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Correspondence

Challenges in detection of AmpC β-lactamases among *Enterobacteriaceae*

Sir,

We read with interest the study by Manoharan *et al*[1]. Detection of AmpC β-lactamases (AmpC) among clinical isolates remains a challenge, as there are no standard guidelines[2]. Cefoxitin resistance is suggestive of AmpC production, but it is not specific as resistance to cefoxitin can also be mediated by certain class A β-lactamases, carbapenemases and decreased production of outer membrane porins[3]. The three-dimensional extract test and phenylboronic acid test are widely used for detection of plasmid mediated AmpC. However, these methods have certain drawbacks. The three dimensional test is laborious, while the phenylboronic acid test lacks specificity as it can inhibit class A *Klebsiella pneumonia*e carbapenemase (KPC) β-lactamase besides AmpC[3]. Moreover, these phenotypic tests cannot differentiate the various families of plasmid-mediated AmpC enzymes and therefore, multiplex PCR has been developed[4]. Though we appreciate the efforts by Manoharan *et al*[1] to evaluate the phenotypic and genotypic methods for detection of AmpC among commonly encountered *Enterobacteriaceae*, some points need clarification:

(1) It is not clear why the authors have used *Pseudomonas aeruginosa* ATCC 27853 as a control strain for quality check of susceptibility testing of members of *Enterobacteriaceae*. Use of *E. coli* ATCC 25922 along with ESBL producing *Klebsiella pneumoniae* ATCC 700603 would have sufficed.

(2) The authors have mentioned that there was no significant difference in susceptibility of the AmpC producers and those that did not produce Amp C. However, as per the data in Table II, 56.6 per cent (112/198) of the AmpC negative isolates and 83.3 per cent (97/114) of the AmpC positive isolates were susceptible to amikacin ($P<0.0001$), suggesting that there is a significant difference between the two groups.

(3) It would be interesting to know the reason for the increased susceptibility of the AmpC positive isolates to amikacin. A note on use of amikacin in clinical practice would have shed some light.

It is mentioned in the results, that a very high proportion (92%) of the AmpC phenotypes was also found to be ESBL producers. But, production of AmpC β-lactamases is known to interfere with the confirmatory test for detecting ESBL producers using combinations of cephalosporins with clavulanic acid (beta-lactamase inhibitor)[5,6]. In an ESBL producing isolate which is also a co-producer of AmpC, the expected enhancement in zone diameter may often not be observed when the cephalosporin is tested in combination with clavulanic acid in the presence of AmpC, which is an inhibitor-resistant beta-lactamase. This inhibitor-based approach is generally considered unreliable for detection of ESBL production in isolates co-producing AmpC, as it is associated with high false negativity[5,6]. Contray to this, it is surprising to know that the authors have detected a high proportion of AmpC producers to be positive for ESBL production by the phenotypic confirmatory test.

(4) It would be useful to know the basis of labelling 79.4 per cent of AmpC producers to be of nosocomial origin.

(5) The phenylboronic acid-cefoxitin disc test has been reported by the authors to have a sensitivity of 72.9 per cent, specificity of 45.4 per cent, with positive predictive value of 49.2 per cent, and negative predictive value of 69.7 per cent, when compared with PCR. However, the authors have performed PCR for detection of AmpC genes only among the isolates positive by phenylboronic acid-cefoxitin disc test. Ideally, calculation of sensitivity and specificity must include PCR among the AmpC negative isolates also to determine the actual number of false negatives and true negatives.

(6) We would suggest that with only 42.1 per cent of strains assigned to genotype by the multiplex PCR described in this study[1], it is not an ideal tool for the detection of plasmid mediated AmpC genes, contrary to the authors' conclusion.

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