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Nalidixic acid resistance predicting reduced ciprofloxacin susceptibility of *Salmonella enterica* serovar Typhi

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ABSTRACT

Objective: To evaluate the applicability of nalidixic acid (Nx) resistance in determining reduced susceptibility to ciprofloxacin (Cp) among *Salmonella enterica* serovar Typhi (*S. Typhi*) clinical isolates. **Methods:** The correlation between Cp MIC (minimum inhibitory concentration) values and that Nx, and between Cp MICs and zone diameter of inhibition (ZDI) around 30- μ g Nx disc for 421 *S. Typhi* isolates were determined by scattergram analysis. The specificity and sensitivity, in determining Cp reduced-susceptibility, of Nx resistance by disk testing and MIC determination were calculated. **Results:** For the test *S. Typhi* isolates the simultaneous presence of Nx-resistance and decreased Cp susceptibility has been recorded. The Nx sensitive *S. Typhi* isolates were sensitive to Cp; the disc diffusion and MIC breakpoints cannot determine Cp resistance. The sensitivity of Nx disc was 100 %, and the specificity was 89.20 % in determining Cp reduced-susceptibility of *S. Typhi* isolates; when MICs of Nx were compared with MICs of Cp, the sensitivity and specificity of the approach were 100 % and 95.95 %, respectively. **Conclusion:** Association between Nx-resistance and reduced susceptibility to Cp was noticed ($P \leq 0.001$) among the *S. Typhi* isolates, and thus Nx-resistance might be an indication of decreased susceptibility to Cp.

1. Introduction

Enteric fever due to the infection of multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi (*S. Typhi*) showing resistance to chloramphenicol, cotrimoxazole and ampicillin has posed a serious therapeutic challenge in many developing countries including India. Fluoroquinolones, including ciprofloxacin (Cp), were found effective against the infection of MDR *S. Typhi*, which were detected sensitive to Cp following the NCCLS (National Committee for Clinical Laboratory Standards) breakpoints [1]. However, there was an emergence of *S. Typhi* isolates causing Cp treatment failure of typhoid fever [2]. These isolates comparatively had higher minimal inhibitory concentrations (MIC) of Cp, though they were found susceptible to Cp by conventional disc diffusion testing and recommended MIC breakpoints [3–5]. Thus, several workers have observed clinical failure to Cp therapy due to infection with nalidixic acid (Nx)-resistant *S. Typhi*, and many workers

considered Nx susceptibility test as a surrogate marker for decreased susceptibility to Cp in *S. Typhi* [6,7].

Based on the fact of enteric fever treatment failures due to the infection of *S. Typhi*, showing in vitro susceptibility to Cp following the NCCLS guidelines, several authors suggested Nx screening test in order to detect Cp resistance of *S. Typhi* isolates [8]. It has been recommended that the *S. Typhi* isolates should be tested for Nx-resistance in order to avoid reporting false susceptibility to fluoroquinolones. In the present study, the relevance of using the resistance to Nx as a marker for decreased Cp susceptibility in *S. Typhi* isolates was evaluated by comparing the MICs of Cp and that of Nx, and MICs of Cp and ZDI obtained around 30- μ g Nx disc. In our collection of *S. Typhi* isolates, Nx susceptibility testing proved both sensitive and specific in screening isolates with decreased Cp susceptibility.

2. Materials and Methods

2.1. *Salmonella Typhi* strains

A total of 421 *S. Typhi* isolates from blood samples of suspected enteric fever patients undergoing treatment

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at the Calcutta School of Tropical Medicine, Kolkata (India) were used in the present study; the control strain used was *Escherichia coli* ATCC 25922.

2. 2. Scattergram analysis

The isolates were tested against nalidixic acid (Nx) and ciprofloxacin (Cp) using 30- μ g disc and 5- μ g disc, respectively, in order to determine the zone diameter of inhibition (ZDI) around the discs for all the isolates. Minimum inhibitory concentration (MIC) values of Nx and Cp for all the isolates were determined by agar dilution method using antibiotic concentrations, Nx (0.25 to 512 μ g/mL) and Cp (0.005 to 2 μ g/mL). The ZDIs obtained around the 30- μ g Nx disc were compared with the ZDIs from around the 5- μ g Cp disc, as well as with the Cp MICs for the isolates by scattergram analysis. The detail of the methods are mentioned elsewhere [9], following Clinical and Laboratory Standards Institute (CLSI, formerly called the National Committee for Clinical Laboratory Standards; NCCLS) criteria [10].

