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ORIGINAL ARTICLE

AmpC Beta Lactamases Among ESBL Producing Escherichia Coli And Klebsiella Pneumoniae- If You Don't Look, You Won't Find.

VANDANA K E *, HONNAVAR P**

ABSTRACT

Amp C beta lactamases confer resistance to a wide variety of beta lactams and pose both diagnostic and therapeutic challenges, as their presence goes undetected in the presence of ESBLs. We evaluated 52 ESBL positive clinical isolates of E.coli (n=41) and K.pneumoniae (n=11) for AmpC production by a phenyl boronic acid disc (PBA) and the 3 dimensional enzyme test (3DET). The PBA method detected 24 (58.5%) and 9 (82%) E.coli and K.pneumoniae isolates as AmpC positive, while detection by the 3DET increased the numbers to 29 (70.7%) and 10 (91%) isolates, respectively. We conclude that a large number (75%) of ESBL producers are also found to produce AmpC and that the PBA disc method is a very useful and reliable method for routine use in laboratories.

Key Words: Amp C beta lacatamase, ESBL, phenyl boronic acid, 3 dimensional enzyme test.

* (MD)Asso. Prof, **Final Yr MSc student Dept of Microbiology, Kasturba Medical College, Manipal, Udupi – 576104 Karnataka. (India)
Corresponding Author
Dr.K.E.Vandana, (M.D.)Asso.Prof.
Dept. of Microbiology, Kasturba Medical College, Manipal, Udupi, 576104, Karnataka, (India). Ph : 0820-2922322, 9902206009
Email:vandanake@yahoo.co.in

Introduction

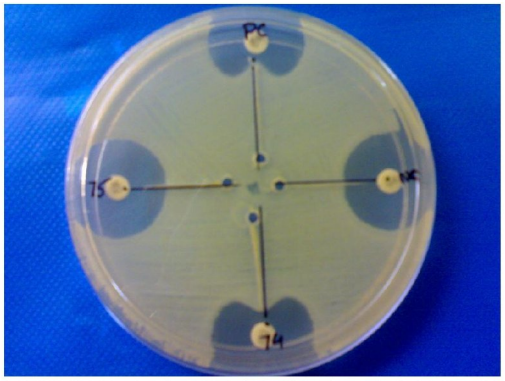
The production of extended spectrum beta lactamases (ESBLs) has been reported in virtually all species of Enterobacteraceae, which greatly complicates the therapy for infections caused by these organisms. However, the frequency of the isolates producing AmpC beta lactamases is largely unknown due to difficulties in the phenotypic detection and confusion about the appropriate reporting convention [1],[2]. Amp C beta lactamase is often misidentified as ESBL. Detecting Amp C isolates is clinically important, not only because of their broader cephalosporin resistance, but also because carbapenem resistance can arise in such strains by further mutations, resulting in reduced porin expression [3],[4] Here, we studied the prevalence of Amp C beta lactamases in E.coli and Klebsiella isolates that produce ESBLs.

Materials and Methods

A total of 52 clinical isolates of ESBL producing E.coli and Klebsiella from a teriary care hospital were analyzed for the production of Amp C beta lactamase. ESBL production was tested by the disc approximation method using Co-amoxiclav and cefotaxime [5]. Amp C production was detected by the boronic acid disc enhancement method [6] and compared with the three dimensional enzyme test (3DET) as a gold standard [7]. In the former method, 2 cefoxitin discs (30µg) were placed on a Mueller Hinton Agar plate lawn inoculated with a 0.5 McFarland turbidity adjusted suspension of the test strain. To one of the discs, 400µg of phenyl boronic acid (Sigma-Aldrich) was added. After overnight

incubation in air at 37° C, the zones of inhibition were measured. Enhancement of 5mm of zone around a cefoxitin disc with PBA, in comparison with a disc with cefoxitin alone, was taken as a positive reaction for Amp C production.

For 3DET, crude enzyme extracts were prepared by freeze thawing centrifuged cell pellets of broth cultures. The enzyme extracts were then inoculated into wells on an MHA plate lawn inoculated with E.coli, ATCC 25922 and a cefoxitin disc was placed on it. Linear slits were made from the well directing towards the disc, 3mm away from it. The plates were read after overnight incubation at 37° C. Any enhanced growth of the indicator strain decreasing the radius of the cefoxitin inhibition zone at the end point of the slit, was considered as positive for Amp C [Table/Fig 1]



(Table/Fig 1) Amp C detection by 3DET method on a lawn culture of E.coli ATCC 25922Legends for Fig 1: PC- Known AmpC positive strain, NC- Amp C negative strain. 74- Test strain positive for Amp C. 75 – Test strain negative for Amp C.

