

Original Research Article

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AmpC Beta Lactamase Detection in *Escherichia coli* and Comparison of Two Phenotypic Methods

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ABSTRACT

Increasing drug resistance due to the production of AmpC β -lactamases and Extended spectrum β -lactamases (ESBLs) is an increasing cause of concern as it has left the clinicians with limited therapeutic options. Aims were detection of AmpC β lactamase in *E. coli* an comparison of the two phenotypic tests- AmpC disk test and Inhibitor based test using phenyl boronic acid. A total of 156 *E. coli* isolates from various clinical samples were collected for the study. These isolates were further subjected to ESBL detection by disc diffusion test, screening for AmpC β -lactamases by cefoxitin disc and confirmation of AmpC β -lactamases by AmpC disc test and Inhibitor based test using phenyl boronic acid. Statistical analysis done by Chi-Square test and Fisher's Exact test. AmpC screening detected 68 isolates as presumptive AmpC producers, of which 82.4% were confirmed to be true AmpC producers by confirmatory tests. Phenotypic confirmation by AmpC disc test and Inhibitor based test detected 20.5% and 35.9% isolates respectively as AmpC producers. Coexistence of AmpC with ESBL was seen among 32(20.5%) isolates. Pure AmpC producers were 15.4%. Pure AmpC producers as well as co-producers of ESBL and AmpC showed multidrug resistance. Pure AmpC producers showed 100% sensitivity to cefepime and imipenem while co-producers showed only 25% sensitivity to cefepime and 87.5% sensitivity to imipenem. The prevalence of AmpC producers among *E. coli* in our study was 35.9%. Pure AmpC producers are 100% susceptible to cefepime and imipenem. The Inhibitor based test is a simple, efficient and better test for the detection of AmpC production.

Keywords

AmpC β lactamase,
Inhibitor based test,
AmpC disc test.

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Introduction

The increasing use of broad spectrum antibiotics has resulted in the development of bacterial resistance. The favourable outcome of common infections in community and hospital settings is threatened by emerging resistant bacteria. The most important single mechanism of resistance to penicillin and cephalosporins in gram positive and gram negative organisms is the production of β lactamase enzymes (Chaudhary *et al.*, 2004).

Extended spectrum β lactamases and AmpC β lactamase production by bacteria are also emerging as the current cause of concern (Singhal *et al.*, 2005). AmpC β lactamases are cephalosporinases that are poorly inhibited by clavulanic acid (Singhal *et al.*, 2005; Sinha *et al.*, 2008). Differentiated from ESBL by their ability to hydrolyse cephamycins as well as other extended spectrum cephalosporins.

Major risk factors for colonization or infection with ESBL, AmpC producing organisms are long term antibiotic exposure, prolonged hospital or ICU stay, nursing home residency, severe illness, residence in institution with high rates of ceftazidime and other 3GC use and instrumentation or catheterization.

For clinical microbiologists detection of AmpC mediated resistance in gram negative organisms poses a challenge as there are no standard guidelines for the detection of this resistance mechanism. Clinical labs need to address this issue as much as detection of ESBL, since they may co-exist and mask each other (Hemalatha *et al.*, 2007). Screening with cefoxitin disc recommended for initial detection (Akujobi *et al.*, 2012). Some phenotypic tests include three dimensional test, AmpC disc test, E test strips (Getzlaff *et al.*, 2011), inhibitor based tests using boronic acid.

However, phenotypic tests cannot distinguish among various families of plasmid mediated AmpC β lactamases with an extended spectrum. For these purposes, the current gold standard for plasmid mediated AmpC β lactamase detection is multiplex PCR (Jacoby, 2009).

This study is undertaken to detect AmpC β lactamase in *Escherichia coli* based on two phenotypic tests, the AmpC disk test and inhibitor based method using boronic acid and the comparison of these two methods.

Materials and Methods

It was a prospective study of all *E. coli* isolates from the relevant clinical samples of in-patients received in the department of Microbiology over a period of one year from January 2012 to December 2012. A total of 156 consecutive, non-repetitive samples of *E.coli* were collected for the study.

