Bacteriology of enteric fever cases at Nepal Police Hospital, Kathmandu

Rai K,[1] Shah PK,[1] Rai G,[2] Rai SK,[3] Rai KR,[3] Gautam AK,[1] Yanagida JI,[4] Ono K,[4] Thapa S[5]

[1]Tri-Chandra Multiple Campus, Ghantaghar, [2]Nepal Police Hospital, Maharajgunj, [3]Shi-Gan International College of Science and Technology / National Institute of Tropical Medicine and Public Health Research, Maharajgunj, Kathmandu, Nepal, [4]Kobe Tokiwa University, Kobe, Japan, [5]Graduate School of Biomedical Sciences, University of North Texas Health Science Center, Fort Worth, Texas, USA.

Corresponding Author: Kamal Rai, Birendra Multiple Campus, Bharatpur, Chitwan, Nepal; email: kamal_bro22@yahoo.com

ABSTRACT

Enteric fever caused by *Salmonella enterica* serotype Typhi and Paratyphi is one of the major public health problems in developing countries attributed to poor hygiene and sanitation. This cross-sectional study was conducted at Nepal Police Hospital, Kathmandu over a period of 5 months from April to August 2013. A total of 928 blood samples collected from patients suspected of enteric fever were included in this study. Blood culture was done in bile broth and then sub-cultured on MacConkey agar. No growth on sub-culture even after the fifth day was regarded as culture negative. Isolates were identified on the basis of their microscopic and colony characteristics, biochemical and sero-typing results. Antibiotic susceptibility test of the isolates was done by Kirby-Bauer disc diffusion method using a panel of commonly used drugs in Nepal. Overall culture positive rate was 17.1% (159/928) with significantly high positive rate (24.8%; 123/495) in the age group of 16-30 years. Positive rate was higher in males (19.7%; 131/665) compared with positive rate (10.6%; 28/263) in females (P=0.01). *Salmonella enterica* serotype Typhi was most common (74.8%; 119/159) followed by *Salmonella enterica* serotype Paratyphi A (25.2%; 40/119). Of the eleven antibiotics used, Ceftriaxone was the most effective (all isolates were sensitive) followed by Amoxicillin (95.6% isolates were sensitive). Altogether 90.5% (15/159) of isolates were sensitive to first line antibiotics namely Ampicillin, Tetracycline, Cotri-moxazole and Chloramphenicol (ATCoC) while the widely preferred Quinolone group of antibiotics were less effective (58.5%, 54.7% and 9.4%, of the total isolates were sensitive to Ciprofloxacin, Ofloxacin and Nalidixic acid, respectively). These findings indicated that enteric fever is still endemic in Kathmandu Valley and awareness with regard to its prevention and control needs to be emphasized.

Keywords: Bacteriology, Enteric fever, *Salmonella*, Kathmandu, Nepal

INTRODUCTION

Enteric fever caused by *Salmonella enterica* serotype Typhi and Paratyphi A is still a major public health problem in developing countries.[1] Serotype Typhi alone causes approximately 21 million cases of enteric fever resulting in 217,000 deaths while 5.4 million cases are estimated to be caused by serotype Paratyphi A worldwide annually.[1] Out of this, more than 90% of morbidity and mortality occurred in Asian countries.[1] This disease is associated with poor hygiene and sanitation resulting in consumption of unsafe drinking water and contaminated food.[1,2]

In Nepal, enteric fever caused by *S. enterica* serotype Typhi and Paratyphi A constitute one of the major public health problems.[2-6] Enteric fever caused by *S. enterica* serotype Paratyphi A has increased considerably during recent years.[3-6] The disease caused by *S. enterica* serotype Paratyphi A is considered to be milder than the disease caused *S. enterica* serotype Typhi. However, it is difficult to distinguish between disease caused by these serotypes on the basis of clinical features.[7-9] Enteric fever cases including focal epidemics are reported even from Kathmandu Valley, where the capital city is located.[3,5,6,10] This is attributed mainly to improper/sub-standard sanitary conditions and fecal contamination of drinking water.[10,11] The antibiotic sensitivity pattern has also changed.[6,10] In this paper, we reported the bacteriological status of enteric fever among police personnel and their family members along with the antibiogram of *Salmonella* isolates.

MATERIALS AND METHODS

A cross-sectional bacteriological study of enteric fever was done during the monsoon (over a period of 5 months from April to August 2013) at Nepal Police Hospital, Maharajgunj, Kathmandu, Nepal. In this study, all clinically suspected cases of enteric fever who gave blood samples for *Salmonella* culture (n=928) were included.

