Disk Diffusion-based Algorithm Improves Detection of *aac(6')-Ib-cr*

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Background. The enzyme AAC(6')-Ib-cr acetylates both aminoglycosides and fluoroquinolones [ciprofloxacin (CIP) and norfloxacin (NOR), but not levofloxacin (LVX)]. Since it confers low level CIP/NOR resistance, bacteria with *aac(6')-Ib-cr* may be categorized as susceptible under the current susceptibility breakpoints. Our aim was to develop an algorithm to improve the phenotypic detection of *aac(6')-Ib-cr*.

Methods. To design the algorithm, we used 105 enterobacteria (A set) with resistance/decreased susceptibility to quinolones previously characterized for plasmid-mediated quinolone resistance genes. To test the performance of the phenotypic algorithm, we used an independent set of 232 enterobacteria (B set) consecutively recovered over a period of 5 days (2007) in 66 hospitals of WHONET-Argentina. Susceptibility tests were done by disk diffusion (DD) and agar dilution (MIC) under CLSI 2012. In the B set, *qepA* was screened by PCR and *aac(6´)-Ib-cr* by allele-specific PCR and DNA sequencing.

Results. In the A set, 24 (23%) isolates had *aac(6')-Ib-cr* (*qepA* not found). Among these, the susceptibility was (DD-MIC): nalidixic acid (NAL), 21%-33%; CIP, 17%-46%; LVX, 92%-88%, and amikacin (AKN) 54%-83%. The distributions of the difference between the diameters of the DD inhibition zones of LVX and CIP ($\Delta_{LVX-CIP}$) were significantly different between isolates with or without *aac(6')-Ib-cr* [median (range), in mm: 8 (3 to 14) *vs* 0 (-4 to 9), respectively, $p<0.0001$, Mann-Whitney Test]. Of note, $\Delta_{LVX-CIP}$ was ≥5 mm in all but 1 (3 mm) of isolates with *aac(6')-Ib-cr* and ≤4 mm in all but 1 (9 mm) of isolates without it, which resulted in 95.8% of sensitivity (Se) and 98.8% of specificity (Sp) for *aac(6')-Ib-cr* detection. In the B set, 13/15 isolates with $\Delta_{LVX-CIP}$ ≥5 mm and 10/217 isolates with $\Delta_{LVX-CIP}$ ≤4 mm had *aac(6')-Ib-cr* (*qepA* not found). However, these 10 isolates were not considered as false negatives since they showed no inhibition zones for CIP and halos ≤10 mm for LVX, resulting in 100% of Se and 99.0% of Sp. Among the 13 isolates with $\Delta_{LVX-CIP}$ ≥5 mm and *aac(6')-Ib-cr*, 3, 3, 11 and 10 strains were susceptible to NAL, CIP, LVX or AKN, respectively.

Conclusions. $\Delta_{LVX-CIP}$ ≥5 mm is a strong predictor for the presence of *aac(6')-Ib-cr* and constitutes a low-cost and easy tool to improve its detection.

Keywords: PMQR; aac(6')-Ib-cr; enterobacteria
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