Resistance to quinolones in *Salmonella infantis* due to overexpression of an active efflux system and a mutation in the *gyrA* gene

Sir: Extensive use of antimicrobials in human and animal treatment, in the latter as growth promoters, has resulted in a considerable increase in resistance to quinolones among *Salmonella*[1]. The aim of this study was to identify the mechanisms of resistance to quinolones in *Salmonella enterica* serovar Infantis clinical isolates in the city of Corrientes (Argentina). For this purpose, six isolates of *Salmonella infantis*, in all probability linked to a foodborne community outbreak, were included in this study. All isolates were recovered from stool specimens over a two-day period and were cultured, identified, and serotyped by standard methods[2]. Antimicrobial susceptibility was tested with disk diffusion assay, according to CLSI (formerly NCCLS)[3] criteria, against nalidixic acid 30 μg, ciprofloxacin 5 μg, and other antimicrobials with therapeutic or epidemiological purposes. To assess the activity of the AcrAB-like efflux pump, in addition to antimicrobial diffusion disk susceptibility, minimal inhibitory concentrations (MICs) of nalidixic acid and ciprofloxacin were performed following the CLSI guidelines[3] by agar dilution test in the presence and absence of Phe-Arg-β-naphthylamide (Sigma, St. Louis, USA) at a concentration of 20 mg/L[4] The quinolone-resistant determining regions (QRDR) of the *gyrA* gene were amplified by PCR and sequenced and analyzed using a method previously described by Boucheron et al[5].

Clonal relationships between the isolates were assessed by ERIC-PCR according to the method described by Beyer *et al*[6] using two parts of the primer ERIC2[7]. PCR products were separated on 2% agarose gels, stained with ethidium bromide, visualized and photographed on an UV transilluminator.

Among the six strains studied, two were nalidixic acid-sensitive (NAL^s) with MICs of 4 and 8 μg/mL, respectively, and four were nalidixic acid-resistant (NAL^r), with MICs ≥ 256 μg/mL. These strains presented decreased susceptibility against ciprofloxacin (MIC = 2 μg/mL). The zone diameters for both antimicrobials increased and the MICs decreased two-fold against nalidixic acid and one-fold against ciprofloxacin when the strains were tested in the presence of AcrAB inhibitor; these differences were minimal or not evident among the nalidixic acid-susceptible strains, which suggested overexpression of an efflux pump inhibited by Phe-Arg-β-naphthylamide (likely to be AcrAB) as a mechanism of resistance. When the QRDR of the *gyrA* gene of each strain was amplified and sequenced, the nalidixic acid-resistant strains presented a mutation in codon Ser-83 of the *gyrA* gene (TCC → TTC), resulting in an amino acid change of Ser to Phe. All strains were susceptible to the remaining antimicrobial agents tested.

The NAL^r isolates presented identical ERIC-PCR profiles, which differed from those of the NAL^s isolates. The technique showed that whereas the NAL^r isolates belonged to the same clone, they did not have an epidemiological relationship with the NAL^s isolates. In addition, there were some differences between the two NAL^s isolates. Nalidixic acid resistance and decreased susceptibility to ciprofloxacin among *Salmonella* isolates is an increasing concern in several countries, even in Argentina, where 4% of human isolates are resistant to nalidixic acid[8-10]. The emergence of multiple resistance mechanisms in *Salmonella enterica* requires constant surveillance among the frequent and unusual serotypes.

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