Original article

Nalidixic acid susceptibility status of *Salmonella enterica* serovar Typhi isolates from Kolkata, India

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Abstract

Introduction and objectives: Typhoid is endemic in India; multidrug resistance of *Salmonella enterica* serovar Typhi has been reported from Kolkata, India, but nalidixic acid (Nx) resistance has not been documented in this part of the world. The aim of this study was to determine the Nx susceptibility for *S. enterica* serovar Typhi isolates (1991-2001) associated with enteric fever in Kolkata, India.

Materials and methods: The clinical *S. enterica* serovar Typhi isolates (n=421) were subjected to Nx (the prototype quinolone, which is used for *in vitro* screening tests for fluoroquinolone resistance) susceptibility testing by disc diffusion and determination of minimum inhibitory concentration (MIC) values. The test results, from the two methods, were compared by scattergram analysis, and the sensitivity and specificity in determining Nx resistance by disc testing, with respect to MIC values, were calculated.

Results: The *S. enterica* serovar Typhi isolates were categorized into resistant, intermediate and susceptible to Nx by disc testing and MIC. The isolates showed year-wise increment of Nx MICs (0.5-256µg/ml) during 1991-2001. High sensitivity (100%) and specificity (92.96%) in determining Nx resistance by disc testing, compared to MIC values were obtained for the isolates.

Conclusion: Increasing trend of Nx resistance, as determined by two *in vitro* methods, among *S. enterica* serovar Typhi isolates was noticed; such Nx resistance may help predict decreased susceptibility to ciprofloxacin (an anti-typhoid fluoroquinolone) among emerging *S. enterica* serovar Typhi in our part of the globe.

Keywords: *Salmonella enterica* serovar Typhi; Disc diffusion; Minimum inhibitory concentration; Nalidixic acid resistance

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Introduction

Enteric fever, caused by the infection of *Salmonella enterica* serovar Typhi, is a global public health problem. The emergence and persistence of multidrug-resistant (MDR) and nalidixic acid (Nx)-resistant *S. enterica* serovar Typhi strains constitute a grave concern, and this is particular in many parts of Asia, where antibiotics are readily available in an unregulated marketplace, and inadequate doses and durations of antibiotics are often used.

Outbreaks of enteric fever due to the infection of *S. enterica* serovar Typhi that are resistant to Nx and showed reduced susceptibility to the fluoroquinolone antibiotics, viz., ciprofloxacin (Cp), have been reported in a number of countries [1]. Based upon the Cp susceptibility status, *S. enterica* serovar Typhi are categorized into fully susceptible, intermediate and resistant to Cp. The isolates fully susceptible to Cp by disc testing, typically have Cp minimum inhibitory concentration (MIC) of ≤0.03 µg/ml, and these are invariably sensitive to Nx [2].

Isolates that are resistant to Cp by disc testing have MIC>1.0µg/ml, and are resistant to Nx [2,3]. The intermediate isolates are susceptible to Cp by disc testing, but have MICs 0.125-1.0µg/ml (decreased susceptibility to Cp), and are usually resistant to Nx [2,4]. Currently, several treatment failures with Cp therapy have been reported due to the infection of *S. enterica* serovar Typhi showing decreased susceptibility to Cp [4,5]. But, it has been reported that the decreased susceptibility to Cp among *S. enterica* serovar Typhi isolates could not be detected by the disc diffusion method; the isolates were found fully sensitive to Cp (5µg disc) having zone diameter of inhibition (ZDI)>21mm, and were resistant to Nx (30-µg disc) [4].

It has also been reported that Nx resistance is a marker for predicting decreased susceptibility (low-level of resistance) to Cp among *S. enterica* serovar Typhi, and also an indicator of treatment failure to Cp [4, 6]. Hence, Clinical Laboratory Standards Institute (previously National Committee for Clinical Laboratory Standards) recommended that all *S. enterica* serovar Typhi isolates should be screened for Nx resistance along with Cp. However, use of Cp discs along with Nx discs is costly and may not be affordable for many laboratories, especially of rural areas, in developing countries including India.

