Susceptibility to patterns of ciprofloxacin among nalidixic acid-resistant *Salmonella* isolates collected in Banepa, Nepal from enteric fever patients

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Abstract

The present study determined the susceptibility to ciprofloxacin of nalidixic acid resistant *Salmonella* (NARS) isolated from enteric fever patients at Scheer Memorial Hospital, Banepa, Nepal, from June 2012 to December 2012. The antimicrobial sensitivity to nalidixic acid and ciprofloxacin was determined using modified Kirby-Bauer disc diffusion and broth dilution method according to the guidelines of the Clinical and Laboratory Standard Institute. *Salmonella* was isolated from 34 out of 992 (3.43%) blood cultures collected during the study period, and 10 (29.4%) isolates were identified as *Salmonella enterica* serotype Typhi, while 24 (70.6%) were identified as *Salmonella enterica* serotype Paratyphi. Out of the total isolates, 31 (91.2%) were nalidixic acid-resistant *Salmonella* (NARS). Among NARS, the minimum inhibitory concentration values for ciprofloxacin ranged from 0.25 to 2 mg/L and were constantly higher than those shown by the nalidixic acid-susceptible *Salmonella*. Therefore, in typhoid *Salmonella* nalidixic acid resistance may be the indicator of decreased susceptibility to ciprofloxacin.

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Introduction

The term *enteric fever*, coined to embrace both typhoid and paratyphoid fever, has been defined as a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteremia.[1] Enteric fever is potentially life threatening systemic illness characterized by high fever and abdominal complaints. Enteric fever is caused by the human adapted pathogens *Salmonella enterica* serotype Typhi (*S.* Typhi) and *S. enterica* serotype Paratyphi *(S. Paratyphi* A, B, C). These organisms are important causes of febrile illness in crowded and impoverished populations with inadequate sanitation that are exposed to unsafe water and food and also pose a risk to travelers who visits the country of endemicity.[2]

Ciprofloxacin is synthetic antimicrobial agent commonly prescribed against enteric fever belongs to second generation 4-quinolones which is also known as fluoroquinolones and acts by inhibiting bacterial Topoisomerase II (DNA gyrase) which is required for DNA supercoiling and Topoisomerase IV which is required for strand separation during cell division.[3] The wide spread use of ciprofloxacin in recent years (2003-2012), several treatment failures have also been reported due to decreased susceptibility of ciprofloxacin.[4,5] Generally, a single mutation (Ser-83 to Phe or Ser-83 to Tyr) in gyr A was associated with reduced susceptibility to ciprofloxacin (MIC 0.125-1 mg/L) and nalidixic acid resistant.[6]

Routinely applied disc diffusion method is not convenient to identify reduced susceptibility of ciprofloxacin among NARS. Therefore, many

literatures suggest that Nalidixic acid resistant (NAR) may be an indicator of reduced susceptibility of ciprofloxacin.[7] This reduced susceptibility (MIC 0.125-1 mg/L) can be identified by evaluating minimum inhibitory concentration (MIC) of ciprofloxacin.[8]

Materials and Methods

Study site and study period

This study was conducted at Scheer Memorial Hospital, Banepa from June 2012 to December 2012.

Study population and ethical approval

The study was conducted on the populations visiting Scheer Memorial Hospital, Nepal who were clinically suspected to have enteric fever, and requested for blood culture and antibiotic susceptibility testing. A total of 992 blood samples from the patients of suspected enteric fever were included in the study. Samples with improper labeling, insufficient blood volume, and inappropriate collection and transport were rejected. This research work was carried out according to ethical guidelines approved by the Bioethics Committee of Scheer Memorial Hospital, Banepa, Nepal and all the study subjects provided their consent to participate in this study.

Sample processing

These samples were collected aseptically using a disposable syringe. The top of the culture bottle was cleaned with iodine immediately before addition of blood. 5 ml of venous blood drawn from adults was added to 50 ml of sterile Brain Heart Infusion (BHI) broth. For children, the ratio used was 3 ml of blood in 30 ml BHI. The clinical history and personal data of the patients were recorded. The BHI broth culture bottles were labeled and incubated at 37°C.

Isolation

Incubated culture bottles were checked daily for turbidity or visual changes (*i.e.* gas formation, discoloration of broth). If any turbidity was seen, then preliminary Gram's staining was performed. If Gram-negative rods were seen, the broth was subcultured on MacConkey agar (MA) and blood agar (BA). If no turbidity or growth on subculture was observed for 7 days the cultures were discarded.

Identification

The bacterial isolates from BA and MA were identified by Gram's staining and standard conventional Biochemical tests. Gram-negative short rods, producing non-lactose fermenting pale yellow colonies on MA and non haemolytic mucoid colonies on BA were subcultured on nutrient agar for biochemical tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was performed by disc diffusion method and the MIC values were determined by broth dilution method.

Statistical analysis

All data were entered into a computerized database (Microsoft Office Excel 2010, Microsoft Corporation). Descriptive analyses were completed and variables were recorded as necessary for statistical modeling using commercially available programs (SPSS 16.0 for Windows, SPSS inc. and WHONET 5.6, WHO).

Results

A total of 34 *Salmonella* were isolated during the study period. Out of these, 10 were *S.* Typhi and 24 were of *S.* Paratyphi. There was an increase in the isolation rate of *S.* Paratyphi compared to *S.* Typhi (Table 1). The suspected patient age was ranged from one day to 88 years old. Out of the total 992 blood samples, 458 (46.17%) were male and 534 (53.83%) were female. The prevalence of bacteremia among male was 4.15% (19/458) and among female was 2.81% (15/534). The difference in growth of organisms in male and female was statistically insignificant (P=0.42) (Table 2). All 34 isolates were sensitive *in-vitro*

Table 1. Frequency of *Salmonella* isolates among different age groups and sex.

