

Detection of AmpC β -lactamase in clinical isolates of *Escherichia coli* among children

Noor-ul-Ain Jameel¹, Hasan Ejaz², Aizza Zafar³, Hafsa Amin⁴

ABSTRACT

Objective: The production of AmpC β -lactamases is one of the common mechanisms of β -lactam drugs resistance. AmpC β -lactamases produce resistance to various clinically important cephalosporins. The purpose of this study was to determine the frequency of AmpC β -lactamase producing *Escherichia coli* and its occurrence in different clinical samples.

Methodology: This cross sectional observational study was conducted during April 2011 to March 2012 in the Microbiology Department of The Children's Hospital and Institute of Child Health, Lahore. A total number of 20,257 pathological samples like blood, cerebrospinal fluid (CSF), urine, pus, endotracheal tube (ETT), pleural fluid, sputum and urinary catheters were analyzed during the study period. *E. coli* were identified using API 20E system and resistance to cefoxitin was used as a screening test followed by disk potentiation as confirmatory test for AmpC β -lactamases.

Results: *E. coli* were isolated in 670 samples out of which 85 (12.6%) were AmpC β -lactamase producers. The gender distribution of patients with AmpC β -lactamase producing *E. coli* was 52 (61.2%) in males and 33 (38.8%) in females. The occurrence rate of AmpC β -lactamase producing *E. coli* in clinical samples was highest in blood 45 (52.9%).

Conclusion: The present study shows moderately high frequency of AmpC β -lactamase producing *E. coli* which may increase morbidity and mortality among children. Earlier detection of AmpC β -lactamases will decrease the morbidity rate of AmpC β -lactamase producing *E. coli* infection.

KEY WORDS: AmpC β -lactamase detection, *Escherichia coli*, children.

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INTRODUCTION

AmpC β -lactamase represents a group of β -lactamases found in various enteric bacteria.¹ AmpC β -lactamases are not inhibited by β -lactamase inhibitors like clavulanic acid and are clinically significant because they resist to a wide variety of β -lactamase inhibitors including α -methoxy- β -lactam such as cefoxitin.^{2,3} These enzymes are mostly produced by *Escherichia coli*, *Klebsiella species* and other Gram-negative bacteria.⁴

E. coli strains can cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhea, urinary tract infections, septicemia and neonatal meningitis.⁵ AmpC producing *E. coli* are resistant to clinically important cephalosporins and this is a major cause of public health concern because

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resistance is increasing both in community acquired and nosocomial infections.^{6,7}

There is no standardized phenotypic method for screening of AmpC β -lactamases. It is assumed that prevalence and occurrence of AmpC producers is underestimated partially because the Clinical and Laboratory Standard Institute (CLSI) does not give any particular recommendation for their detection. Presently, the procedures which have been recommended by the CLSI to detect AmpC producing strains involve an initial 3-dimensional test with a cefoxitin disk.⁸ AmpC activity can be detected when screening antibiogram indicates that activity of cefoxitin and third generation cephalosporin (excluding cefepime) is reduced.⁹ One method for detecting AmpC β -lactamase is inhibitor based method using boronic acid which has been reported to be an effective inhibitor of AmpC β -lactamase.¹⁰

The aim of this study was to determine the frequency of AmpC β -lactamase producing *Escherichia coli* and its occurrence in different clinical samples.

METHODOLOGY

This cross sectional observational study was conducted at the Microbiology Department of The Children's Hospital and Institute of Child Health Lahore, Pakistan, from April 2011 to March 2012. A total number of 20, 257 pathological samples like blood, urine, CSF, pus, ETT, pleural fluids, sputum and urinary catheters were collected from various wards. The samples were cultured on solid media as MacConkey and Blood agar. Cystine Lysine Electrolyte Deficient Medium (CLED) was used only for urine cultures. *E. coli* were identified by colonial morphology, Gram's stain, catalase test, oxidase test and API 20E system (bioMerieux). A seven digit number generated on the basis of various biochemical reactions of API 20E system was checked by API 20E software to confirm *E. coli*.¹¹

A suspension of each bacterial strain was made according to the 0.5 McFarland turbidity standard and an even lawn of bacteria was made on Muller Hinton agar 90mm petri plate. Cefoxitin was used as screening test for AmpC β -lactamase production. In

accordance with the CLSI criteria isolates resistant to cefoxitin were selected for further processing by disk potentiation method using 3-amino phenyl boronic acid (APB).^{12,13} To perform this method 300 μ g of APB was added to the 30 μ g commercial disks of ceftazidime and cefotaxime. The disks of ceftazidime and cefotaxime were placed on Muller Hinton agar plate along with and without APB at a distance of 30mm and incubated for overnight at 37°C. The diameter of zone of inhibition around the ceftazidime and cefotaxime disks with APB was compared with that of ceftazidime and cefotaxime disks without APB for the confirmation of AmpC β -lactamase production. An increase of ≥ 5 mm in zone diameter of disk with APB was interpreted as positive result for AmpC β -lactamase production.

