

Extended Spectrum and AMPC Beta-lactamases in the Enterobacteriaceae Isolated from Pediatric Urinary Tract Infection Cases

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Urinary tract infection is the third most leading cause of infection in pediatric age group, causing high morbidity and mortality. Resistance to broad spectrum beta lactams mediated by extended spectrum beta lactamase and AmpC beta lactamase enzymes is an increasing problem worldwide. Unfortunately the ESBL producers often also have resistance determinants to other antibiotic groups, leaving an extremely limited range of effective agents. Study was conducted on 100 non repetitive mid stream urine samples collected in a sterile container from pediatric patients suspected of urinary tract infection. All enterobacteriaceae isolates were subjected for detection of ESBL and AmpC beta-lactamases. 72 enterobacteriaceae were isolated in the study. Among them ESBL producers were 47(65.27%), pure AmpC producers were 23(31.94%) and 31(43.05%) were co-producers (ESBL+AmpC). In the present study, E.coli was the commonest isolate causing UTI in children with highest production of Amp C. Klebsiella spp showed highest production of ESBL. Simple disc tests can be used to detect ESBL and Amp C beta lactamases especially where resources for molecular detection are not available. Many of these beta-lactamases disseminate rapidly being plasmid mediated. Hence it is necessary that rapid detection of these enzymes should be done and immediate control measures should be implemented to prevent their dissemination.

Key words: ESBL, Amp C, Resistance, UTI.

Urinary tract infection is the third most leading cause of infection in paediatric age group, causing high morbidity and mortality.¹ Antimicrobial resistance showed by different uropathogens is one of the barricades that might hinder a successful treatment.² Resistance to broad spectrum beta lactams mediated by extended spectrum beta lactamase and AmpC beta lactamase enzymes is an increasing problem worldwide.³ Extended spectrum beta lactamase are beta lactamases capable of conferring bacterial resistance to the penicillins, first, second and third generation cephalosporins and aztreonam and

which are inhibited by beta lactamase inhibitors such as clavulanic acid.⁴ ESBLs belong to group 2be of Bush's functional classification.⁵ AmpC beta lactamases confer resistance to wide variety of beta lactam drugs including cefoxitin, narrow, expanded and broad spectrum cephalosporins, beta lactamase inhibitor combinations and aztreonam.⁶

E.coli, Klebsiella spp and Proteus spp are commonest organisms which cause urinary tract infection in paediatric age group. Presently more than 1000 beta-lactamases exist in the gram negative bacteria, the most important being extended spectrum beta-lactamases(ESBLs), AmpC beta-lactamases.⁷ They are increasing rapidly and becoming a major problem in the area of infectious diseases. Unfortunately the ESBL producers often also have resistance determinants to other antibiotic groups, leaving an extremely limited

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range of effective agents. A delay in appropriate therapy can cause severe complications.⁸

In view of the above concern, and as a simple disc based protocol using cefoxitin non susceptibility as a screening tool, followed by the Tris EDTA method for confirmation, detects Amp C with 95% sensitivity and 98% specificity,⁹ this study has been taken up.

MATERIALS AND METHODS

Inclusion criteria

Children aged between 3 and 14 years, suspected of having UTI were included in this study.

Exclusion criteria

Children who had undergone bladder catheterisation or had received antibiotics within 48 hours prior to attending the hospital were excluded from the study.

The guidelines of institutional Ethical committee were followed during the study.

A total of 100 non repetitive mid stream urine samples were collected in a sterile container. The samples were processed within half an hour of collection. Urine was cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar by semi quantitative culture method, using a 0.001ml calibrated wire loop and incubated at 37°C for 24 hours. Identification of bacterial growth was done by standard microbiological and biochemical techniques.⁸

All enterobacteriaceae isolates were subjected for detection of ESBL and AmpC beta-lactamases.

ESBL Detection (according to CLSI 2014 guidelines).¹⁰

Screening test (Disc diffusion method)

Cefpodoxime(10µg), Ceftazidime(30µg), Aztreonam(30µg), Cefotaxime(30µg) and Ceftriaxone(30µg) were used. Zone diameters were measured in mm. Zones above as indicated in CLSI guidelines were taken as screen test positive for ESBL production.

Confirmatory test (Combined Disc diffusion method)

- 1) Ceftazidime(30µg) and Ceftazidime-clavulanic acid(30µg/10µg) and
- 2) Cefotaxime(30µg) and Cefotaxime-clavulanic acid(30µg/10µg) were used.

A 5mm increase in zone diameter for either of these tested in combination with clavulanic acid VS its zone when tested alone was a confirmed ESBL

AmpC detection

Screening test (Cefoxitin disc test)¹¹

Isolates with zone diameter for cephoxitin (30µgms) disc less than or equal 18mm were taken as screen positive.

Confirmatory Test (Tris EDTA disc test)¹²

Tris EDTA was used to permeabilize the bacterial cell and release β-lactamase into external environment. AmpC discs were prepared in house.

