitivity report are essential to avoid recurrent and chronic infections as well to prevent late complications like renal cortical scarring as a precedence to chronic renal failure.

Materials and Methods: The study was carried out in samples received from January 2022 to June 2022 to assess the antibiotic sensitivity pattern of Uropathogenic *E. coli* in pediatric patients and also to ascertain the prevalence of extended spectrum beta lactamase (ESBL) in these infections. Four hundred and thirty-two clean catch midstream urine samples were collected from pediatric patients during the study period and after exclusions, 129 samples of monobacterial growth of more than 100,000 cfu/mL were selected for the study. Out of these 129 g negative *bacterial* isolates, 78 isolates were identified as *E. coli*., these were subjected to disc diffusion based antibiotic sensitivity testing using the Kirby Bauer method and CLSI 2021 guidelines. Accordingly antibiotics Ampicillin (10 μ g), Gentamicin (10 μ g), Ciprofloxacin (5 μ g), Levofloxacin (5 μ g), Cotrimoxazole (25/1.25 μ g), Amoxicillin-clavulanic acid (20/10 μ g), Piperacillin/Tazobactum (100/10 μ g), Norfloxacin (10 μ g), Amikacin (30 μ g), Ceftazidime + clavulanic acid (20/10 μ g), Cefuroxime (30 μ g), Cefotaxime (30 μ g), Imipenem (10 μ g), Doripenem (10 μ g), and Meropenem (10 μ g) were tested. Screening of ESBL producing *E. coli* was done with combined disc diffusion method using ceftazidime and ceftazidime-clavulanic acid in combination.

Results: Out of the 78 *E. coli* isolates, the screening for ESBL producers revealed that 50 isolates were ESBL positive. The antibiogram of 28 non-ESBL producers revealed that most isolates were resistant to Penicillins and cephalosporins. The non-ESBL producers showed sensitivity to Piperacillin-Tazobactum (100%), Carbapenems (100%), Nitrofurantoin (100%), Amikacin (96.4%), Ciprofloxacin (75%), Levofloxacin (78.5%), and Norfloxacin (75%). On the other hand, the ESBL producers were resistant to Penicillin, Beta lactams + inhibitors, Cephalosporins, Aminoglycosides, Tetracyclines, Folate pathway inhibitors, and Fluroquinolones.

Conclusion: UTI is a common health problem in children and is an important cause of morbidity and mortality. The effect of ESBL producers is very much evident in the sensitivity pattern wherein the Amoxycillin-Clavulanic acid reduces from 71.4% to 0% among ESBL producers. In the same way, Piperacillin-Tazobactum reduces from 100% to 52%, Amikacin reduces from 96.4% to 64%, and cephalosporins become totally ineffective in the ESBL producing strains. Every healthcare institution must develop its own antimicrobial treatment policy based on the culture and sensitivity report prevailing in the past 6 months. As per the antimicrobial stewardship programs in health-care facilities in low-and middle-income countries advised by the World

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Health Organization, these policies need to be reassessed at least once in 6 months to know the pattern of emerging resistance as well as to decide about the use of antibiotic recycling for the better usage of available antibiotics for the treatment of UTI.

Key words: AMR, Antibiotic resistance, *Escherichia coli*, Paediatric urinary tract infections, Renal scarring, Urinary tract infections, Uropathogenic *Escherichia coli*, Uropathogens

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INTRODUCTION

Urinary tract infections (UTI) are one of the most common infections in children. The infections can escalate very quickly to severe infections in the absence of a reliable history and non-specific examination findings. The prevalence varies with age. The prevalence of UTI is high in young infants, toddlers, and older adolescents. Female children are more commonly affected due to the shorter urethra and increased chance of *bacterial* entry from the gastrointestinal system due to anatomical proximity. In the same manner, uncircumcised male children also have a higher incidence of UTI due the higher bacterial skin flora concentration under the nappy in infancy as well as the surface area of foreskin which can act as a nidus for infection. As the infant grows up, toilet training, and voluntary holding, bladder stasis may act as factors predisposing the risk of UTI. Any condition that impairs the flow of urine or stasis may lead to development of UTI. These include neurogenic bladder, urogenital abnormalities, and altered immune function. These may lead to recurrent UTI leading to increase in hospital admissions and economic burden.^[1]

REVIEW OF LITERATURE

UTI poses a major risk to growing children. If the UTI are not identified and treated properly, the developing renal cortex in children is vulnerable to renal scarring resulting in hypertension and chronic renal failure in later years of life.^[2] As far as the data available from studies conducted in Kerala, the age group most affected by UTI is between 0 and 6 years.^[3] The risk factors identified apart from the anatomical and pathological factors include uncircumcised boys, constipation, worm infestation, enuresis, wiping from back to front, and urethral instrumentation.^[4] The most common *bacterial* involved in causation of UTI happens to be *Escherichia coli* followed by *Klebsiella*, Coagulase negative *Staphylococcus, Citrobacter, Enterococcus, Acinetobacter* spp, Proteus vulgaris, and *Staphylococcus aureus*.