2. 3. Determination of specificity and sensitivity

The specificity and sensitivity, in determining the reduced susceptibility to Cp, of Nx resistance by disc testing and MIC value determination were calculated using formulae:

% sensitivity = true resistant / (true resistant + false sensitive) \times 100, and % specificity = true sensitive / (false resistant + true sensitive) \times 100 [11].

2. 4. Statistical analysis

The association between Nx-resistance and decreased Cp susceptibility was analyzed statistically following t-test, in order to compare the difference between mean MIC values of Cp and Nx for Nx-resistant (by disc diffusion) *S. Typhi* isolates. A *P*-value of \leq 0.001 was considered significant.

3. Results

The MIC histogram of Cp for *S. Typhi* isolates (n=421) showed three patterns of distribution with a range of 0.0075–1.25 μ g/mL for Cp (Figure 1). The isolates were divided into three populations based on Nx-susceptibility according to the NCCLS recommendations: with the Cp MICs ranging from 0.075 μ g/mL to 1.25 μ g/mL for the Nx-resistant population, from 0.05 μ g/mL to 0.075 μ g/mL for isolates showing intermediate susceptibility to Nx, and from 0.0075 μ g/mL to 0.075 μ g/mL for Nx-sensitive population.

There was an association between increased Cp MICs and Nx resistance based upon ZDI obtained around 30- μ g Nx disc for the isolates. Of the 157 isolates classified as Nx-resistant *S. Typhi* according to the NCCLS recommendation (ZDI \leq 13 mm), there were 125 with Cp MICs in between 0.1 μ g/mL and 1.25 μ g/mL, thus showing reduced susceptibility to Cp; the remaining 32 isolates had Cp MICs 0.025 – 0.075 μ g/mL. The MIC range of Cp for 253

Nx-sensitive *S. Typhi* was 0.0075–0.075 μ g/mL indicated sensitivity of the isolates to Cp. Eleven *S. Typhi* isolates, which showed intermediate susceptibility to Nx (ZDI 16 mm), had Cp MICs 0.025–0.075 μ g/mL.

Table 1.

Mean MICs (μ g/mL) of Nx and Cp for Nx-resistant and Nx-sensitive *S. Typhi* isolates

Agents	<i>S. Typhi</i> isolates	
	Nx-resistant	Nx-sensitive
Nalidixic acid (Nx)	112.5 \pm 78.58* (32–256)	6.18 \pm 4.14 (0.5–16)
Ciprofloxacin (Cp)	0.63 \pm 0.38* (0.075–1.25)	0.033 \pm 0.02 (0.0075–0.075)

**P* < 0.001 compared to Nx-sensitive *S. Typhi* isolates. Values are mean \pm S.E. Figures in the parentheses are the MIC ranges.

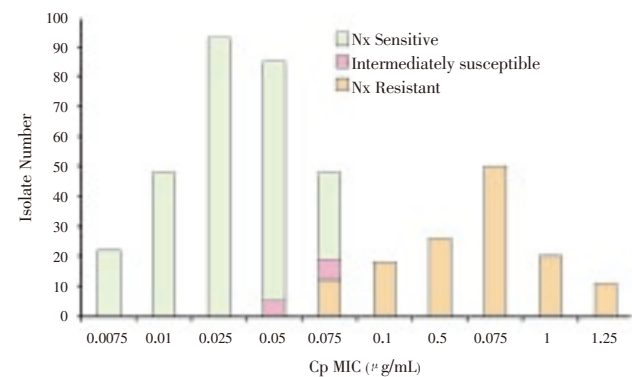


Figure 1. Ciprofloxacin (Cp) minimum inhibitory concentration (MIC) histogram for 421 *S. Typhi* isolates. Cp MICs are plotted on the x-axis, and the numbers of isolates are plotted on the y-axis. Columns of different colours indicate the patterns of nalidixic acid (Nx) susceptibility for the isolates.