Herein, we assess the Nx susceptibility of *S. enterica* serovar Typhi isolates by disc testing as well as MIC determination in order to predict the Cp susceptibility status among the isolates, and Cp treatment failure. The aim of the present study was to calculate the percent sensitivity and specificity in determining Nx resistance by disc testing, with respect to MIC values, and in turn to assess the applicability of the method in determining Cp resistance of *S. enterica* serovar Typhi.

Materials and methods

Bacterial strains

A total of 421 *S. enterica* serovar Typhi isolates obtained, during 1991-2001, from enteric fever outbreak as well as sporadic cases associated for treatment with the Calcutta School of Tropical Medicine, India were used in the study [7]. The control strain used was *Escherichia coli* ATCC 25922.

Nalidixic acid susceptibility testing

The susceptibility testing to Nx (the prototype quinolone, which is used for *in vitro* screening tests for Cp resistance) for the *S. enterica* serovar Typhi isolates was performed by disc diffusion method of Bauer *et al*. [8], and interpretation of the

results was in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) [9]. The Nx MICs for all the isolates were determined by agar dilution method following NCCLS guidelines [10]. The Nx (disc and powder) used in the study was obtained from Hi-Media, India.

Scattergram analysis and determination of specificity and sensitivity

The ZDIs obtained around the 30-µg Nx disc was compared with the MICs of the antibiotic for the isolates by scattergram analysis. The specificity and sensitivity in determining Nx resistance by disc testing, with respect to MIC values, were calculated using formulae: %sensitivity= true resistant / (true resistant + false sensitive)×100, and % Specificity=true sensitive/(false resistant +true sensitive)×100 [11].

Results

The Nx susceptibility test results for 421 *S. enterica* serovar Typhi isolates are as follows. Disk diffusion susceptibility test grouped 421 isolates into three categories: Nx-resistant (n=157; 37.4%), intermediately susceptible to Nx (n=11; 2.6%) and Nx-sensitive (n=253; 60%). By MIC determination, among 421 isolates, 137 (32.54%) were resistant, 12 (2.86%) intermediately susceptible, and 272 (64.6%) sensitive to Nx.

The Nx MICs for 421 *S. enterica* serovar Typhi isolates are shown in figure 1. The sensitive isolates (n=272, 64.6%) showed MICs 0.5-16µg/ml, while the MICs for resistant isolates (n=137; 32.54%) were between 32µg/ml and 256µg/ml. The remaining 12 (2.86%) isolates, showing MIC 24µg/ml, were intermediately susceptible.

Year wise increment of MICs of Nx for the isolates (1991-2001) is represented in the figure 2. Nx MICs for the isolates of 1991-1994 were 0.5-16µg/ml. Range of MICs was 8-32µg/ml during 1995-1997. The values were between 16µg/ml and 256 µg/mL during 1998-2001. This clearly indicated the upward shifting of Nx MICs among the *S. enterica* serovar Typhi isolates.

Among 157 Nx-resistant isolates (ZDI ≤13mm) by disc testing, 137(87.3%) showed Nx MICs ≥32µg/ml, and for the remaining 20 isolates, Nx MICs were between 16µg/ml and 24µg/ml (Fig. 3). Out of 11 isolates, which were intermediately susceptible to Nx (ZDI 16mm) by disc testing, two showed Nx MIC of 24µg/ml, and the remaining nine showed Nx MICs between 8µg/ml and 16µg/ml. Nx MICs for 253 Nx-sensitive isolates (ZDI ≥19mm) were ≤ 16µg/ml.