RESULTS

E. coli were isolated from 670 samples while the rest of samples showed no growth or growth of other bacterial species. AmpC β -lactamases were detected in only 85 (12.6%) isolates of *E. coli*, while 585 (87.3%) isolates were non AmpC producing strains (Table-I).

The number of AmpC β -lactamase producing *E. coli* isolated from male and female patients was 52 (61.2%) and 33 (38.8%), respectively. High numbers of AmpC β -lactamase producing *E. coli* were isolated from blood 45 (52.9%) while less numbers were isolated from other clinical samples (Table-II). Information regarding ward location was also collected for 85 AmpC β -lactamases producing *E. coli* isolates: 37 from Neonatal Nursery Unit, 13 from Medical Care units, 7 from Intensive Care Unit, 5 from Emergency Ward, 3 from Oncology Ward, 2 from Outpatient Unit, 2 from Surgery Ward and 12 from other units.

Table-II: Site of isolation of AmpC β -lactamase producing *E. coli*

Site of isolation (n/%)	No of AmpC producing <i>E. coli</i>	
	Males	Females
Blood (45/52.9)	35	10
Urine (21/24.7)	8	13
Pus (6/7)	2	4
CSF (3/3.5)	2	1
ETT (3/3.5)	2	1
Urinary Catheters (3/3.5)	1	2
Sputum (2/2.4)	1	1
Pleural Fluids (2/2.4)	1	1
Total (85)	52	33

Table-I: Frequency of AmpC β -lactamase strains among *E. coli*.

<i>Escherichia coli</i> (n=670)	Number	Percentage
AmpC β -lactamase	85	12.6
Non AmpC	585	87.3

DISCUSSION

AmpC β -lactamase producing organisms are major clinical concern because they are usually resistant to all β -lactam drugs, except for cefepime, ceftiofene and carbapenems.^{14,15} Detection of any type of AmpC β -lactamases is a challenge to the clinical microbiologists. There are no guidelines in place for efficient detection of AmpC β -lactamases by CLSI. The accurate detection of AmpC β -lactamases is important to improve the clinical management of infection and to collect sound epidemiological data.¹⁶ In our study frequency of AmpC β -lactamase producing *E. coli* is 12.6%. A study carried out at a tertiary care hospital in Rawalpindi reported a high frequency of 45% AmpC β -lactamase producing *E. coli*.¹⁷ A study conducted in India reported 58.5% frequency of AmpC β -lactamase producing *E. coli* by boronic acid disk method.¹⁸ High frequency of 43.6% AmpC producing *E. coli* was also reported in a study at Medical Centers in Taiwan.¹⁹ In another study from US veterans medical centers reported only 13 (1.6%) AmpC β -lactamases out of 683 *E. coli* isolates.²⁰ It seems that the frequency of AmpC β -lactamase producing *E. coli* varies in different regions of world.

The frequency of AmpC β -lactamases producing *E. coli* was 61.2% in males and 38.8% in females. The rate of incidence of AmpC β -lactamases producing *E. coli* was found to be higher in males as compared to females. A study from Canada reported high frequency of AmpC β -lactamases producing *E. coli* infections in females 78%.²¹ The reason for this is not known but it could be concluded that isolation of AmpC β -lactamases producing bacteria is not gender dependent.

Occurrence of AmpC β -lactamase producing strains of *E. coli* was different in various specimens. In present study, high occurrence of AmpC β -lactamase producing *E. coli* was found in blood samples (52.9%) followed by urine (24.7%). Mulvey et al reported AmpC β -lactamase producing *E. coli* strains in a high rate from urine (77.5%) than the blood (8.6%) samples while the rest of samples like wound (5.7%), abscess (0.47%), tracheal secretions (4.6%) and others (2.8%) showed less occurrence.²² Similarly, AmpC β -lactamase producing *E. coli* were isolated in a high frequency from urine (78.5%) in a study carried out in Canadian hospitals.²¹ In another study carried out in 5 children hospitals of China reported occurrence of AmpC producing strain of *E. coli* in various samples like sputum 49.4%, urine 32.7%, blood 10% and respiratory secretions 10%.²³

These studies showed high occurrence of AmpC β -lactamase producing *E. coli* from urine while in our study AmpC β -lactamase producing *E. coli* were isolated in high number from blood. According to our results high number of AmpC β -lactamase producing *E. coli* were isolated from neonates who are usually at high risk of bacteremia. The highest frequency of AmpC β -lactamase producing *E. coli* from blood in our setup shows high occurrence of bloodstream infections than urinary infections.

We detected moderately high number of AmpC β -lactamase producing *E. coli* using disk potentiation which is a simple and reliable method for detection of AmpC β -lactamases. In conclusion, Microbiology laboratories can play an important role in earlier detection of AmpC β -lactamase producing organisms which could significantly reduce the risk of treatment failure and can help to generate sound epidemiological data. Measures should be taken to stop the spread of AmpC producing strains among the patients and hospital environment.

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