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Original Article

Bacteriological profile, antibiotic sensitivity pattern, and detection of extended-spectrum β-lactamase in the isolates of urinary tract infection from children

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Abstract

Background: Appropriate use of antibiotic in children with urinary tract infection (UTI) is essential so as to curb the spread of drug-resistant organisms. **Objectives:** To study the bacteriological profile and antibiotic sensitivity pattern in children with UTI and to determine the prevalence of extended-spectrum β-lactamase (ESBL) producers of *Escherichia coli* and *Klebsiella* species. **Methods:** This prospective study was conducted from October 2010 to September 2011. The children between age group of 3 and 14 years who attended Pediatric Department at a tertiary care hospital were included in the study. Single midstream urine specimen was collected from each patient with suspected UTI. **Result:** Out of 184 urine samples with suspected UTI, 122 children had culture-proven UTI. Of 122 cases, 81 (66.39%) cases were seen in females. The most common organism isolated was *E. coli* (50%) followed by *Klebsiella pneumoniae* (23.77%) and *Enterococcus* species (8.19%). *E. coli, Klebsiella* spp., *Citrobacter freundii,* and *Proteus mirabilis* responded better to nitrofurantoin (NIT) (76.8%). Ceftriaxone (79.12%), cefotaxime (74.8%), and cefixime (71.7%) showed higher sensitivity as compared to ceftazidime (63.4%) and cefoperazone (CPZ) (59.4%). Among aminoglycosides, amikacin (82.4%) had a better response as compared to gentamicin (64.6%). Out of 61 *E. coli* and 31 *Klebsiella* species, 35 (38.04%) were ESBL producers. The sensitivity of these organisms to imipenem was 100% with a good response to meropenem, CPZ-sulbactam, and piperacillin-tazobactam. **Conclusion:** *E. coli* and *Klebsiella* spp. were the most common isolates and many of them were ESBL producers. NIT seemed to be a reasonable alternative to cephalosporins for the treatment of UTIs in children. Carbapenems were found to be effective in ESBLs and non-ESBL producing uropathogens and can be considered as reserve drugs.

Key words: *Antibiotics, Children, Drug resistance, Organisms, Urinary tract infection*

Urinary tract infection (UTI) is the third most leading cause of infection in pediatric patients causing high morbidity and mortality [1]. Since it presents with non-specific symptoms in young children, it sometimes goes undiagnosed. It results into complications such as renal scarring, hypertension, and chronic renal failure [2]. Thus, improperly treated UTI in children is a cause for both clinical and public concern.

Diagnostic approach to UTI is based on both clinical and laboratory evaluation. Practically microscopic analysis alone is enough for diagnosis of UTI along with clinical correlation. However, sensitivity, specificity, and predictive values of this test vary according to the patient population studied. Thus, urine culture still remains a gold standard.

The prevalence of extended-spectrum β-lactamase (ESBL) among clinical isolates varies from country to country and from institution to institution. Over the last few decades, there were outbreaks of ESBLs has been observed worldwide, which pose a great threat to commonly used cephalosporin [2]. In the United States, the occurrence of ESBL production in Enterobacteriaceae ranges from 0 to 25%, depending on the institution, with the national average being around 3% [3]. Its prevalence is around 21.7% in Europe [4]. Elsewhere in Asia, the percentage of ESBL production in *Escherichia coli* and *Klebsiella pneumoniae* varies from 4.8% in Korea to 8.5% in Taiwan and up to 12% in Hong Kong [5]. In India, the prevalence of ESBL phenotypes in *E. coli, K. pneumoniae,* and *Salmonella* species is >61%, >55%, and 3-8%, respectively [6]. It is interesting that specific ESBLs appear to be unique to a certain country or region. For example, TEM-10 has been responsible for several unrelated outbreaks of ESBL-producing organisms in the United States [3].

Hence, there is a need to have an early diagnosis of UTI to reduce morbidity, mortality and also need to emphasize on appropriate antibiotic selection so as to curb the spread of

drug-resistant bugs. In view of above concern, this study has been taken up.

MATERIALS AND METHODS

This prospective study was conducted from October 2010 to September 2011 in the Department of Pediatrics and Microbiology of a tertiary care teaching hospital. The study was carried out after obtaining prior approval from the Ethical Committee. An informed consent was taken from patient's caretakers. Children between 3 and 14 years of age who have admitted to pediatric ward from October 2010 to September 2011 with a clinical diagnosis of UTI were included. The diagnosis was made in the presence of a history of fever with any of the symptoms such as dysuria/excessive cry while micturition, increased frequency, burning micturition, or suprapubic pain/flank pain. Those children who had undergone bladder catheterization or had received antibiotics within 48 h prior to attending the hospital and samples which grew more than one type of organism was considered as contaminated and hence, excluded from this study.