Antimicrobial susceptibility of test isolates against aminoglycosides, cephalosporins, monobactums, beta lactam and beta lactamase inhibitor combinations, fluoroquinolones and carbapenems, were tested using the Kirbey Bauer disc diffusion method according to CLSI standards [8].

Results

Of the total 52 isolates, E.coli (n=41) and K.pneumoniae(n=11) were obtained from urine (50) and pus (2). Out of 41 E.coli strains, 24 (58.5%) strains were found to produce Amp C by the PBA disc method, while the 3DET method detected 5 more Amp C positive isolates (29, 70.7%). Of 11 K.pneumoniae strains, 10 (91%) strains were found to be positive by the 3DET method that detected one extra strain as compared to the PBA method (82%) [Table/Fig 2].All the 52 isolates were resistant to cefoxitin. The sensitivity and specificity of the PBA method were found to be 85% and 100% respectively, when compared to the 3DET method. AST results of these strains are given in [Table/Fig 3] . Meropenem was the only drug which was found to be effective against all these isolates, followed by cefoperazone sulbactum, piperacillin tazobactum and amikacin. Three isolates were susceptible to cefipime, all of which were positive for Amp C. All were uniformly resistant to fluoroquinolones. The overall prevalence of Amp C in ESBL producing isolates was 75%.

(Table/Fig 2) Comparison of two methods in Amp C detection.

Isolates (n)	PBA method n positive(%)	3DET method n positive (%)
E.coli(41)	24 (58.5)	29 (70.7)
K.pneumoniae(11)	9 (82)	10 (91)
Total (52)	**33 (63.5)**	**39 (75)**

(Table/Fig 3) Susceptibility results of Amp C positive isolat

Antimicrobial agent	Susceptible n (%)
Amikacin	13 (33)
Aztreonum	1 (3)
Cefipime	3 (8)
Cefoperazone sulbactum	29 (74)
Cotrimoxazole	4 (10)
Genatmicin	7 (18)
Netilmicin	10 (26)
Meropenem	39 (100)
Piperacillin-tazobactum	20 (52)

Discussion

Organisms overexpressing Amp C beta lactamases are a major clinical concern because these are usually resistant to all beta lactam drugs, except for cefepime, cefpirome and carbapenems [9], [10]. In contrast to ESBLs, they hydrolyse cephamycins and are not inhibited by beta lactamase inhibitors. Costitutive overexpression of Amp C occurs, either by deregulation through the mutation of the Amp R gene in the chromosome or by

acquisition of a transferable AmpC gene on a plasmid or on another transferable element commonly called as plasmid mediated amp C beta lactamase [10],[11]. The origin of Amp C in E.coli is chromosomal, although recently, plasmidic Amp C also has been isolated. K.pneumoniae harbours only plasmid mediated Amp C. Detection of any type of Amp C beta lactamase is a challenge to clinical microbiologists. There are no guidelines in place for efficient detection by CLSI guidelines. Phenotypic variations in the bacterial expression of the enzymes make the task of laboratory detection more complicated. Clinical implications of plasmid encoded amp C mediated resistance have to be addressed more cautiously. There are no guidelines for detection of this resistance mechanism and yet, there is as much need for clinical laboratories to address this issue as there is for the detection of ESBLs. The accurate detection of plasmid mediated amp C is important to improve the clinical management of infection and to provide sound epidemiological data [2].

There is a paucity of data from Indian laboratories on the coexistence of multiple beta lactamases in individual isolates. Studies from various parts of India have reported the prevalence of Amp C in clinical isolates of Enterobacteria as varying from 2.2% to 20.7 % [12], [13]. However, these studies were designed to estimate the prevalence of AmpC among all the clinical isolates of Enterobacteria. Our study highlights the importance of appropriate detection methods for Amp C enzymes in those isolates which are already designated to be ESBL positives, as the coexistence of different classes of beta lactamases in a single bacterial isolate poses a challenge, both in diagnosis and therapy. Use of a cefoxitin disc is useful in screening for Amp C. However, we observed that 13 cefoxitin resistant isolates did not produce Amp C, which may be attributed to other resistance mechanisms like porin channel alterations in these isolates. Though the isolates showed in vitro susceptibility to cefoperazone sulbactum and piperacillin tazobactum combinations, their clinical usefulness was doubtful as AmpC was not inbibited by beta lactamase inhibitors.

Conclusion

In the present study, we found a large number of ESBL producing E.coli and K.pneumoniae strains, which also produced AmpC beta lactamase (75%). Though the 3DET test is the gold standard for Amp C detection, it is labour intensive and cannot be done routinely on all clinical isolates. The PBA disc method is simple and reliable for detection of this resistance mechanism. Microbiology laboratories should proceed to detect multiple beta lactamases in individual isolates which are already designated as ESBL producers, so that the appropriate therapy can be chosen for patient management, and also so that sound data can be generated on resistance mechanisms and hospital infection epidemiology.

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