Most common isolates of pediatric bacteriuria belong to the Gram-negative coliform group of *bacteria* which ascend the urinary tract. *E. coli* have specific properties like the fimbriae which help the *bacteria* attach to uroepithelial surface thereby allowing the *bacteria* to overcome host defenses easily.^[5] Other factors that help in causation of urinary tract infection include α -hemolysin, M hemagglutinin, endotoxin, cytotoxic necrotizing factor 1, K capsular antigen, a rigid cell wall, serum resistance ability due to the outer membrane protein TraT, aerobactin which supports growth by chelating iron, and adhesive capacity. Upper urinary tract infection involves the kidneys and ureters.^[6] The three different types of

adhesins identified on uropathogenic E. coli include Type 1 pili (or fimbriae), Pfimbriae, and X-adhesins These adhesins facilitate adherence of the bacteria to mucosal receptors in the uroepithelium in spite of the flushing action of urine flow.^[7,8] Once the uroepithelium is invaded, an intracellular biofilm is formed.^[7] The biofilm can protect the uropathogenic E. coli from the host immune system. The patients with upper urinary tract infection present with abdominal pain, loin tenderness along with fever, anorexia, vomiting, lethargy, and malaise. Lower urinary tract involves the bladder and urethra and thus the symptoms are more localized like lower abdominal pain, dysuria, urinary frequency, and urgency. In younger patients, the classical signs are absent and thus the differentiation between upper and lower UTI becomes less obvious.^[9] The clinical symptoms may vary from mild dysuria to life-threatening urosepsis. Fortunately, severe infections are less common and if they do occur, they are more commonly seen in neonates.^[9-11] UTI is more common cause of occult infections in infants. Pediatric population can also become victims of short-term morbidity with poor oral intake and dehydration apart from the rare case cases of perinephric abscess formation. In some cases, the spread of uropathogens can occur through the hematogenous route as well. Meningitis can also occur with hematogenous spread to cerebrospinal fluid and is more predominant in infants.

Long-term morbidity can occur following UTI. It has been documented that 15% of children will have evidence of renal scarring following single incidence of urinary tract infection. This scarring becomes important if it leads to renal dysfunction, hypertension, and chronic kidney disease (CKD).^[12] In the absence of renal abnormalities or recurrent UTI, the risk of CKD is seen to be minimal.^[13] There is a genetic predisposition to recurrent UTI and renal scarring Genes that have been shown to predispose patients to recurrent UTI and renal scarring include angiotensin-converting enzyme insertion/deletion gene, Interleukin (IL)-8 receptor CXCR1 and CXCR2 genes, IL-10-1082 A/G gene, heat shock protein 72 gene, transforming growth factor- β 1 gene, toll-like receptor pathway genes, and vascular endothelial growth factor gene.^[14-19]

In view of the above-mentioned morbidity and long-term complications, effective diagnosis and treatment of UTI gain importance. As far as the diagnosis is concerned, there are challenges as the pediatric age patients cannot explain the symptoms and is based on the observation of the parent and good clinical examination. The next challenge comes with sample collection and it is very difficult to obtain a clean catch midstream urine sample (CCMSU). The urinary screening with microscopy or dip stick methods is not effective in ascertaining the diagnosis of UTI. In the given scenario, it is imperative for every healthcare institution to develop an antibiotic policy based on the culture and sensitivity pattern so that an effective empiric therapy can be instituted in cases of pediatric UTI.

MATERIALS AND METHODS

The study was conducted at Azeezia institute of Medical Sciences and Research, Kollam district of Kerala State during the period of January 2022–June 2022 to assess the antibiotic sensitivity pattern of uropathogenic *E. coli* among the isolates from urinary samples of pediatric patients reporting to the pediatric department hospital with complaints or signs and symptoms suggestive of UTI.

