

Original Research Article

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Assessment of Amp C Beta-lactamase Production by Various Methods in Urinary Isolates of *Escherichia coli* from a Tertiary Care Teaching Hospital

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ABSTRACT

The current study was aimed to assess the percentage of Amp C Beta-lactamase and Extended spectrum beta-lactamase (ESBL) plus Amp C Beta-lactamase co-producer strains among urinary isolates of *E. coli* by disc diffusion method and to compare the evaluation of the AmpC detection by the phenotypic method using Phenyl Boronic acid and Tris-EDTA. A cross-sectional study conducted on a total of 200 non-repetitive, consecutive, urinary isolates of *E. coli*. The study was conducted in the Department of Microbiology of S.R.M. Medical College, Hospital and Research center, Kattangulathur. Out of the 200 isolates, a total of 47% strains were identified as the multi-drug resistant (MDR). The proportion of ESBL producing strains were 41%. Further, among the 84 clinical isolates subjected to evaluation for AmpC production, 52.38% were positive for AmpC production and 45.23% isolates were ESBL and AmpC co-producers, respectively. Of these AmpC producers (n=44), 26 (59.09%) and 18 (40.90%) isolates were identified by phenylboronic acid and TRIS-EDTA methods, respectively. Extended spectrum beta-lactamase, AmpC Beta-lactamase production, and its coexistence does exist among MDR strains of *E. coli*. Further, this study concludes that phenylboronic acid method could be an efficient alternative for the detection of AmpC.

Keywords

AmpC Beta-lactamase, Phenylboronic acid, *Escherichia coli*, Multidrug resistance.

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Introduction

The production of β -lactamase is the principal mechanism by which Gram-negative bacteria resist the action of β -lactam antibiotics (Paniagua-Contreras *et al.*, Sood *et al.*, 2012). In recent decades, there has been an alarming rise in infections caused by bacteria that express extended-spectrum β -lactamases (ESBLs) (Sood *et al.*,

2012; Mittal *et al.*, 2014). Many Enterobacteriaceae organisms also produce broad-spectrum cephalosporinases (AmpC) by intrinsic chromosomally encoded mechanism (Harris *et al.*, 2015). But recently, high-level Amp C AmpC production is also increasingly being reported in new species like *Escherichia coli* via plasmid acquisition (Jaurin *et al.*, 1981;

Tracz *et al.*, 2007). This Plasmid-mediated Amp C AmpC can coexist with ESBL enzymes in the same host (Taneja *et al.*, 2008; Tracz *et al.*, 2007). These ESBL and Amp C AmpC co-producers pose a special challenge to susceptibility testing as they make interpretation of various assessment methods less reliable and increases the probability of false susceptibility reporting to beta-lactamases (Harris *et al.*, 2015; Paltansing *et al.*, 2015; Yang *et al.*, 2007).

Even though various laboratory methods have been evolved in recent years, the validity and reliability of these methods are quite variable. No standard guidelines have evolved to recommend any particular method in a given setting (Manoharan *et al.*, 2012; Hassan *et al.*, 2013). Considering the wide geographical variation in the occurrence of Amp C AmpC production and resource availability, it is of vital importance to identify simple, quick, valid and cost-effective laboratory methods suitable for resource-limited settings like India (Tracz *et al.*, 2007; Ahmed *et al.*, 2015).

Several methods have been proposed to assess the Amp C AmpC production by microorganisms. These methods broadly include disc diffusion methods, agar based agar-based methods like cefoxitin agar method (Gupta *et al.*, 2012; Handa *et al.*, 2013). phenotypic inhibitor based methods like phenyl boronic acid (Hassan *et al.*, 2013; Sageerabano *et al.*, 2015; Shanthi *et al.*, 2012). and the three-dimensional extraction test (3DET) (Kumar *et al.*, 2014).

The validity and reliability of these tests are quite variable and often put the clinical microbiologist in a dilemma in recommending appropriate anti-microbial

agents key β -lactam resistant isolates, especially third-generation cephalosporins (Drinkovic *et al.*, 2015).

Considering the lack of proper recommendations and scarcity of the studies in this vital area and also the ever increasing incidence of multidrug-resistant multidrug-resistant strains of *E. coli*, the present study has been undertaken to fill this knowledge gap.

