## Authors' response

Sir,

We thank Joseph and Mathias for their correspondence with reference to our recent publication[1] and provide below explanations to the queries raised.

(1) *Pseudomonas aeruginosa* ATCC 27853 has been used to quality check amikacin E test strip as per manufacturers E test product insert guidelines (AB bioMerieux, Solna Sweden). The above strain has been used as a negative control to quality check Triple Sugar Iron Agar (TSI) test[2] which is widely used to differentiate members of *Enterobacteriaceae*.

(2) The reason for the significant difference in amikacin susceptibility between cefoxitin resistant Amp C positive and negative strains is unclear and needs further elucidation. In general, in our multicentric study amikacin susceptibility across all centers remains high. This may be because it is used sparingly as a therapeutic option possibly due to its side effects (nephrotoxicity) and availability (lack of oral form). Further, in India due to high levels of ESBL mediated Gram-negative resistance in recent years[3], broad spectrum carbapenem use is preferred as the first empiric choice particularly in treatment of nosocomial infections.

(3) Our laboratory was the reference center for the multicentric ESBL surveillance study funded by Indian Council for Medical Research. All the study isolates were screened for ESBL production by disc diffusion[4] and results confirmed by E test ESBL strips, only such isolates were finally classified as ESBL positives. Further, all study isolates from blood infections were PCR tested for the presence of ESBL genes as per protocol published previously[5]. In our experience, we have found the concordance between the CLSI[4] recommended disc diffusion and E test to be very high (>95%) with excellent correlation to the PCR based approach for confirming ESBL presence among all study isolates including the ESBL-AmpC co-producers.

(4) In this study, nosocomial infection was defined as infection that occurs within 48-72 h post admission with corresponding microbiology culture positivity. The ICMR-ESBL study collected data via a detailed patient questionnaire with regard to prior history of hospitalization or surgery or dialysis or residence in a long term care facility. Further, presence of permanent indwelling catheter or a pericutaneous medical device (tracheotomy tube, gastrostomy tube or Foley's catheter) was also noted. Based on the information provided study isolates were categorized as either nosocomial or hospital associated and community acquired.

(5) In the absence of a reliable phenotypic method for screening and confirming AmpC producers we have used the PCR approach to cover six families of plasmid AmpC genotypes that are reported to be commonly produced[6]. Complete characterization of plasmid Amp C genotypes would have meant detection of individual subtypes under genotype family. One of the study objectives was to evaluate a step-wise protocol which could be implemented in a diagnostic laboratory with no PCR facility for plasmid AmpC detection. Therefore, an initial cefoxitin screening (step 1) followed by phenylboronic acid- cefoxitin combined

disc diffusion (step 2) was done and results of step 2 were compared with PCR. It is known that cefoxitin resistance can also be caused by hyperproduction of chromosomal Amp C, carbapenemases or porin loss. Against this background, it would be very difficult to interpret and differentiate the basis of a phenylboronic acid- cefoxitin and PCR negative result among cefoxitin resistant strains. Therefore, in this study we have compared the performance of the phenylboronic acid-cefoxitin positive results against PCR and provided specificity and sensitivity. We will be testing a larger collection of cefoxitin resistant isolates and planning sequencing studies to determine individual plasmid AmpC genotypes, a comprehensive comparison, and analysis of the different detection and confirmation methods will be undertaken during this study.

(6) Based on the study findings we concluded that the multiplex PCR based approach will be an important tool for the detection of plasmid mediated Amp C genes. However, given the complexity of the plasmid mediated AmpC genotype distribution and absence of a standard detection and confirmation protocol a multi-step approach of phenotypic screening followed by molecular based confirmation will need to be done, this was one of the important conclusions of the study.

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## References

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