All samples were processed as per standard procedure (Winn *et al.*, 2006; Crichton, 2006). Gram staining was done to observe for pus cells and gram negative bacilli. Culture was done on MacConkey agar and blood agar. All cultures were incubated at 37⁰C for 24 hours.

Next day, the organism was identified by colony morphology, Gram stain, motility test and basic routine biochemical reactions using standard laboratory procedures.

Antimicrobial susceptibility test was carried out by modified Kirby-Bauer disk diffusion method as per current CLSI guidelines. The antibiotic susceptibility profiles against gentamicin, co-trimoxazole, cefoxitin, ceftazidime, ceftriaxone, cefepime, imipenem were studied (Clinical and laboratory standard institute, 2007).

AmpC screening was done using cefoxitin disc. A zone of inhibition of >18mm was taken as susceptible. Cefoxitin resistant *E. coli* were considered as probable AmpC producers. Confirmation of AmpC production was by the confirmatory tests, the AmpC disc test and inhibitor based test with phenyl boronic acid (Coudron *et al.*, 2000).

Phenyl boronic acid (PBA) solution was prepared by dissolving 120g of phenyl boronic acid in 3ml dimethyl sulfoxide and 3ml of sterile distilled water. 20 μ L of this solution was added on to a cefoxitin disc and kept for drying for 30 minutes. Mueller Hinton Agar was inoculated with a 0.5 McFarland turbidity suspension of test strain. Disc containing cefoxitin (30 μ g) and cefoxitin with PBA discs was placed on the agar. After overnight incubation in air at 37⁰C, the zone of inhibition was measured. A difference in zone size of >5 mm between the disc containing cefoxitin with PBA compared to the disc containing cefoxitin alone was

considered indicative of AmpC production (Philippon *et al.*, 2002; Yagi *et al.*, 2005; Pitout *et al.*, 2010).

AmpC disk test is based on the use of Tris-EDTA which permeabilises the cell to release beta lactamases. Tris-EDTA discs were prepared by adding 20 µL of a 1:1 mixture of saline and 100X Tris-EDTA solution. The Mueller-Hinton agar plate was inoculated with a lawn culture of cefoxitin - susceptible *E. coli* ATCC 25922. A cefoxitin disc was placed on the agar. Tris-EDTA disc inoculated with several colonies of test organism was placed almost touching the cefoxitin disc. The plate was then incubated overnight at 37°C. An indentation or flattening of zone of inhibition indicated inactivation of cefoxitin. Absence of a distortion indicated no significant inactivation of cefoxitin.

Results and Discussion

Among the 156 isolates of *E. coli*, AmpC β lactamase production was seen in 56(35.9%) isolates.

AmpC screening test for 156 isolates, 68(43.6%) showed reduced susceptibility to cefoxitin with zone size <18mm. Therefore, 68(43.6%) isolates were considered as presumptive AmpC producers.

AmpC screen test showed 68(43.6%) isolates to be positive for AmpC production. Confirmatory tests done on these showed 56(82.4%) to be AmpC producers.

The confirmatory tests done were the AmpC disc test and the inhibitor based test. AmpC disc test showed 32 of the screen positive isolates to be AmpC producers whereas inhibitor test showed 56 of 68 to be AmpC producers. All the 156 isolates were also subjected to confirmatory tests of which

AmpC disc test detected 20.5% and inhibitor test detected 35.9% of the AmpC producers.

Comparison of the confirmatory tests for AmpC detection was done by two methods. This showed that all isolates that were positive by AmpC disc method were also positive by inhibitor method whereas inhibitor based method showed 56 isolates to be AmpC producers.

All AmpC producers were showing multiple drug resistance, 100% resistance was seen with amoxicillin-clavulanic acid, ceftazidime, ceftriaxone and piperacillin. Susceptibility was high with cefepime and imipenem, 48(85.7%) sensitivity was seen to cefepime and 44(78.6%) to imipenem (Figure 1).

Despite the discovery of ESBL and AmpC β-lactamases atleast a decade ago, there remains a low level of awareness of their importance. Many clinical labs have problems in detecting ESBLs and AmpC β-lactamases.

Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions. Inappropriate use of cephalosporins in clinical practice has led to the increased prevalence of ESBL and AmpC enzymes among gram negative bacteria (Shoorashetty *et al.*, 2011).