Materials and Methods

The study was a cross-sectional study conducted on a total of 200 non repetitive non-repetitive, consecutive, urinary isolates of *E. coli*. The study was conducted in the Department of Microbiology of S.R.M. Medical College, Hospital and Research centre, Kattangulathur. The study was conducted between the period of January 2011 to January 2012. Initially, all the samples were subjected to wet mount and Gram Staining. Then samples were cultured on Cysteine Lactose Electrolyte Deficient Agar (CLED) and standard biochemical tests were done for identification of *E. coli*. The identified isolates were subjected to antibiotic susceptibility testing as per CLSI guidelines using ATCC *E. coli* 25922 strain.

All strains, which were resistant to the second generation Cephalosporin were screened for extended spectrum beta-lactamase (ESBL) production by disk diffusion method using Cefoxitin (30 μ g), Ceftriaxone (30 μ g), Ceftazidime (30 μ g), Ceftazidime/Clavulanic acid (30/10 μ g), on Mueller Hinton Agar. The reference zone for Ceftriaxone <25mm, for Ceftazidime <22mm and \leq 27mm for Ceftazidime/Clavulanic acid, according to CLSI guidelines.

All the second generation Cephalosporin resistance isolates were screened for Amp C, beta-lactamase disk diffusion method using Cefoxitin (30µg), Ceftazidime (30µg), Ceftazidime/Clavulanic acid (30/10 µg). The diameter of a zone of inhibition was noted and interpreted according to the CLSI guidelines. All these strains were also assessed for ESBL and Amp C co-production by disc diffusion method.

All isolates, which were shown to be Amp C producers in disc diffusion method were assessed for Amp C production by the phenotypic method, using phenyl boronic phenylboronic acid. Peptone water suspension of clinical isolates equivalent to 0.5 Mc Farland Standard was prepared. A sterile swab was dipped into the suspension and inoculated (lawn culture) on the Mueller-Hinton Mueller-Hinton Agar was made. Plate surface was allowed to dry.

A disk containing 30µg of Cefoxitin and another Cefoxitin disc (30µg) impregnated with 400µg of Phenyl Boronic Phenylboronic Acid were placed on the agar. Plates were incubated at 37°C for 18-24 hours. Isolates demonstrating a zone diameter around the disk containing Cefoxitin and Boronic Acid ≥ 5 mm than the zone diameter around the disk containing Cefoxitin alone were considered as Amp C producer.

Subsequently, Amp C producing isolates were assessed for Amp C production by using Tris EDTA-Amp C disk test. A Lawn culture of *E. coli* ATCC 25922 was prepared on Mueller Hinton Agar plate. Sterile disk (6mm) was moistened with Tris-EDTA Tris-EDTA (20µl) and inoculated with several colonies of the test organism.

The inoculated disk was then placed beside a Cefoxitin disk (almost touching) on the

inoculated plate. The plates were incubated at 35°C for 18-24Hrs. A positive test appeared as the flattening or indentation of the Cefoxitin inhibition zone.

Results and Discussion

A total of 200 isolates of *Escherichia coli* which were consecutive, non-duplicate samples isolated were included in the study. The age distribution of isolates showed that all age groups are being affected by *E. coli*. The proportion of participants in the paediatric age group (below 15 years) was 6%, in adolescent and adult age group up to 45 years was 49.5%. About 2.5% of participants were between 46 to 60 years and the remaining 225 were above 60 years.

The proportion of females (73%) was much higher than the proportion of males (27%) (Table 1).

Out of the 200 isolates, the proportion of multidrug resistance strains) resistant to third generation cephalosporin was 47% (95% CI 40.08% to 53.92%). The proportion of ESBL producing strains was 41% (95% CI 34.18% to 47.82%) (Table 2).

Among the 84 clinical isolates were subjected to evaluation of Amp C production by disc diffusion method, 52.38% (95% CI 41.7% and 63.06%) were positive for Amp C production and 45.23% (95% CI 34.59% to 55.87%) isolates were ESBL and Amp C co-producers (Table 3).

All the 44 Amp C producers by disc diffusion method were evaluated by phenylboronic acid and TRIS-EDTA methods to confirm Amp C production. The phenylboronic acid method has identified 26 (59.09%) as Amp C producers and TRIS-EDTA method has identified 18 (40.90%) isolates as Amp C producers (Table 4).

