

## ANTIBIOGRAM OF SALMONELLA SPP ISOLATES IN KATHMANDU, NEPAL

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### ABSTRACT

#### INTRODUCTION:

Drug resistant *Salmonella* spp. is endemic in several Asian countries. Nalidixic acid-resistant *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A show reduced susceptibility to fluoroquinolones and have resulted in a rise in treatment failures. Over the past few decades, nalidixic acid-resistant *Salmonella* spp have emerged in Nepal as well.

#### MATERIAL & METHODS:

This is a retrospective study that aims to provide a more recent antibiogram of *S. Typhi* and *S. Paratyphi A* isolates in Kathmandu. Between Poush, 2071 and Ashwin, 2072 (December 16, 2014 to October 17, 2015), 186 culture positive cases of enteric fever were diagnosed at the Civil Service Hospital. Upon isolation of *S. Typhi* or *S. Paratyphi A*, antimicrobial susceptibility testing was performed with amoxicillin, azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, nalidixic acid, ofloxacin and tetracycline.

#### RESULTS:

This study shows a much higher frequency of nalidixic acid-resistance in Kathmandu than previously reported; 95.7% in *Salmonella enterica* serovar Paratyphi A and 86.5% in *Salmonella enterica* serovar Typhi. The rates of ciprofloxacin- and ofloxacin-resistance were over 50% in both serovar.

#### CONCLUSION:

In Nepal, it is necessary to reevaluate the use of fluoroquinolone therapy and introduce feasible alternatives so as to curb treatment failures.

**KEY WORDS:** *S. Typhi*, *S. Paratyphi A*, nalidixic-resistance, fluoroquinolones

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## INTRODUCTION

Enteric fever is endemic and a major cause of febrile illness in Nepal. The disease is mainly transmitted through contaminated food and water. It thrives in environments of poor sanitation and inadequate supply of clean water<sup>1</sup>. In addition to *Salmonella enterica* serovar Typhi (*S. Typhi*), *Salmonella enterica* serovar Paratyphi-A (*S. Paratyphi*) has emerged as a major causative organism in the past decade<sup>2,4</sup>. The incidence of paratyphoid cases has been reported to be higher than the incidence of typhoid cases in Kathmandu<sup>4</sup>.

Nalidixic acid-resistant strains of *S. Typhi* have been reported since the early 1990's in Asia. These strains have shown reduced susceptibility to ciprofloxacin in various countries, for instance, Vietnam, Japan and Tajikistan<sup>5-7</sup>. Moreover, it has been suggested that such strains are endemic in neighbouring countries, India and Pakistan<sup>8</sup>. Nalidixic acid-resistant *S. Paratyphi A* isolates in India have also shown reduced susceptibility to ciprofloxacin<sup>9</sup>. Recent evidence shows a high rate of nalidixic acid-resistant strains of *S. Typhi* and *S. Paratyphi A* in Nepal as well<sup>10,11</sup>. In Kathmandu, the rate of nalidixic acid-resistance in *S. Paratyphi A* strains has been reported to be higher than in *S. Typhi* strains<sup>11</sup>.

Furthermore, a 2006 study conducted in Kathmandu revealed that patients infected with either serovar presented with indistinguishable clinical syndromes, dismissing the assumption that *S. Paratyphi A* causes a milder disease<sup>12</sup>. With the increasing incidence of paratyphoid cases in Nepal and the higher prevalence of drug resistance among these cases, an increase in the rate of treatment failures may be a concern<sup>11</sup>. In Nepal, *S. Typhi* and *S. Paratyphi A* isolates have been susceptible to fluoroquinolones in the past, however, this is no longer true<sup>12,13</sup>. This retrospective study aims to provide a more recent antibiogram of *S. Typhi* and *S. Paratyphi A* isolates in Kathmandu.

## MATERIAL & METHODS

The Civil Service Hospital in Kathmandu provides affordable medical services to the inhabitants of Kathmandu Valley and the surrounding area. The microbiology laboratory at this hospital is equipped with culture facilities for the diagnosis of bacterial infections. Between Poush, 2071 and Ashwin, 2072 (December 16, 2014 to October 17, 2015), 186 culture positive cases of enteric fever were diagnosed between at this location. The BACTEC™ FX blood culture system was initially used to detect microbial growth. Positive samples were then incubated in MacConkey agar and blood agar for 24

hours. Upon isolation of *S. Typhi* or *S. Paratyphi A*, antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method. All *S. Typhi* and *S. Paratyphi A* isolates were tested for susceptibility with amoxicillin, azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, nalidixic acid, ofloxacin and tetracycline.

Medical data were obtained from MiDAS, an electronic medical records software used by the laboratories at the Civil Service Hospital. Excel 2013 for Windows and IBM SPSS Statistics 20 for Windows were used for data analysis.

## RESULTS

Of the 182 patients that presented with enteric fever, 88 were infected with *S. Typhi* (48.4%), and 94 were infected with *S. Paratyphi A* (51.6%). Two *S. Typhi* and two *S. Paratyphi A* cases were excluded from the study because the complete antibiogram for these cases were not available. A higher rate of nalidixic acid resistance was seen in *S. Paratyphi A* isolates than in *S. Typhi* isolates (95.7% vs. 86.5%). The rates of ciprofloxacin- and ofloxacin-resistance were similar in both serovars. Amoxicillin-resistance was more common in *S. Paratyphi A* isolates when compared to *S. Typhi* isolates (68% vs. 24.7%). All *S. Typhi* isolates were susceptible to ceftriaxone, chloramphenicol and tetracycline while all *S. Paratyphi A* isolates were susceptible to ceftriaxone, chloramphenicol and cotrimoxazole. Susceptibility to chloramphenicol and cotrimoxazole has increased from rates seen in 2002<sup>12</sup>. Antibiogram of *S. Typhi* and *S. Paratyphi A* isolates are shown in Table 1 and Table 2 respectively.