Table 1. ESBL screening test positive and confirmatory test positive isolates

Organism	Total number of isolates	ESBL screening positive	ESBL confirmatory positive
<i>E.coli</i>	46	42(91.30%)	30(65.21%)
<i>Klebsiella spp</i>	22	20(90.90%)	15 (68.18%)
<i>Citrobacter spp</i>	2	02(100%)	01(50%)
<i>Proteus spp</i>	2	02(100%)	01(50%)
Total	72	66 (91.66%)	47 (65.27%)

Table 2. Amp C screening test positive and confirmatory test positive isolates

Amp C	Total number of isolates	Amp C screening positive	Amp C confirmatory positive
<i>E.coli</i>	46	37(80.43%)	15(32.60%)
<i>Klebsiella spp</i>	22	16(72.72%)	07(31.81%)
<i>Citrobacter spp</i>	2	02(100%)	01(50%)
<i>Proteus spp</i>	2	02(100%)	0(0%)
TOTAL	72	56 (77.77%)	23(31.94%)

20µl of 1:1 mixture of sterile saline and 100X Tris EDTA was applied to the sterile filter paper discs and allowed to dry and stored at 2-8°C. Discs were rehydrated with 20 microlitre saline, 8 to 10 colonies of test strain was applied to the disc. A lawn culture of *E.coli* ATCC -25922 was done on Muller-Hinton Agar plate. A cephoxitin disc(30µg) was placed on this inoculated plate. The inoculated AmpC disc was placed almost touching the cephoxitin disc surface in contact with agar surface. The plates were then incubated overnight. Flattening or indentation of the cephoxitin inhibition zone in the vicinity of the test disc was taken as a positive test. A test showing an undistorted zone was considered negative.

RESULTS

Out of 100 urine samples, 16 samples showed no growth. 84 samples showed growth including, 46(54.76%) *E.coli*, 22(26.19%) *Klebsiella pneumoniae*, 5(5.9%) *Enterococcus* spps, 3(3.57%) coagulase negative staphylococci, 2(2.38%) *Citrobacter* spp, 2(2.38%) *Acinetobacter* spp, 2(2.38%) *Proteus* spp, 2(2.38%) *Pseudomonas* spp. Only 72 enterobacteriaceae were included in the study. Among them total ESBL isolates were 47(65.27%), pure AmpC producers were 23(31.94%) and 31(43.05%) were co-producers (ESBL+AmpC).

DISCUSSION

In the present study, *E.coli* was the commonest isolate causing UTI in children with highest production of ESBL and AmpC followed by *Klebsiella* spps.

Detection of AmpC production in pathogens might be important for ensuring effective antibiotic therapy since the presence of an AmpC beta lactamase seems to result in therapeutic failure when broad spectrum cephalosporins are used.¹³

AmpC beta lactamases are cephalosporinases and can be differentiated from other ESBLs by their ability to hydrolyse cephamycins.¹⁴

In our study prevalence of ESBL was 65.27% and *Klebsiella* spps were predominant ESBL producer, compared to a study done by M.S Kumar et al with prevalence of 19.8% and *E.coli*

as predominant ESBL producer.¹⁵

In a study done by Dechen C Tsering et al, 34.03% gram negative bacilli were ESBL producers and showed sensitivity of 100% for ESBL detection test.¹⁶

In a study done by Vidya Pai et al, 31.1% were ESBL producers, and 27.8% showed AmpC production with commonest organism being *Citrobacter*, compared to our study with 31.94% AmpC producers and *E.coli* as the highest AmpC producer.¹⁷

In another study conducted by Vinita Ravat et al,¹⁸ total ESBL production was seen in 39% isolates, 25% were ESBL and AmpC co-producers where as in the present study 43.05% were co-producers.

In a study done by Silke Polsfuss, the sensitivity of AmpC detection by Cefoxitin disc test was 97.4%, and specificity was 78.7%.¹¹

In another study by Supriya Upadhyay et al, 64.6% *E.coli* isolates were AmpC producers which is very high compared to the present study.¹⁹

In a study Rajkumar Manojkumar Singh, showed sensitivity of 93.44% for ESBL detection by combined disc test.⁴

In a study done by Amutha Chelliah et al, ESBL was detected in 57.14% isolates of *E.coli* and AmpC was detected in 6.1% of isolates.²⁰

In the present study, *E.coli* was the commonest isolate causing UTI in children with highest production of Amp C. *Klebsiella* spps showed highest production of ESBL. The prevalence of ESBL was 65.27% prevalence of AmpC was 31.94% our hospital, which is significantly high. Simple disc tests can be used to detect ESBL and Amp C beta lactamases especially where resources for molecular detection are not available. Many of these beta-lactamases disseminate rapidly being plasmid mediated. Hence it is necessary that rapid detection of these enzymes should be done and immediate control measures should be implemented to prevent their dissemination.

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