Detection of AmpC- β lactamase enzymes by AmpC disc test

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Microbes have many mechanisms of unresponsiveness to antimicrobial agents that may arise in them over months or years. Among them degradation of antibiotics by enzymes is one of the common methods of bacterial resistance particularly in *Enterobacteriaceae*.^{1,2} At least 400 different types of β -lactamases from clinical isolates have been discovered. Broadly they are of three types: extended spectrum beta lactamases (ESBLs), Amp C β -lactamases and carbapenemases. AmpC type beta lactamases are cephalosporinases and hydrolyze penicillins, cephalosporins, except the fourth-generation compounds and monobactams.^{3,4,5} AmpC β -lactamase producing bacterial pathogens may cause a major therapeutic failure if not detected and reported in time. In addition, plasmid-mediated AmpC β -lactamases are associated with false in vitro susceptibility to cephalosporins. Thus, β -lactamase producers should be detected routinely before administering β -lactam drugs to the patient. The benefits include better patient outcome and avoidance of inappropriate therapy.⁶ At present there is no gold standard to detect the presence of plasmid-mediated AmpC β -lactamases. Usually a cephamycin (cefotaxime) is used as a screening method since cephamycin-resistance is typical to AmpC β -lactamases. Although it is highly sensitive yet lacks specificity; as cefotaxime resistance points to many resistance mechanisms: porin loss, AmpC or metallo β -lactamase (carbapenemase) production. Strains with AmpC

β -lactamases are resistant to β -lactam/ β -lactamase inhibitor combinations a feature that differentiates them from ESBL's as long as there is no concomitant production of an ESBL.⁷

For phenotypic confirmation of the production of AmpC β -lactamases, tests commonly used are: three dimensional test, modified three dimensional test, modified three dimensional spot inoculation test, AmpC disc test using Tris-EDTA, Modified Hodge test, cefotaxime agar medium (CAM) test, double disc synergy test, E-test and inhibitor based test using boronic acid.⁸ Occasionally Modified Hodge test gives false negative results with AmpC producers that test positive with Tris-EDTA AmpC disc test, because Tris-EDTA makes the bacterial wall permeable leading to release of β -lactamase into the environment.⁹

In present study AmpC disc test was used to detect AmpC enzyme production. One hundred and fifty isolates that were resistant to cefotaxime and one or more third generation cephalosporin antibiotics or showed antagonism between cefotaxime and cefotaxime discs were subjected to AmpC disc test. Out of these, maximum were *E. coli* 75 (50%) followed by *Klebsiella* spp. 38 (25.3%), *Acinetobacter* spp. 25 (16.7%), *Enterobacter* spp. 7 (4.7%). Apart from these 2 (1.4%) isolates of *Citrobacter* spp. and *Pseudomonas* spp. each and 1 (0.7%) isolate of *Serratia* spp. were also present. These were further tested for AmpC beta lactamase production by AmpC disc test.

AmpC disc test detected 35 (23.3%) isolates to be AmpC positive. Out of these, 15 (42.9%) were *E. coli*, 10 (28.6%) *Acinetobacter*, 6 (17.1%) *Klebsiella*, 3 (8.6%) *Enterobacter* and 2 (5.7%) were *Citrobacter* species.

In AmpC beta lactamase positive isolates, 100% resistance

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was seen to ampicillin + salbactam, 88.9% of isolates were resistant to ceftriaxone, 82.2% to ceftazidime, and 97.8% of the isolates exhibited resistance to cefotaxime. Also 40% isolates were resistant to co-amoxiclav. Lower resistance pattern was seen for piperacillin + tazobactam (11.1%) and cefipime (22.2%).

For fluoroquinolones a higher resistance pattern was seen, with 100% isolates being resistant to ciprofloxacin, 66.7% being resistant to levofloxacin and 80% to ofloxacin. In addition 80% isolates were found resistant to gentamicin, 68.9% to amikacin. Co-trimoxazole resistance was seen in 71.1% isolates. However lower resistance patterns were seen for nitrofurantoin (18.2%), with none of the isolates being resistant to polymyxin-B and tigecycline.

Early detection of changing resistance patterns is very important in preventing the dissemination of resistant bacteria and modifying the treatment strategies. To prevent the spread of AmpC beta-lactamase producing strains, hospitals must have a robust infection control committee with appropriate hospital antibiotic policy that is updated regularly. Based on the results of this study, fluoroquinolones, gentamicin, amikacin, and cotrimoxazole need to be used cautiously in patients suspected of being infected with AmpC Gram negative bacteria as high level resistance was seen against these antibiotics or they should be used only if found susceptible. Beta lactam combinations like piperacillin + tazobactam and co-amoxiclav and cephalosporins like cefipime can go a long way in the management of these cases.

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