Antibiotic	Antibiogram of <i>S. Typhi</i> isolates		
	Susceptible n (%)	Resistant n (%)	Intermediate n (%)
Amoxicillin	53 (59.6)	22 (24.7)	14 (15.7)
Azithromycin	68 (76.4)	3 (3.4)	18 (20.2)
Ceftriaxone	89 (100)	0 (0)	0 (0)
Chloramphenicol	89 (100)	0 (0)	0 (0)
Ciprofloxacin	13 (14.6)	49 (55.1)	27 (30.0)
Cotrimoxazole	87 (97.8)	2 (2.2)	0
Nalidixic Acid	11 (12.4)	77 (86.5)	1 (1.1)
Ofloxacin	13 (14.6)	48 (53.9)	28 (31.5)
Tetracycline	89 (100)	0 (0)	0 (0)

Table 1. Antibiogram of *S. Typhi* isolates.

Antibiotic	Antibiogram of <i>S. Paratyphi A</i> isolates		
	Susceptible n (%)	Resistant n (%)	Intermediate n (%)
Amoxicillin	23 (24.5)	64 (68.0)	7 (7.5)
Azithromycin	57 (60.6)	7 (7.5)	30 (31.9)
Ceftriaxone	94 (100)	0 (0)	0 (0)
Chloramphenicol	94 (100)	0 (0)	0 (0)
Ciprofloxacin	7 (7.5)	49 (52.1)	38 (40.4)
Cotrimoxazole	94 (100)	0 (0)	0 (0)
Nalidixic Acid	4 (4.3)	90 (95.7)	0 (0)
Ofloxacin	6 (6.4)	49 (52.1)	39 (41.5)
Tetracycline	93 (98.9)	1 (1.1)	0 (0)

Table 2. Antibiogram of *S. Paratyphi A* isolates.

## DISCUSSION

The ability to achieve high concentrations in macrophages, bowel, bile and the urinary tract coupled with easy administration (i.e. oral) has made fluoroquinolones the drugs of choice for the treatment of enteric fever<sup>9</sup>. Nalidixic acid-resistant *Salmonella* spp isolates show a decreased clinical response to fluoroquinolones<sup>14</sup>. There is increasing evidence that while AST may demonstrate the minimum inhibitory concentration of *Salmonella* spp isolates to fall within the susceptibility range, this result may not be indicative of clinical response<sup>14</sup>. Hence, it has been recommended that nalidixic acid-resistance be used as an indicator for clinical response to fluoroquinolones, and that short course-short fluoroquinolone therapy be avoided for the treatment of enteric fever caused by nalidixic acid-resistant isolates<sup>15,16</sup>.

This study found a higher rate of nalidixic acid-resistance in *Salmonella* spp isolates than previously reported in Kathmandu<sup>11,12</sup>. Consistent with previous evidence, *S. Paratyphi A* was more likely to be nalidixic acid-resistant when compared with *S. Typhi*<sup>11,12</sup>. While nalidixic acid-resistance should be used as an indicator for the prescription of fluoroquinolones, AST is not widely available in Nepal, and ciprofloxacin and ofloxacin are widely used for the treatment of enteric fever<sup>11,12</sup>. Moreover, antimicrobials are available from pharmacies without a prescription. These factors may be involved in the spread of drug-resistant *Salmonella* spp isolates in Kathmandu. Among other methods to control enteric fever, vaccination has been widely used worldwide. Immunization against *S. Typhi* does not provide effective cross-protection against *S. Paratyphi A*, and there are no licenced vaccines against *S. Paratyphi A*<sup>17</sup>. Although annual vaccination of school children in Thailand against *S. Typhi* has

been effective in decreasing the incidence of overall typhoid fever, a decrease in the incidence of *S. Paratyphi A* was not reported<sup>18</sup>. With the high prevalence of *S. Paratyphi A* in the community, it is not clear whether immunization would be an effective option in Kathmandu. Further studies are required to evaluate the feasibility of mass vaccination against enteric fever in Nepal.

Along with an improvement in a consistent supply of clean water and sanitation, enteric fever-specific education across various population such as school children, food handlers, pharmacists, and pharmacy attendants would be beneficial in the control of enteric fever in Nepal. Particularly, education for pharmacy attendants regarding the risks of antimicrobial sales without prescriptions could be helpful in reducing the indiscriminate use of these drugs.

## CONCLUSION

The limited number of health facilities that offer AST; the widespread and indiscriminate use of fluoroquinolones; and inadequate supply of clean water and poor sanitation in crowded urban settings in Kathmandu is a major concern with respect to the spread of drug resistant strains of *Salmonella* spp. With the spread of drug resistant strains, treatment failure has been reported from around world. The rate of nalidixic acid-resistant *S. Typhi* and *S. Paratyphi A* is alarmingly high in Kathmandu, much higher than previously reported. Moreover, more than 50% of both *Salmonella* serovars showed resistance to ciprofloxacin and ofloxacin. The emergence of untreatable typhoid and paratyphoid fever may soon be a reality in Kathmandu.

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