

High Level of Resistance to nalidixic acid in *Salmonella enterica* serovar Typhi in Central India

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Abstract

Background: Fluoroquinolones are the drugs of choice for the treatment of typhoid fever. But the recent increase in minimum inhibitory concentration (MIC) values of ciprofloxacin in *Salmonella* Typhi may result in delayed response and serious complications. Nalidixic acid resistance has been used as an indirect evidence of increased minimum inhibitory concentration for ciprofloxacin in *Salmonella* Typhi.

Methods: We evaluated the isolates received at the National *Salmonella* and *Escherichia* Centre for nalidixic acid and ciprofloxacin susceptibility using standard methods. Minimum inhibitory concentrations have also been evaluated.

Results: Ninety-six percent of the isolates were found to be nalidixic acid resistant while all isolates were found to be ciprofloxacin sensitive. The difference between minimum inhibitory concentration values of ciprofloxacin for nalidixic acid resistant and nalidixic acid sensitive isolates was found to be statistically significant.

Conclusion: The study may be helpful in revising treatment strategies for the infections caused by nalidixic acid resistant *Salmonella* Typhi in the country.

Keywords: nalidixic acid, resistance, *Salmonella* Typhi, India

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Introduction

Typhoid fever is a major health problem in developing countries such as India [1] and is also a potential threat to developed nations in an era of increasing immigration and global operation. Fluoroquinolones have been considered to be the empirical therapy of choice against MDR-ST [2]. However, towards the end of last decade, treatment failures with ciprofloxacin have been reported due to infection with nalidixic acid resistant *Salmonella* Typhi which were found to be susceptible to ciprofloxacin in disc diffusion tests. These strains exhibited a higher minimum inhibitory concentration (MIC) of ciprofloxacin [3]. The present study was undertaken to assess the nalidixic acid resistant strains of *Salmonella* Typhi circulating in the central west part of the country.

Materials and Methods

Fifty *Salmonella* Typhi isolates received from 14 hospitals of Maharashtra during the year 2008 at the National *Salmonella* and *Escherichia* Centre (National Reference Laboratory), Central Research Institute, Kasauli, India, constituted the material for the study. It should be noted that samples from

different hospitals were not collected strictly in accordance with the protocol required for any epidemiological study.

Ready-made media and biochemicals (Hi Media Lab, Pvt. Ltd., Mumbai, India) were procured in their dehydrated form. All isolates were identified by conventional biochemical tests [4] and confirmed by serotyping [5] using standard *Salmonella* agglutinating sera (Seiken Laboratories, Tokyo, Japan). Antibiotic susceptibility patterns of the isolates were determined according to CLSI guidelines and interpretative criteria [6]. Minimum inhibitory concentrations were determined by agar dilution test [7] using purified antibiotic powders (Hi Media Laboratories, Pvt. Ltd., Mumbai, India).

Results and Discussion

Out of fifty isolates of *Salmonella* Typhi received at the National *Salmonella* and *Escherichia* Centre, 48 (96%) were found to be resistant to nalidixic acid (NARST) with MIC values of > 480 mg/L and only 2 (4%) isolates were found to be sensitive to nalidixic acid (NASST) with MIC values of 7.5 mg/L (Table 2). When these isolates were further tested against ciprofloxacin, all were found to be sensitive to

Table 1. Details of the isolates.

Period of isolation	Age of patients (yrs)	Place of isolation	Source
January	3 - 25	Maharashtra	Blood
March	5 -22	Maharashtra	Blood
April	5 - 30	Maharashtra	Blood
June	10 -25	Maharashtra	Blood
August	5 - 44	Maharashtra	Blood
September	1.5 - 26	Maharashtra	Blood
November	5 - 30	Maharashtra	Blood

* P-value < 0.05 for NARST vs. NASST; compared by independent t test

ciprofloxacin with MIC values ranging between 0.029 and 0.625 mg/L, which is considered to be in the susceptible range as per CLSI definition (susceptible if $\leq 1\mu\text{g/ml}$) (Table 2).

The present study reports high frequency of NARST (96%) (Table 2), which is higher in comparison to the earlier studies (60-78%) [8,9,10] conducted in the country. No seasonal distribution has been found and the strains were found to be present throughout all seasons of the year (Table 1) depicting the risk of infections with these strains at any time. Almost every age group (Table 1) has been found to be infected with these strains.

The mean MIC of ciprofloxacin was found to be significantly higher for NARST as compared to that of NASST (Table 2, $P = 0.0213$). In accordance with earlier studies [10], the sensitivity of nalidixic acid resistance as a surrogate marker for identifying isolates with increased levels of MIC of ciprofloxacin was found to be good in the present study.

However, non-typhoidal *Salmonella* strains have been reported from southeast Asia that are susceptible to nalidixic acid but show reduced susceptibility to ciprofloxacin [11]. This phenomenon occludes the use of nalidixic acid resistance determination by the disk diffusion method as a tool to screen for decreased ciprofloxacin susceptibility in non-typhoidal *Salmonella*. This resistance pattern

may possibly be due to the presence of an efflux pump mechanism [12,13] or mutations at locations other than *gyrA* [11]. Fluoroquinolone resistance in non-typhoidal *Salmonella* has emerged as a threat to the health community in the past few years [14,15,16]; therefore, invasive infections with serious complication caused by non-typhoidal *Salmonella* should be treated with increased caution to avoid treatment failures [17].

Association of nalidixic acid resistant *Salmonella* Typhi with severe clinical illness and treatment failures with fluoroquinolones is a matter of concern for the medical community and an alarming increase in the frequency of nalidixic acid resistance in *Salmonella* Typhi in the country aggravates the scenario. Use of ciprofloxacin may delay the initiation of appropriate antibiotic therapy and thereby may lead to increased morbidity due to very high frequency of NARST in the country.

Conclusion

The frequency of nalidixic acid resistance has been increasing for the past decade. In light of the endemicity of the disease and the prevalence of nalidixic acid resistant *Salmonella* Typhi in the country, health authorities should take appropriate steps to formulate therapeutic strategies.

Table 2. Antimicrobial susceptibility to nalidixic acid and ciprofloxacin.

Antibiotic susceptibility	NARST	NASST
Number of isolates	48	2
Nalidixic acid MIC range (mg/L)	>480	7.5
Ciprofloxacin susceptibility	Sensitive	Sensitive
Ciprofloxacin MIC range (mg/L)	0.32±0.2*	0.019±0.0*

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