All non-repetitive midstream urine samples obtained during the study period were included in the study. A total of 432 CCMSU samples were received in the microbiology laboratory during the study period. These samples were subjected to wet mount examination and were inoculated to 5% sheep blood agar and MacConkey agar plates. The plates were kept for incubation at 37°C for 18–24 h. After 24 h of incubation at 37°C 255, samples did not show any evidence of *bacterial* growth and were reported as No Growth. Twenty-eight samples showed presence of more than three types of *bacterial* growth which also corroborated with the wet mount findings and were not processed further. After exclusions, totally 149 samples of monobacterial growth of more than 10⁵cfu/mL were considered for the study. Twenty samples grew Grampositive organisms such as S. aureus, Staphylococcus epidermidis, Streptococcus spp., and Enterococcus spp. One hundred and twenty-nine isolates were Gram-negative bacilli out of which 51 isolates were Klebsiella pneumoniae and remaining were Citrobacter spp., Enterobacter, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, and Acinetobacter spp. Seventy-eight isolates were E. coli and these isolates were considered for the present study.

Processing of Samples

All samples were cultured on culture enriched and selective media by semi-quantitative method. Samples were inoculated on 5% sheep blood agar plate and Mac Conkey agar plate by streaking using sterile calibrated wire loop and incubated aerobically for 18–24 h at 37°C. Samples which showed monobacterial significant grown (>10⁵ CFU/mL) were included in this study. Isolation and identification of isolates were based on their morphology in gram staining, cultural characteristics, and biochemical reactions.^[20] Antibiotic susceptibility testing of all isolates was performed by Kirby–Bauer's disc diffusion method and interpretation of the results was done based on CLSI 2021. *Bacterial* suspension was made and compared to 0.5 McFarland turbidity standards in peptone water. Antibiotic discs (Himedia Laboratories Pvt. Ltd. Mumbai)

used were Ampicillin (10 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Cotrimoxazole (25/1.25 µg), Amoxicillin-clavulanic acid (20/10 µg), Piperacillin/Tazobactum (100/10 µg), Norfloxacin (10 µg), Amikacin (30 µg), Ceftazidime (30 µg), Ceftazidime + clavulanic acid (20/10 µg), Cefuroxime (30 µg), Cefotaxime (30 µg), Imipenem (10 µg), Aztreonam (30 µg), Tetracycline (30 µg), Nitrofurantoin (300 µg), Tobramycin (10 µg), Colistin (10 µg), Etrapenem (10 µg), Doripenem (10 µg), and Meropenem (10 µg).

Procedure

Antibiotic sensitivity testing was done using Kirby-Bauer method. Discs were applied aseptically. Gap of 24 mm centre-centre was ensured as per CLSI guidelines. Plates were Incubated at $35 \pm 2^{\circ}$ C and examined after a minimum of 16–18 h.

Screening for Extended Spectrum Beta Lactamase (ESBL) Isolates

Screening of ESBL producing E. coli according to CLSI guidelines, strains showing zone of inhibition of $\leq 22 \text{ mm}$ for Ceftazidime and/or ≤17 mm for Cefpodoxime and/or ≤27 mm for Cefotaxime were considered for confirmation test for ESBL. ESBL producing E. coli isolates were the subcultured into sterile nutrient agar plates and incubated for 24-48 h. The isolated single colonies were used for further comparative studies. ESBL production among potential ESBL producing isolates was confirmed phenotypically using combined disc diffusion method. Comparison of the zone of inhibition was made for the Ceftazidime (30 μ g) versus that of the Ceftazidime disc in combination with clavulanic acid $(30/10 \,\mu g)$, placed 25 mm apart (center to center). A difference in the inhibition zone diameter of \geq 5 mm for a combination disc versus ceftazidime disc alone confirmed ESBL production (Phenotypic Confirmatory Disc Diffusion Test).^[21]

RESULTS

Out of the 78 *E. coli* isolates, the screening for ESBL producers revealed that 50 isolates were ESBL positive. The antibiogram of 28 non-ESBL producers revealed that most isolates were resistant to penicillins and cephalosporins. The non-ESBL producers showed sensitivity to Piperacillin-Tazobactum (100%), Carbapenems (100%), Nitrofurantoin (100%), Amikacin (96.4%), Ciprofloxacin (75%), Levofloxacin (78.5%), and Norfloxacin (75%). On the other hand, the ESBL producers were resistant to Penicillin, Beta lactams + inhibitors, Cephalosporins, Aminoglycosides, Tetracyclines, Folate pathway inhibitors, and Fluroquinolones [Table 1].

DISCUSSION

UTI is a common health problem in children and is an important cause of morbidity and mortality. Bacteria play a major role in these infections and among the bacteria, Gram-negative organisms like E. coli are the most common uropathogens causing infections. The anatomical factors play a major role in causation of infection whereby the bacteria in the gastrointestinal tract find an easy way to enter into the renal system and cause infection assisted by the virulence factors present in the bacteria. The issues involving the diagnosis of pediatric UTI include non-reporting of symptoms by preverbal children. Parents may notice lethargy, irritability, poor feeding, and vomiting. Fever may or may not be present. The change in odor or color of urine may be missed in nappy wearing children. Older children may be able to report clinical symptoms precisely and may be confirmed with clinical examination and culture using the CCMSU sample. In patients in whom the sample collection is doubtful may be subjected to invasive methods of sample collection including catheterized sample or using supra pubic aspiration of urine.