Sex of Patients	Name of organism	Age group of patients (years) <15	15-29	30-44	45-59	≥60	Total
Female	*S.* Typhi	4	1	1	0	1	7
	S. Paratyphi	2	2	1	2	1	8
	Total	6	3	2	2	2	15
Male	*S.* Typhi	3	0	0	0	0	3
	S. Paratyphi	6	3	3	2	2	16
	Total	9	3	3	2	2	19

S. Typhi, *Salmonella* Typhi; *S.* Paratyphi, *Salmonella* Paratyphi.

Table 2. Frequency of growth on blood culture.

Growth	Male	Female	Total	Statistics
Positive	19	15	34	
Negative	439	519	958	P=0.42
Total	458	534	992	

to ciprofloxacin. The sensitivity was checked using 5 μg ciprofloxacin discs on Mueller-Hinton agar by modified Kirby-Bauer disc diffusion method (Table 3). Among total 34 isolates of *Salmonella*, 31 (91.18%) were resistant to nalidixic acid (Table 4). Out of 3 nalidixic acid sensitive isolates, 2 isolates were *S.* Paratyphi and 1 isolate was *S.* Typhi. The MIC value of ciprofloxacin among nalidixic acid sensitive *Salmonella* (NASS) was in the range of (0.03125-0.0625) mg/L and among nalidixic acid resistant *Salmonella* (NARS) was in the range of (0.25-1) mg/L except 2 isolates. The association between NARS and reduced susceptibility of ciprofloxacin was statistically significant (P<0.001) (Table 5).

Discussion

Enteric fever continues to be a major health problem in Nepal. Its treatment still remains a challenge due to the rapid emergence of antibiotic resistance in the causative strains. Low isolation rate of *Salmonella* (3.43%) was found in this research. The use of antibiotics prior to blood collection may possibly explain the low isolation rate of *Salmonella* in Nepal, since antibiotics are frequently prescribed even for mild cases of fever.[9,10] The numbers of *S.* Paratyphi (41.18 %) were comparatively higher than *S.* Typhi. Increasing prevalence of *S.* Paratyphi has been reported from several studies worldwide including Nepal.[11] The proportional increase of *S.* Paratyphi infections has been attributed to changing clinical attitude to investigate mild fever cases for enteric fever, changing host susceptibility, change in virulence of the organisms, and widespread use of quinolones antibiotics against *S.* Typhi in the past decade.[12] In this study, the percentage of NAR *S.* Typhi was 90% and NAR *S.* Paratyphi was 91.7%. High incidence of NARS isolates, ranging from 73.3 to 94.9%, was described from different studies held in Nepal.[4,10,13] Nalidixic acid screening has been suggested to detect and predict decreased ciprofloxacin susceptibility in enteric fever causing *Salmonella*.[14,15] In our study, all 34 NARS isolates were found to be sensitive to ciprofloxacin by routine disc diffusion tests. This is in accordance with a regional study that found that the isolates with decreased *in vivo* susceptibility to ciprofloxacin appear susceptible with routine disc diffusion tests.[16]

Conclusions

Enteric fever still remains one of the most common clinical diseases, especially in rainy seasons in Nepal, where *Salmonella* Paratyphi is the most prevalent causative serovar. Inability to identify reduced susceptibility to ciprofloxacin by the standard disk diffusion techniques urges for the revision of the current guidelines. NARS screening test may be a practical surrogate to the determination of ciprofloxacin MIC value and could be used as an indicator of decreased susceptibility to ciprofloxacin in order to avoid treatment failure.

Table 3. Antimicrobial-resistant patterns of *Salmonella* isolates.

Antibiotics used	Resistant isolates (%)	
	S. Paratyphi (n=24)	*S.* Typhi (n=10)
Nalidixic acid	22 (91.67)	9 (90)
Ciprofloxacin	0	0
Gentamicin	0	0
Ofloxacin	0	0
Co-trimoxazole	2 (8.33)	4 (40)
Ampicillin	4 (16.67)	5 (50)
Cefixime	0	0
Ceftriaxone	0	0
Tetracycline	2 (8.33)	3 (30)
Chloramphenicol	2 (8.33)	4 (40)

S. Paratyphi, *Salmonella* Paratyphi; *S.* Typhi, *Salmonella* Typhi.

Table 4. Nalidixic acid susceptibility pattern of *Salmonella* isolates.

Isolates	NARS	NASS	Total	Statistics
S. Typhi	9	1	10	
S. Paratyphi	22	2	24	P=0.791
Total	31	3	34	

NARS, nalidixic acid-resistant *Salmonella*; NASS, nalidixic acid-sensitive *Salmonella*; *S.* Typhi, *Salmonella* Typhi; *S.* Paratyphi, *Salmonella* Paratyphi.

Table 5. Minimum inhibitory concentration of ciprofloxacin in nalidixic acid-sensitive and acid-resistant *Salmonella* isolates.

MIC of ciprofloxacin (mg/L)	NASS isolates	NARS isolates	Statistics
≤0.03125	2 (66.67)	0	
0.0625	1 (33.33)	0	
0.125	0	0	
0.25	0	6 (19.35)	P<0.001
0.5	0	19 (61.29)	
1	0	4 (12.9)	
2	0	2 (6.45)	
Total	3 (100)	31 (100)	

MIC, minimum inhibitory concentration; NASS, nalidixic acid-sensitive *Salmonella*; NARS, nalidixic acid-resistant *Salmonella*.

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