After taking all precautions, a freshly voided clean catch midstream urine sample was collected in a commercially available sterile and wide-mouthed plastic container meant for urinalysis and urine culture. Urine was cultured on cytokine lactose electrolyte deficient agar using a 0.001 ml calibrated wire loop and incubated at 37°C for 24 h. Urine wet mount examination and Gram-stain of an uncentrifuged sample were also done and examined for the presence of pus cells and organisms.

Next morning, the isolates were identified by colony morphology, Gram-stain, motility test, and routine biochemical reactions using standard laboratory procedures [7,8]. In cases, where Gram-negative bacilli were isolated, colony count of more than 10^5/ml of a single organism was considered diagnostic of UTI [9]. Samples which grew more than one type of organism (multiple growths) were considered as contaminated and were not taken as positive for infection.

Antibiotic sensitivity was put up by the Kirby-Bauer disk diffusion method using commercially available antibiotic discs (HiMedia) following the Clinical Laboratory Standard Institute (CLSI) guidelines [10]. *E. coli* and *Klebsiella* spp. were screened for ESBL production by disk diffusion method with ceftazidime (CAZ) and ceftriaxone disks as recommended by CLSI Guidelines. Phenotypic confirmation was done by double disk diffusion test and phenotypic confirmatory disk diffusion test.

Statistical Analysis

Relationships between variables were analyzed using Chi-square test, Fisher's exact test. $p<0.05$ was considered statistically significant. The analysis was done by the SPSS version 17.0 statistical software.

RESULTS

During 1-year of the study period, a total number of 184 children between age group of 3 years and 14 years with suspected UTI were evaluated. Out of these patients, 122 children were diagnosed to have UTI. The most common organism isolated was *E. coli* (50%), followed by *K. pneumonia* (Fig. 1).

About 60% of *E. coli* were sensitive to third-generation cephalosporins. Among aminoglycosides, amikacin (AK) was the most sensitive antibiotic (65.57%). *K. pneumoniae* was equally sensitive (65.51%) to nitrofurantoin (NIT) and AK followed by gentamicin (GEN) (58.62%) (Table 1).

Acinetobacter baumannii isolates were found to be highly sensitive (100%) to meropenem (MRP) and imipenem (IPM). It was highly resistant to norfloxacin (100%), ciprofloxacin (87.5%), and GEN (87.5%). *Pseudomonas aeruginosa* isolates were sensitive to most of the antibiotics. However, numbers of patients were few to give conclusive results (Fig. 2).

Enterococcus species were 100% sensitive to vancomycin, linezolid, and teicoplanin. It was most resistant to

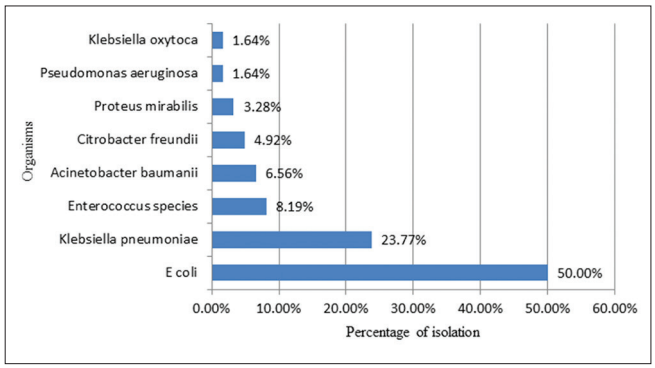

Figure 1: Organisms isolated on culture

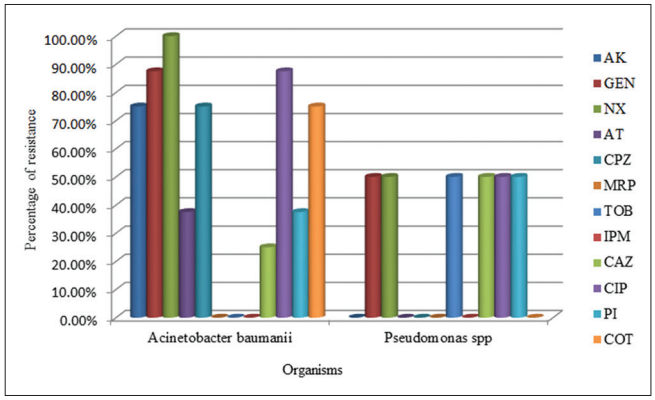

Figure 2: Resistance pattern of non-lactose fermenters to antibiotics. AT: Aztreonam, CPZ: Cefoperazone, MRP: Meropenem, TOB: Tobramycin, IPM: Imipenem, CIP: Ciprofloxacin, PI: Piperacillin