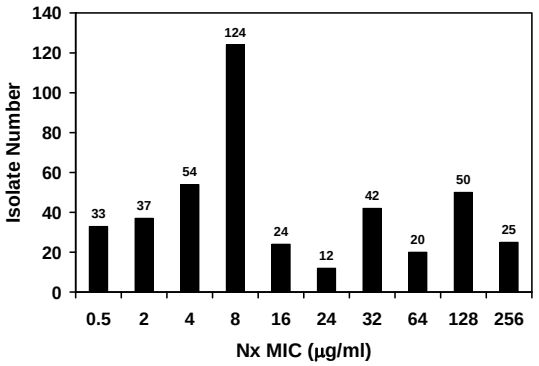

Fig. 1: Minimum inhibitory concentration (MIC) of nalidixic acid (Nx) for 421 *S. enterica* serovar Typhi isolates. The NCCLS MIC breakpoints for Nx susceptibility and resistance are ≤ 16 µg/ml and ≥ 32 µg/ml, respectively

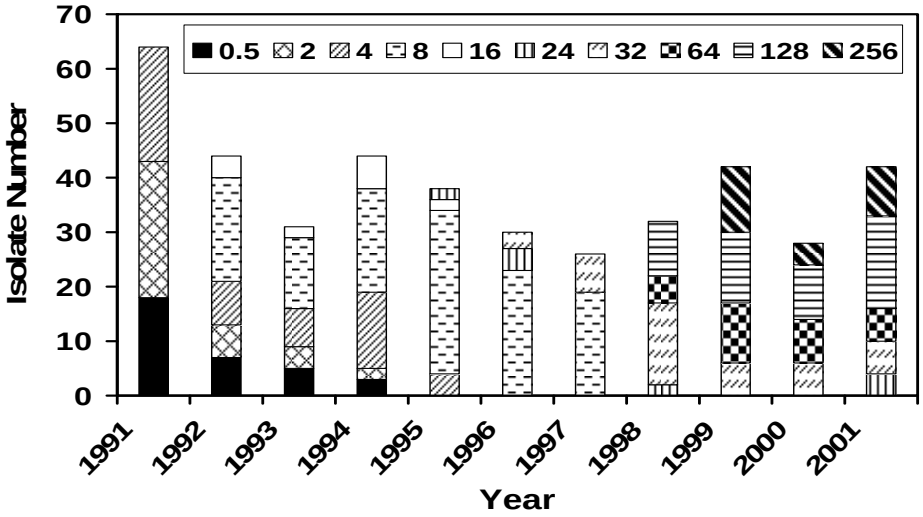

Fig. 2: Year wise (1991-2001) distribution of *S. enterica* serovar Typhi isolates with increasing trend of MICs to nalidixic acid (Nx). Values within the graphic indicate the MICs of Nx (µg/ml) for the isolates

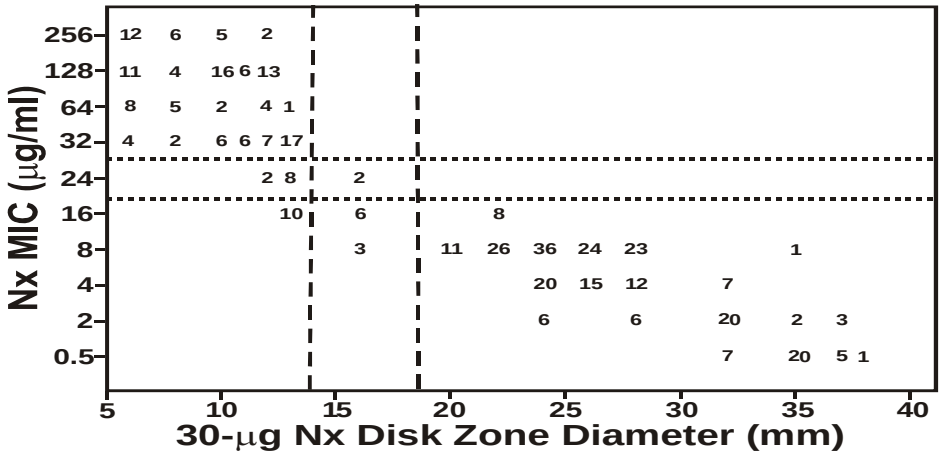

Fig. 3: Scattergram for 421 *S. enterica* serovar Typhi isolates comparing the minimum inhibitory concentration (MIC) values of nalidixic acid (Nx) with the zone diameter of inhibition (ZDI) around 30-µg Nx discs processed by the NCCLS methods. The broken vertical lines represent the interpretative ZDI (susceptible at ≥19 mm, resistant at ≤13 mm) and dot horizontal lines represent MIC break points (susceptible at≤16µg/ml, resistance at≥32µg/ml) suggested for Enterobacteriaceae. The numbers within the graphic indicate the number of *S. enterica* serovar Typhi isolates

