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Performance of the Pefloxacin and Ciprofloxacin-based Susceptibility Tests on the Detection of Low-level Quinolone Resistance Mechanisms (LQRM) in Enterobacteria

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Background: The EUCAST has proposed the use of a pefloxacin 5- μ g disk (PEFd) to improve the screening of decreased ciprofloxacin (CIP) susceptibility (DCS) in *Salmonella* spp. (SAL). Aim: to evaluate the performance of the susceptibility tests with CIP and PEFd on LQRM detection in a set of previously characterized clinical isolates of SAL and other enterobacteria (ETB).

Methods: 70 isolates with LQRM (21 SAL, 49 ETB) and 25 full quinolone susceptible strains (7 SAL, 18 ETB) were used. LQRM comprised mutations associated with quinolone resistance (MAQRs) in *gyrA* and plasmid-mediated quinolone resistance mechanisms [several *qnrB* alleles, *qnrS1* and *aac(6')-Ib-cr*], either alone or combined. Two ETB with MAQRs in both *gyrA* and *parC* but with DCS were also included. Antibiotic susceptibility testing was done by disk diffusion (DD) with nalidixic acid (NAL), CIP and PEFd and agar dilution (AD) with CIP, under the CLSI guidelines.

Results: the distribution of the DD halos (mm) and MICs (μ g/ml) of LQRM isolates were, respectively [median (range)]: NAL 12 (6 – 15), 64 (16 – >512); CIP 26 (23 – 32), 0.5 (0.25 – 1); PEFd 17 (15 – 22), in the SAL subset, and NAL 16 (6 – 26), 32 (2 – >512); CIP 21 (11 – 32), 1 (0.03 – 8); PEFd 20 (6 – 29), in ETB. None of the SAL LQRM isolates was susceptible (S) by PEFd or AD with CIP [EUCAST or CLSI breakpoints (BP)]. Similarly, 5% (1/21) of the SAL LQRM isolates were S by DD with CIP (CLSI BP). In contrast, 29% (14/49) of the ETB LQRM isolates resulted S by PEFd while 47% (23/49) and 51% (25/49) were S by DD with CIP (EUCAST or CLSI BP, respectively). Of note, 3/4 isolates with AAC(6')-Ib-cr alone were S by PEFd probably because this enzyme does not acetylate the drug. The failure in ETB LQRM detection was critical for AD with CIP: 41% (20/49) and 80% (39/49) were S under the EUCAST or CLSI BP, respectively. The use of PEFd in the ETB subset resulted in 72% sensitivity (Sen) and 100% specificity (Spe) for LQRM detection. We found that the use of a susceptibility PEFd BP ≥ 27 mm (≤ 26 mm, resistant) resulted in 94% Sen and 100% Spe in the ETB subset (both parameters remained 100% in SAL).

Conclusions: In SAL, PEFd and both DD and AD with CIP showed good performances on LQRM detection. In ETB, PEFd was a better LQRM detector than DD or AD with CIP. The BP of PEFd and CIP are well established for SAL but they should be revised for ETB.