Blood samples (4-5 ml from adults and 2-3 ml from children) were collected aseptically by clean venipuncture. The collected samples were inoculated into 45 ml sterile bile broth and incubated at 37^{0}C. Sub-culture was done on MacConkey agar on the following

days (for up to 5 days to confirm culture negative). The isolates were identified by microscopic and colonial characteristics, biochemical reactions and sero-typing. Serotyping was done using commercially available polyvalent and monovalent anti-sera (Denka Seiken Co. Ltd, Tokyo, Japan).

All *Salmonella* isolates were subjected to antibiotic susceptibility test (AST) employing Kirby-Baur disc diffusion technique following the Clinical Laboratory Standard Institute (CLSI) guidelines. AST was done using commonly used antibiotics, namely, Ampicillin, Cotri-moxazole, Tetracycline, Chloramphenicol, Ciprofloxacin, Ofloxacin, Nalidixic acid, Ceftriaxone, Azithromycin, Cefexim and Amoxycillin. *Escherichia coli* ATCC 25922 strain was used as a reference strain for quality control.

RESULTS

Of the total 928 blood samples processed, 159 (17.1%) were culture positive. *S. enterica* serotype Typhi was isolated from three-fourth of the cases (74.8%; 119/159) while *S. enterica* serotype Paratyphi A was isolated from the remaining one-fourth (25.2%; 40/159). Mixed growth of *Salmonella* species was not observed. The highest culture positive rate (24.8%) was seen in the age group of 16-30 years, followed by children below 16 years (14.7%) and in the older population above 45 years (6.1%). Lowest culture positive rate (5.4%) was seen in the age group 31-45 years (Table 1). These differences were statistically significant ($p<0.05$). Positive rate in males (19.7%; 131/665) was significantly higher compared with positive rate in females (10.6%; 28/263) ($P<0.05$). The highest blood culture positive rate (n=57; 35.8%) was observed in the month of August (peak monsoon), followed by June (n=52; 32.7%), July (n=29; 18.2%), May (n=11; 6.9%) and lowest in April (most dry month) (n=8; 5.0%) (Fig. 1).

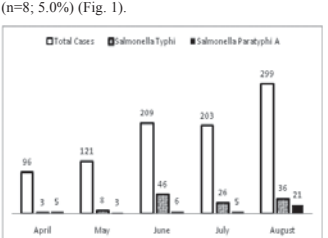

Fig. 1: Monthly distribution of suspected enteric fever and *Salmonella* culture positive cases

Table 1: Blood culture positive rates in different age groups of suspected enteric fever cases

Age group (Yrs)	Total n	Positive n	%	P value
< 16	129	19	14.7	P=0.01
16 - 30	495	123	24.8	
31 - 45	255	14	5.4	
> 45	49	3	6.1	
Total n	928	159	17.1	

Of the 11 different drugs used for AST, Ceftriaxone was the most effective drug (all 159 isolates tested were sensitive i.e. 100% sensitive). The second most effective antibiotic was Amoxycillin (96.6% of *S. enterica* serotype Typhi and 92.5% of *S. enterica* serotype Paratyphi A were sensitive). Altogether, 89.9% of *S. enterica* serotype Typhi and 90.0% of *S. enterica* serotype Paratyphi A isolates were sensitive to the first line antibiotics, namely, Ampicillin, Tetracycline, Cotri-moxazole and Chloramphenicol (ATCoC). On the other hand, altogether 90.5% isolates were resistant to Nalidixic acid (89.9% *S. enterica* serotype Typhi and 92.5% *S. enterica* serotype Paratyphi A were resistant) whereas 50.4% and 15.0% of total *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi A isolates were resistant to Ciprofloxacin, respectively. A total of 53.8% *S. enterica* serotype Typhi and 20% of *S. enterica* serotype Paratyphi A were resistant to Ofloxacin (Table 3).

Table 2: Blood culture positive rates according to gender

Sex	Total n	Positive n	%	P value
Male	665	131	19.7	P=0.01
Female	263	28	10.7	
Total	928	159	17.1	

DISCUSSION

Enteric fever remains one of the major public health problems in developing countries and is attributed to poor sanitation/personal hygiene and consumption of unsafe drinking water.[1,12] In Nepal, an outbreak of enteric fever occurs every year attaining its peak in the monsoon.[2,3,5,13] Though, the present study was not conducted throughout the year, a higher number of cases were observed during the monsoon (June to August) than in summer (April). This correlated well with the high level of contamination of drinking water during the rainy season.[12,14] Such seasonal variation has also been reported by other studies.[15,16]