There was a correlation between MICs of Cp and that of Nx for the *S. Typhi* isolates with simultaneous presence of Nx-resistance and decreased Cp susceptibility. All 125 *S. Typhi* isolates for which the Cp MICs were \leq 0.1 μ g/mL were resistant to Nx (MICs 32–256 μ g/mL). The 12 Nx-resistant isolates showed Cp MICs 0.075 μ g/mL. Out of 12 isolates, which were intermediately susceptible to Nx (MIC 24 μ g/mL), 5 showed Cp MIC of 0.05 μ g/mL, and 7 of 0.075 μ g/mL. All 253 sensitive isolates (Nx MIC \leq 16 μ g/mL) showed Cp MICs of \leq 0.075 μ g/mL.

The mean MICs of Nx and Cp for the isolates are shown in the Table 1. The differences in the MIC of Cp for the two study groups (Nx-resistant and Nx-sensitive) were statistically significant (*P* < 0.001) supporting the association between Nx-resistance and decreased Cp susceptibility.

4. Discussion

Emergence of Nx resistant *S. Typhi* and reports of infection with *S. Typhi* strains with increased resistance to Cp from typhoid-endemic areas have generated concern that strains resistant to Cp may become more prevalent [12]. Capoor et al. [13] reported from New Delhi (India) Nx resistant *S. Typhi* isolates, of which 98.9 % had decreased susceptibility (MIC \geq 0.125–1 μ g/mL) to Cp. Lynch et al. [14] reported from

the United States the Nx resistant S Typhi isolates, among which 97 % had decreased susceptibility to Cp (MIC > 0.12 μ g/mL). Threlfall *et al.* [15] reported that strains resistant at 0.125 μ g/mL but susceptible at 1 μ g/mL are regarded as exhibiting low-level resistance or decreased susceptibility to this antimicrobial, whereas those resistant at 1 μ g/mL are regarded as showing high-level resistance. In an agar dilution breakpoint method, as has been discussed by elsewhere [9], the levels of Cp incorporated into the media are 0.125 μ g/mL and 1 μ g/mL. According to this method, strains resistant at 0.125 μ g/mL but susceptible at 1 μ g/mL are regarded as exhibiting low-level resistance or decreased susceptibility to this antimicrobial, whereas those resistant at 1 μ g/mL are regarded as showing high-level resistance.

It has been suggested that among S. Typhi isolates resistance to Nx may be an indicator of decreased susceptibility to Cp [6,7]. The *Salmonella enterica* serotype Paratyphi A also showed reduced susceptibility to Cp (MIC 0.75 μ g/mL) and was resistant to Nx [16]. Based on the MICs of Cp and ZDI around 30- μ g Nx disc for 421 isolates tested in our study, screening for Nx resistance (ZDI \leq 13 mm) led to the detection of 125 isolates for which the Cp MICs were \geq 0.1 μ g/mL. When this MIC (\geq 0.1 μ g/mL) was used as a breakpoint of decreased Cp susceptibility in this study, the sensitivity of Nx disc was 100 %, and the specificity was 89.20 % based on the fact that 32 of the 296 isolates having Cp MICs 0.0075–0.075 μ g/mL (\leq 0.075 μ g/mL) also had Nx ZDI \leq 13 mm. When MICs of Nx were compared with MICs of Cp for 421 isolates, screening for Nx resistance led to the detection of all 125 isolates with decreased Cp susceptibility (MIC \geq 0.1 μ g/mL). In addition, 12 of the 296 isolates having Cp MICs \leq 0.075 μ g/mL were also included into this category. Therefore, sensitivity of the approach was 100 %, and specificity was 95.95 %, and there was a correlation between resistance to Nx and reduced susceptibility to Cp (P-value of \leq 0.001).

Nx resistance determined by the disc diffusion method may be an indication of decreased susceptibility to Cp [6,7]; in addition, we recorded a correlation between resistance to Nx (MIC, \geq 32 μ g/mL) and reduced susceptibility to Cp (MIC \geq 0.1 μ g/mL). This enables physicians to make informed decisions regarding appropriate class, dosage, and duration of antibiotic therapy for patients infected with S. Typhi showing reduced susceptibility to Cp. Although this older antibacterial is rarely used for treatment, resistance to Nx can be a marker for decreased susceptibility to Cp, because the use of CLSI breakpoints of resistance to Cp in S. Typhi at \geq 4 μ g/mL has been suggested to obscure the true occurrence of resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

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