Discussion

Among *S. enterica* serovar Typhi isolates, Nx-resistance has been reported following disc diffusion by several authors from different parts of the world including India [12-14]; however, Nx- resistance determined by MIC is scanty in India [13, 15]. We report here the MICs of Nx for 421 *S. enterica* serovar Typhi isolates, and verify the correlation between MIC test results and ZDI obtained around 30-µg Nx disc in order to determine Nx-resistance of *S. enterica* serovar Typhi isolates. A total of 137 *S. enterica* serovar Typhi isolates, in the present study, showed Nx-resistance by MIC based on the NCCLS criteria, which recommended ≥32µg/ml of MIC for Nx-resistance. These 137 *S. enterica* serovar

Typhi isolates that exhibited Nx MICs 32-256μg/ml were Nx-resistant by disc testing too, showing ZDI of ≤13mm (the NCCLS recommended ≤13mm ZDI for Nx resistance). Also, interesting enough the isolates in the present communication showed a year wise increment of Nx MICs starting from 0.5 μg/ml during 1991 to 256 μg/ml in the year 2001.

Following disc testing as well as MIC determination, we for the first time from Kolkata, India, obtained *S. enterica* serovar Typhi isolates showing intermediate susceptibility to Nx. In our study, 12 isolates (MIC 24μg/ml) grouped into intermediate susceptible category by MIC; of these, 10 showed Nx resistance by disc testing (ZDI 12-13mm). On the other side, by disc diffusion 11 isolates were intermediately susceptible to Nx (ZDI 16 mm), and of these 11 isolates, nine fell in to susceptible category by MIC determination (Nx MICs 8-16 μg/ml). Only two isolates showed intermediate susceptibility by both methods exhibiting Nx MIC of 24μg/ml, and ZDI of 16mm. Kapil *et al.* [6] isolated *S. enterica* serovar Typhi strains from New Delhi, India that showed intermediate susceptibility to Nx by disc diffusion.

Hakanen *et al.* [16] originally described the Nx resistance as an indicator of the decreased susceptibility to Cp among *S. enterica* isolates. Nx susceptibility has been validated as a screening test for reduced susceptibility to Cp, and has been associated with the increased Cp MICs among the *S. enterica* serovar Typhi isolates that in turn is related to the treatment failure with Cp therapy [16-18]. In the present study, of 272 *S. enterica* serovar Typhi, which were sensitive by MICs (≤16μg/ml), 10 isolates were included in the resistant category by disc diffusion (ZDI 13mm), plus another 10 isolates, out of 12 intermediates by MICs (24μg/ml), were resistant by disc diffusion (ZDI 12-13 mm). Therefore, among the total 421 *S. enterica* serovar Typhi isolates, 157 (37.3%) were Nx-resistant by disc diffusion. So, disc diffusion test results, following NCCLS guidelines, when compared with the MIC test values, as has been reported in this study, showed 100% sensitivity and 92.96% specificity in the determination of Nx-resistance among *S. enterica* serovar Typhi.

Conclusion

Thus, it can be recommended that clinicians should follow Nx disc testing for all *S. enterica* serovar Typhi isolates in order to detect Nx resistance as treatment failure, with Cp therapy, might occur in typhoid fever cases with Nx-resistant *S. enterica* serovar Typhi, and any isolate showing Nx resistance should be reported as the case with decreased susceptibility (increased resistance) to Cp suggesting switch over to another antibiotic for proper treatment.

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