Table 1: Antibiotic sensitivity pattern of Enterobacteriaceae

Organisms	Frequency of resistance of isolates to antibiotics (%)											
	AMP	COT	GEN	AK	NA	NIT	NX	CAZ	CTR	CTX	CPZ	CFM
Escherichia coli	75.04	72.13	60.65	34.42	62.29	39.34	63.93	42.6	37.7	39.3	40.9	39.34
Klebsiella pneumoniae	79.31	68.96	41.37	34.48	58.62	34.48	58.62	48.3	41.4	44.8	44.8	51.72
Klebsiella oxytoca	50.00	0	0	0	0	0	0	50.0	0	0	50.0	50.00
Citrobacter freundii	66.66	50.00	50.00	16.66	33.33	16.66	33.33	16.6	0	16.6	16.6	0
Proteus mirabilis	50.00	25.00	25.00	0	0	25.00	50.00	25.0	25.0	25.0	50.0	0

AMP: Ampicillin, COT: Co-trimoxazole, GEN: Gentamicin, AK: Amikacin, NIT: Nitrofurantoin, NX: Nalidixic acid, CAZ: Ceftazidime, CTR: Ceftriaxone, CTX: Cefotaxime, CPZ: Cefoperazone, CFM: Cefixime

amoxicillin-clavulanic acid (70%), NIT (60%), and high-level aminoglycoside-resistant GEN (50%).

Out of 92 *E coli* and *Klebsiella* species, 35 (38.04%) were ESBL producers. Of these 35 ESBL producers, 23 cases (65.71%) were *E coli* (Table 2).

Sensitivity to IPM was 100% in both *E coli* and *Klebsiella* spp. and with a good sensitivity pattern to MRP, cefoperazone (CPZ)-sulbactam, and piperacillin-tazobactam (PIT). However, *Klebsiella* spp. was most resistant (66.66%) to amoxicillin-clavulanic acid (Table 3).

Table 2: Prevalence of ESBL producers of *Escherichia coli* and *Klebsiella* species

Organisms	ESBL (%)	Non-ESBL (%)
Escherichia coli (n=61)	23 (37.7)	38 (62.3)
Klebsiella spp. (n=31)	12 (38.7)	19 (61.3)
Total (n=92)	35	57

ESBL: Extended-spectrum β-lactamase

Table 3: Antibiotic sensitivity pattern of ESBL producers

Organisms	Sensitivity of ESBL producers to antibiotics (%)				
	AMC	CFS	PIT	MRP	IPM
Escherichia coli	52.17	65.21	60.86	95.62	100
Klebsiella spp.	33.33	66.66	58.33	91.66	100

CFS: Cefoperazone-sulbactam, PIT: Piperacillin-tazobactam, MRP: Meropenem, IPM: Imipenem

DISCUSSION

In the present study, the most common organism isolated from patients with UTI was *E coli* (50%) followed by *K. pneumoniae* (23.77%) and others. Similarly, Boloor et al. [2] observed *E coli* (53%) as the most predominant uropathogen followed by *P. aeruginosa* (9.8%) and *K. pneumoniae* (7.4%). They have isolated *Staphylococcus* in 4.9% and Group B *Streptococci* in 1.6% cases. Taneja et al. [11] and Mashouf et al. [12] have also detected *Staphylococcus* (1.7%) and Coagulase negative *Staphylococcus* (3.2%), respectively. Unlike we have not been able to isolate *Staphylococcus* spp. or any other Gram-positive organisms except for *Enterococcus faecalis.*

From our study, it was observed that *E. coli, Klebsiella* spp., *Citrobacter freundii,* and *Proteus mirabilis* responded better to NIT (76.8%) and third-generation cephalosporins. Among aminoglycosides, AK (82.4%) had the better response as compared to GEN (64.6%). Poor response was seen with ampicillin (AMP) (35.6%); whereas, co-trimoxazole (COT) (56.8%) and norfloxacin (58.9%) had an average response. Thus, physicians can still continue to prescribe NIT, cefixime, norfloxacin, and COT in outpatients as these drugs are available in oral forms. Susceptibility pattern to the above-mentioned antibiotics did not show a major variation among ESBL and non-ESBL producers.

