Dear Editor,

The AAC(6')-Ib (aminoglycoside 6'-N-acetyl transferase type Ib) is an enzyme found in a wide variety of Gram-negative pathogens that confer resistance to tobramycin, kanamycin and amikacin by acetylation. The AAC(6')-Ib enzyme presents significant microheterogeneity at the N-termini, and there are more than 45 nonidentical, genetically stable variants reported from Enterobacteriaceae. While some variants conserve similar properties, others show dramatic differences in specificity, including aac(6')-Ib-cr that acetylates ciprofloxacin. This may lead to dual antibiotic selection. Since studies reporting the prevalence of these enzymes are predominantly polymerase chain reaction (PCR) based, they do not report the prevalence of isolates co-harboring two different variants of aac(6')-Ib. Hence, the exact prevalence of the “cr” variant within the aac(6')-Ib gene pool is not established in our clinical setting. Therefore, the current study was done to estimate the prevalence of aac(6')-Ib-cr.

A total of 200 clinical isolates of Enterobacteriaceae isolated from different tertiary care centres in and around Chennai were included for this study. These isolates were identified to be Escherichia coli (63%), Klebsiella pneumoniae (22%), Enterobacter aerogenes (4.5%), Citrobacter freundii (4%), Klebsiella oxytoca (2%), Citrobacter koseri (1.5%), Proteus mirabilis (1%), Proteus vulgaris (0.5%), Morganella morganii (1%) and Providencia stuartii (0.5%) by conventional biochemical methods. PCR amplification targeting all the variants of aac(6')-Ib was done as per published method. Wild-type variants have the restriction site for the enzyme BtsC1 (New England Bio Labs). Since there is a TGG to CGG mutation at codon 102 in the “cr” variant, it does not possess the BtsC1 restriction site. Hence, the PCR amplicons were digested with BtsC1 and the fragments were visualised in agarose gel electrophoresis. Strains harbouring wild-type variants of aac(6')-Ib gene shows fragments of 272 bp and 210 bp on gel electrophoresis and those which harbour aac(6')-Ib-cr gene shows a single undigested DNA fragment of 482bp. The strains that harbour both the variants simultaneously show three fragments of 482 bp, 272 bp and 210 bp of DNA on the gel electrophoresis [Figure 1]. ATCC BAA 2146 K. pneumoniae was used as a positive control.

PCR assay to confirm the presence of the “cr” variant was done according to published method using ATCC BAA 2146 as the positive control and a sequenced wild-type variant of the aac-(6')-Ib gene as the negative control (KF744024) obtained in our study.

The results are given in Table 1.

The findings of our study indicate that aac(6')-Ib-cr is

![Figure 1: BtsC1 digestion of aac(6')-Ib polymerase chain reaction amplicons. L1- Strain harbouring both the aac(6')-Ib-cr variant and the wild type aac(6')-Ib gene; L2- wild type aac(6')-Ib gene digested by BtsC1; L3- aac(6')-Ib-cr not digested by BtsC1; L4-100 base pair marker](http://www.ijmm.org)

### Table 1: Polymerase chain reaction amplification and BtsC1 restriction digestion to detect aac(6')-Ib variants among Enterobacteriaceae

<table>
<thead>
<tr>
<th>Changes at 304 nucleotide of 102 codon</th>
<th>Distribution of aac(6') Ib variants among enterobacterial isolates (n=125)</th>
<th>Number of fragments of DNA gel electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGATGG aac(6')-Ib</td>
<td>One fragment (482 bp)</td>
<td>Three fragments (482 bp, 210 bp)</td>
</tr>
<tr>
<td>GGACGG aac(6')-Ib-cr</td>
<td>Two fragments (272 bp, 210 bp)</td>
<td></td>
</tr>
<tr>
<td>GGA (T/C) GG aac(6')-Ib-cr and aac(6')-Ib</td>
<td>Three fragments (482 bp, 272bp, 210 bp)</td>
<td></td>
</tr>
</tbody>
</table>
the predominant \textit{aac(6')-Ib} variant in our clinical settings that might be due to its association with other prevalent resistance genes and their co-selection.

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\textbf{Conflicts of interest}

There are no conflicts of interest.

\textbf{References}