In this study of 156 isolates of *E. coli*, 56(35.9%) were detected as AmpC producers (Figure 1). Similar results were reported in studies by Shubha (37.5%), Sinha *et al.*, (37.5%) and Peter Getzlaff *et al.*, (41%) among *E. coli* isolates.

Varying results in other studies, 7% by Singhal *et al.*, 52.2% by Rudresh (2011), 76% by Shanti (2012), showed the prevalence of AmpC production among *E. coli* isolates.

Based on screening test with cefoxitin disc, we identified 68(43.6%) isolates as possible AmpC producers. These 68 isolates were further subjected to confirmatory tests for AmpC production. Fifty six (82.4%) were confirmed to be AmpC producers by confirmatory tests (Table 1 and 2). This indicates that the screening test has good specificity.

In a study by Rajini and others (2008) 226(80%) isolates were found to be cefoxitin screen positive but only 165 (58.5%) isolates were confirmed to harbour AmpC enzyme. Maximal incidence of AmpC production was found among *E. coli* (70%) followed by *K. pneumoniae* (56.7%).

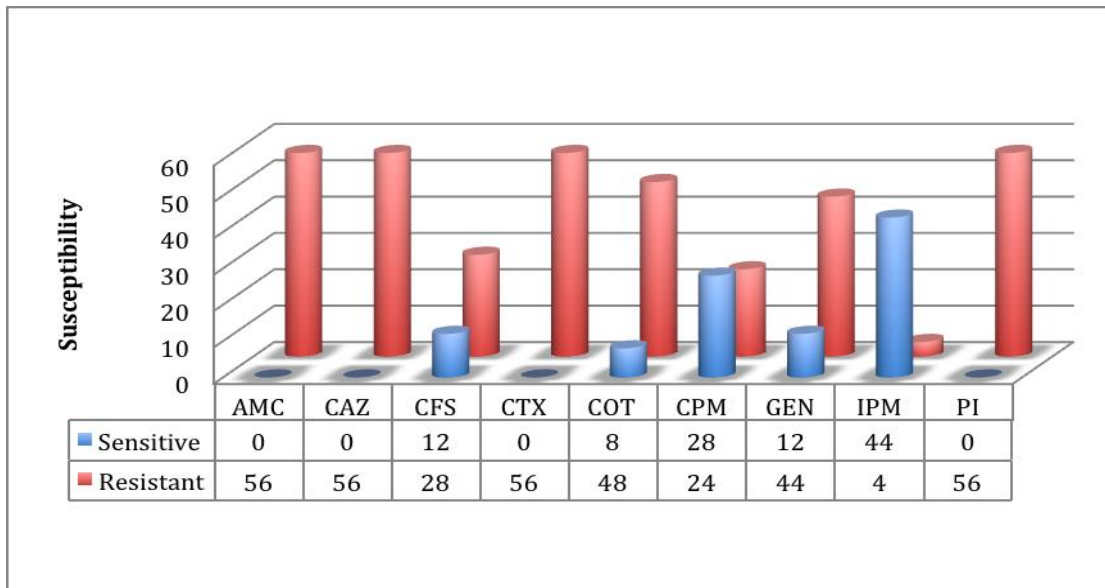
Table.1 Results of AmpC screening test

AmpC Screen	Frequency (%)
Positive	68 (43.6)
Negative	88(56.4)
Total	156(100)

Table.2 AmpC detected by AmpC screen

AmpC screen results	No.
AmpC screen positives	68
Confirmed AmpC producers	56
AmpC negatives	12

Figure.1 Bar diagram for antibiotic susceptibility among 56 AmpC producers



Study done by Rudresh (2011) 80(93.02%) isolates with cefoxitin resistance were AmpC producers.

Sometimes cefoxitin screen negative organisms also harbour AmpC β -lactamase. Manchanda *et al.*, (2003) reported 39% as

AmpC producers which were screen negative. Sowe subjected all 156 isolates to the confirmatory tests irrespective of the results of the screening tests.

Increasing incidence of drug resistance highlights the need to establish newer simple and effective methods for its detection like the inhibitor based test for the detection of AmpC beta lactamase production.

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