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Detection of AmpC β-lactamases production in *Acinetobacter* species by inhibitor (disk) based & modified three dimensional (enzyme extraction) methods

Sir,

Among the nosocomial infections caused by Gram-negative bacteria, the *Acinetobacter* spp. is one of the established[1] and predictable opportunistic pathogens in immunocompromised patients[2,3]. AmpC β-lactamases are class C or group I cephalosporinases and non susceptible to alpha methoxy β- lactams such as cefoxitin or cefotetan. The detection of these β-lactamases is clinically significant because these confer resistance to narrow, extended and broad spectrum cephalosporins as well as β-lactam/β-lactamase inhibitor combinations[4]. This study was undertaken to detect the presence of AmpC β-lactamase in clinical isolates of *Acinetobacter* species by two phenotypic methods.

A total number of 136 non repetitive cefoxitin resistant clinical isolates of *Acinetobacter* spp. were obtained during January to December 2012 in the microbiology laboratory, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India. The isolates were identified only to the Genus level and speciation was not done. The zone size ≤18 mm around the cefoxitin disc was used as a screening test for the presence of AmpC β-lactamase production[5].

All cefoxitin resistant isolates were studied for the presence of AmpC enzyme by the modified three dimensional method[5]. In this method three kinds of results were recorded. Isolates that showed clear distortion of zone of inhibition of cefoxitin were considered as strong AmpC producers. Isolates with no distortion were considered AmpC non producers and isolates with little distortion were considered as weak or intermediate AmpC producer.

The inhibitor (disc) based disc method[6] was performed to confirm the AmpC producers. The test culture was swabbed on Mueller-Hinton agar (Hi-media, Mumbai) plates and cefoxitin (30 μg) and cefoxitin/boronic acid (30/400 μg) discs were placed at a distance of 20 mm from centre to centre. An increase of >0.5 mm around cefoxitin/boronic acid compared to cefoxitin alone was considered positive for the presence of AmpC production[6].

Among the 136 isolates screened, 82 (60.29%) were positive for the AmpC β-lactamase production by the inhibitor (disc) method. Of the total 136 isolates, 84 (61.76%) were strong AmpC producers, 16 (11.76%) intermediate or weak AmpC producers, and 36 (26.47%) were negative for the AmpC producers by the modified three dimensional (enzyme extract) method.

The isolates harbouring AmpC β-lactamases are shown to be largely restricted to the hospitalized patients only[5,7,8]. The CLSI (Clinical and Laboratory Standards Institute) guidelines[9] do not indicate the screening and confirmatory tests for detecting AmpC β-lactamases in *Acinetobacter* species. The modified three dimensional[5] test is a confirmatory test for detecting both inducible and non inducible AmpC β-lactamases but is technically demanding. In case of inhibitor method using boronic acid with cefoxitin is simple, and this test is sensitive to detect the plasmid mediated AmpC β-lactamases[10,11] as well as similar to the ESBL confirmatory test[5]. Among the cefoxitin resistant Gram-negative isolates, Sasirekha[12] reported 20.4 per cent positive for AmpC production whereas Manoharan *et al*[13] reported 36.5 per cent positivity. In a study form Kolkata, 32.77 per cent of isolates were reported positive for ampC by Amp disk test[14]. Several other studies have also reported AmpC β-lactamase positive *Acinetobacter* spp.[14-17].

In this study, 84 (61.76%) and 82 (60.29%) isolates were determined as AmpC producers by modified three dimensional and boronic acid inhibitor methods, respectively. When the two phenotypic methods were compared, the inhibitor method failed to detect the presence of AmpC in only two isolates; 38.23 per cent of cefoxitin resistant isolates were negative for AmpC production by both methods. The resistance to cefoxitin can also be mediated by certain class A beta lactamses, carbapenemases and decreased production of outer membrane porins[18]. In a study from Turkey, more positives (89.76%) were observed by three dimensional method than by boronic acid disk test (85.03%)[19]. However, Bakthavatchalu *et al*[20] reported higher percentage of positives (93%) of AmpC producers by boronic acid inhibitor test than by the three dimensional method (91%). Lee *et al*[21] compared modified Hodge test with boronic acid test and EDTA disk test to evaluate the presence of AmpC beta lactamase and reported the combination-disk test with boronic acid as a sensitive and efficient test for detecting AmpC producers.

In conclusion, our findings suggest that the inhibitor based disc method can be used in routine clinical microbiology laboratories to confirm the presence of AmpC in *Acinetobacter* species.

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