As the chances of renal injury and CKD is more in children, it is essential to treat *bacterial* urinary tract infection as per the local guidelines and sensitivity patterns as the susceptibility can vary significantly in different regions. In our study, out of the Gram-negative bacteriuria accounting to 129 isolates, 78 were *E. coli*. Among the *E. coli*, 28 isolates were non-ESBL producers and 50 were ESBL producers accounting to 64.1%. The implication of the increase in ESBL producers results in increased antibiotic

Table 1: Comparison of antibiotic sensitivity of	
uropathogenic Escherichia coli isolates	

Class	Antibiotics	Escherichia coli	ESBL Escherichia coli
		<i>n</i> =28	<i>n</i> =50
Penicillin	Ampicillin	35.7	0.0
β Lactam+Inhibitor	AMOX-CLAV	71.4	0.0
	PIP-TAZ	100	52.0
Cephalosporins II	Cefuroxime	39.2	0.0
III	Cefotaxime	35.7	0.0
	Ceftazidime	39.2	0.0
Carbapenams	Imipenam	100	72.0
	Meropenam	100	50.0
Aminoglycosides	Gentamicin	53.5	32.0
	Tobramycin	64.2	20.0
	Amikacin	96.4	64.0
Tetracyclins	Tetracycline	67.8	32.0
Fluroquinolones	Ciprofloxacin	75.0	44.0
	Levofloxacin	78.5	28.0
	Norfloxacin	75.0	42.0
Folate pathway (-)	Cotrimoxazole	53.5	48.0
Nitrofurans	Nitrofurantoin	100	86.0

Values mentioned are in percentages (%)

drug resistance leading to clinical treatment failures and increased chances of reinfection and recurrent UTI. The effect of ESBL producers is very much evident in the sensitivity pattern wherein the Amoxycillin-Clavulanic acid reduces from 71.4% to 0% among ESBL producers. In the same way, Piperacillin-Tazobactum reduces from 100% to 52%, Amikacin reduces from 96.4% to 64% and cephalosporins become totally ineffective in the ESBL producing strains. The results in our study as well as the study conducted by Lok Bahadur Shrestha *et al.*^[22] emphasize on the importance of screening for UTI with culture and sensitivity in pediatric infections.

As per the Standard treatment guidelines issued by the Indian Academy of Pediatrics for the year 2022 has suggested Cefixime, Amoxicillin or co-amoxiclay, Cephalexin or Cefadroxil as oral antibiotics. In patients requiring parenteral antibiotics Amikacin, Gentamicin, Cefotaxime, or Ceftriaxone may be tried for UTI. As for those children requiring prophylaxis for prevention of UTI, Cotrimoxazole, Nitrofurantoin, Cephalexin, or Cefadroxil may be used.^[23] However, in India, it would be ideal to rely on culture and sensitivity reports in view of higher antimicrobial resistance pattern, the markedly increasing ESBL produced among Enterobacteriaceae, the difficulties in reporting and diagnosis of UTI are to be considered. It is worthwhile to be noted that every healthcare institution must develop its own antimicrobial treatment policy based on the culture and sensitivity report prevailing in the past 6 months. As per the antimicrobial stewardship programs in health-care facilities in low- and middle- income countries advised by the World Health Organization, these policies need to be reassessed at least once in 6 months to know the pattern of emerging resistance as well as to decide about the use of antibiotic recycling for the better usage of available antibiotics for treatment of UTI.

CONCLUSION

Urinary Tract Infections is one of the most common infections in children and is an important cause of morbidity and mortality. The aetiology is multi-factorial and urinary culture & sensitivity testing is mandatory for identification of the uropathogen. In our study the effect of ESBL producers is very much evident in the sensitivity pattern wherein the Amoxycillin-Clavulanic acid reduces from 71.4% to 0% among ESBL producers. In the same way Piperacillin-Tazobactum reduces from 100% to 52%, Amikacin reduces from 96.4% to 64% and cephalosporins become totally ineffective in the ESBL producing strains. Every healthcare institution must develop its own antimicrobial treatment policy based on the culture and sensitivity report prevailing in the past six months. As per the antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries advised by the World Health Organization these policies need to be reassessed at least once in six months to know the pattern of emerging resistance as well as to decide about the use of antibiotic recycling for the better usage of available antibiotics for treatment of UTI.

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