Table.1 Socio-demographic profile of study population (n=200)

Age group	Frequency	Percent
I. Age group		
Up to 15 years	12	6.0
16 to 45 years	99	49.5
46 to 60 years	45	22.5
Above 60 years	44	22.0
Total	200	100.0
II. Gender		
Male	54	27%
Female	146	73%

Table.2 Description of ESBL and Amp C AmpC production among the clinical isolates (n=200)

Parameter	Frequency	percentage	95% CI	
			Lower	Upper
Number of Multidrug-resistant stains	94	47%	40.08%	53.92%
Number of ESBL producing strains	84	41%	34.18%	47.82%

Table.3 Description of ESBL and Amp C AmpC production among the clinical isolates (n=84)

Parameter	Frequency	percentage	95% CI	
			Lower	Upper
Number of strains producing AmpC by disk diffusion method	44	52.38%	41.7%	63.06%
Number of ESBL and AmpC co-producers by disk diffusion method	38	45.23%	34.59%	55.87%

Table.4 Description of ESBL and Amp C AmpC production among the clinical isolates (n=44)

Parameter	Frequency	percentage
Number of AmpC producing strains by Phenyl boronic acid	26	59.09%
Number of strains producing Amp C by TRIS -EDTA	18	40.90%

In recent times, many studies across the globe and India have reported a sharp rise in ESBL producing strains of *E. coli*. But there are wide variations across the settings in the reported prevalence of ESBL producing strains. Of late various studies across the world and also in India have reported a substantial increase in ESBL producing strains of *E. coli*. But in the prevalence of ESBL producing strains it has been reported that there are wide variations across the settings. Recently a study was carried out in a tertiary care teaching hospital in south India, and we found out in our study that there was a high percentage of ESBL isolates (41%), but prior to our study some reporters have shown the rate of 23.83% by Sood *et al.*, (2012). 34.42% by Akram *et al.*, 38.5% by Soltani *et al.*, and 31.3% by Jadhav *et al.*, they have reported to be slightly lower when compared to the present study. Whereas some studies of Bajpai *et al.*, Grover *et al.*, etc. have reported to have a similar proportion (41.6% and 42.2%, respectively) of ESBL among urinary isolates of *E. coli*.

On the contrary, a much higher proportion of ESBL ranging from 53.4 % have been reported by many other authors like by Ahmed *et al.*, two-thirds by Eshwarappa *et al.*, But on the whole, an important fact is being insisted by most of these studies is that a higher resistance pattern among these ESBL producing strains have been evolved when compared to non-ESBL producing strains.

The proportion of Amp C producers was 52.38% (41.7% and 63.06%) among ESBL producing strains 4 and 45.23% (34.59%) were ESBL and Amp C co-producers have been reported in the current study. Grover *et al.*, have reported coexistence of ESBL and Amp C Beta-lactamase producers was detected in 9.9% (26/262) of the isolates. Only 9.2% of isolates to be Amp C producers and most of them to ESBL and Amp C co-producers have been reported by Hemalatha *et al.*, in their study. By disc diffusion method, Phenylboronic acid and TRIS- EDTA yielded 59.09% and 40.90% positive results for Amp C production among 44 Amp C producers have reported Prevalence of Amp C production by inhibitor based method using boronic acid (IBM) at 40% and 39% and modified three-dimensional test (M3D) respectively have been reported by Handa *et al.*, Hassan *et al.*, by standard three-dimensional enzyme extract assay have reported the proportion of Amp C producers to be 62.8%, and also concluded that among various methods tested, boronic acid disk test to be was highly sensitive (88%) and specific (92%), compared to other methods. Saad *et al.*, evaluated the sensitivity and specificity of disc disk approximation test compared to three-dimensional extract and reported a sensitivity of 88% and specificity of 92%, with an overall diagnostic accuracy of 90.24%. Thus a routine implementation of disc disk approximation methods for the evaluation of Amp C production is

recommended pertaining to these finding findings.

In conclusion, simple methods to detect Amp C production, such as disc diffusion method and phenylboronic acid method are useful in identifying Amp C producing strains of *E. coli*.

The yield of these simple methods is comparable to more complicated methods like TRIS-EDTA.

Recommendations

Assessment of Amp C production by simple methods like phenylboronic acid should be part of routine evaluation in assessing antibiotic susceptibility of Multidrug-resistant strains of *E. coli*. This can minimize the chances of false susceptibility reporting and can have a huge positive impact on treatment outcomes.

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