Further, ESBL producing *E. coli* and *K. pneumoniae* has shown 100% sensitivity to imipenem and with a good sensitivity pattern to MRP, CPZ-sulbactam, and PIT. However, *Klebsiella* spp. was most resistant (66.66%) to amoxicillin-clavulanic acid. *P. aeruginosa* was isolated in only two cases. Hence, it was difficult to assess overall sensitivity pattern. In both the cases, organisms were sensitive to AK, aztreonam, CPZ, MRP, and IPM. One of the isolates was resistant to fluoroquinolones, PI, and GEN.

A. baumannii showed the better response to carbapenems (100% sensitive). CPZ and CAZ had almost similar sensitivity pattern (75%). Aminoglycosides were seen to be quite ineffective. *E. faecalis* isolates were found to be 100% sensitive to vancomycin, linezolid, and teicoplanin. Norfloxacin was found to be sensitive in about 60% of the cases. Nearly 40-50% isolates were sensitive to NIT and high-level GEN. Almost 70% of the organisms were resistant to amoxicillin-clavulanic acid.

There is a variation in frequency and sensitivity pattern among ESBL producing *E. coli* and *Klebsiella* spp. isolates. Some studies show a higher percentage of ESBLs producing *E. coli* isolates and others *Klebsiella* spp. As reported by Allen and colleagues, out of 1636 isolates of *E. coli* from children in Canada, 736 (45%) were resistant to AMP, 514 (31.4%) were resistant to COT, and 22.2% were resistant to both [13]. In the

present study, 73.4% isolates of *E. coli* were resistant to both, which is quite high. This, in turn, suggests the limited role of inexpensive first line agents in our set up.

Hasan et al. [14] conducted a study in New Delhi and stated multi drug resistance (MDR) was most commonly associated with *Enterococci* spp. (77.8%) followed by *P. aeruginosa* (65.1%), *E. coli* (52.9%), *K. pneumoniae* (48%), and *Staphylococcus aureus* (38.8%). Drugs which retained their effectiveness for Gram-negative bacilli were AK (47.3%), norfloxacin (33%), and cefotaxime (CTX) (42%). They have also tested susceptibility to lincomycin (86.1%) and netilmicin (35.4%) which we could not test in our study. Gram-positive cocci showed sensitivity to vancomycin and teicoplanin (100% each) and norfloxacin (41%). This was similar to the present study.

Shobha et al. [15] in Manipal, Karnataka, reported that 27.39% *K. pneumoniae* isolates and 25.39% *E. coli* were ESBL producers by the phenotypic confirmatory method. All ESBL producers were found to be susceptible to AK and IPM, which was in concordance with the present study.

Tankhiwala et al. [16] evaluated ESBLs in urinary isolates in Nagpur and observed 82% of bacterial uropathogens were resistant to COT and 79.2% to AMP. However, some isolates were susceptible to NIT (62.5%), CTX (58.7%), and norfloxacin (44.9%). AK was found to be the drug of choice in *P. aeruginosa* (85.7%). Nearly, 73.5% of Gram-negative bacilli were resistant to nalidixic acid. However, in the present study, these drugs were found to show better susceptibility pattern thus, giving a reasonable option for treatment.

In contrast to the present study, Khurana et al. found a higher percentage of ESBL producer *K. pneumoniae* isolates (38.5%) as compared to *E. coli* (24.7%) [17]. Furthermore, there was no significant difference between ESBL producers and non-ESBL producers in the susceptibility to non-beta lactam agents except for GEN.

One limitation of our study was that we could not get the Ethical Committee clearance for suprapubic aspiration to collect a urine sample. Therefore, we could not include children <3 years. Though suprapubic aspiration is ideal, midstream urine sampling is more practicable as well as possible in children above 3 years of age after proper training of caretakers.

CONCLUSION

NIT seemed to be a reasonable alternative to cephalosporins for the treatment of UTIs in children. Third-generation cephalosporins still showed a good response in non-ESBLs. AK and carbapenems were found to be effective in ESBLs and non-ESBL producing uropathogens and can be considered as reserve drugs. However, *Klebsiella* spp. was most resistant to amoxicillin-clavulanic acid. Prevalence of ESBLs producers and presence of MDR indicates the rampant use of antibiotics, necessitating implementation of effective antibiotic policy.

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