Table 3: AST pattern of *Salmonella* isolates (Total n=159)

Antibiotic used	*S. enterica* **Typhi (n=119)**		*S. enterica* **Paratyphi A (n=40)**	
	Sensitive (%)	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**
Ceftriaxone	119 (100.0%)	0 (0.0%)	40 (100.0%)	0 (0.0%)
Amoxycillin	115 (96.6%)	4 (3.4%)	37 (92.5%)	3 (7.5%)
Cefixime	108 (90.8%)	11 (9.2%)	38 (95.0%)	2 (5.0%)
Chloramphenicol	107 (89.9%)	12 (10.1%)	37 (92.5%)	3 (7.5%)
Ampicillin	107 (89.9%)	12 (10.1%)	36 (90.0%)	4 (10.0%)
Cotrimoxazole	107 (89.9%)	12 (10.1%)	36 (90.0%)	4 (10.0%)
Tetracycline	107 (89.9%)	12 (10.1%)	36 (90.0%)	4 (10.0%)
Ciprofloxacin	59 (49.6%)	60 (50.4%)	34 (85.0%)	6 (15.0%)
Ofloxacin	55 (46.2%)	64 (53.8%)	32 (80.0%)	8 (20.0%)
Azithromycin	43 (36.1%)	76 (63.9%)	18 (45.0%)	22 (55.0%)
Nalidixic acid	12 (10.1%)	107 (89.9%)	3 (7.5%)	37 (92.5%)

In this study, nearly one-fifth (17.1%) of the blood cultures were positive. This was higher than most of the results reported from Nepal and other Asian countries.[3,5,6,13,17] However, culture positive rates higher (23.1% and 27.3%) than our findings have also been reported by other investigators.[8,18] Such discrepancies in the prevalence rate might be attributed to the difference in sample size, age of the study population and the timing of the study (only rainy season or covering the whole year). Higher culture positive rate found in this study can be attributed to the study period which was during the months preceding and during the monsoon (April to August). High culture positive rates during this period have also been observed by other investigators.[3,9,13]

In this study, *S. enterica* serotype Typhi was predominant (74.8%) whereas *S. enterica* serotype Paratyphi A was isolated in one-fourth (25.2%) of the samples. The isolation rate of *S. enterica* serotype Typhi found in this study was higher compared to most studies conducted in Nepal except Prajapati *et al* which reported 83% *S.* Typhi and 17% *S.* Paratyphi A isolation rate.[13] However, the reported isolation rate of *S. enterica* serotype Typhi from Nepal ranges from 34.6% to 72.0%.[3,5,6,7,10,18,19] These previous studies have reported *S. enterica* serotype Paratyphi A isolation rate ranging from 33% to 65.4 %. Of these, two reports have shown *S. enterica* serotype Paratyphi A as the predominant isolate and this has been assumed to be due to food-borne transmission.[3,5] However, our study along with most others found *S. enterica* serotype Typhi to be predominant.[6,7,10,13,18,19] In general, *S. enterica* serotype Typhi is considered to be mainly transmitted through contaminated water while *S. enterica* serotype Paratyphi A is mainly transmitted through contaminated foods.[20] However, it was interesting to observe variations in the dominant type of isolate even though all of these studies were conducted in the Kathmandu Valley. The reason why these kinds of discrepancies have occurred remains to be elucidated.

Blood culture positive rate was significantly high in young and active age group (16-30 years) in this study. Higher positive rates in this age group have also been reported by other researchers from Nepal.[6,21] However, some of the previous studies in Nepal and other Asian countries have reported the highest culture positive rate and complications in children (<15 years) and adolescents.[3,8,9,13,12,17] Higher blood culture positive rate observed in young and active age group might be due to the exposure of police personnel to the environment from where they might have acquired the infection. In this study, most of the patients were infected by *S. enterica* serotype Typhi (which is believed to be transmitted through water) and this correlated well with the high rate of fecal contamination of drinking water in Kathmandu Valley and other parts of Nepal.[14]

In our study, a third generation Cephalosporin (Ceftriaxone) was found to be the most effective (sensitive to all isolates). This was in agreement with previous findings from Nepal.[3,5,6] However, some researchers have reported Ceftriaxone resistant *Salmonella* isolates as well.[8,13] Thus, health workers must be alert regarding the rationale use of this antibiotic in order to prevent development of drug resistance.

Nalidixic acid resistant *Salmonella* isolates were relatively high in our study. However, this finding was in accordance with most studies conducted in Nepal.[3,6,8,10,18,22] Most importantly, we found a high number of *Salmonella* isolates resistant to the widely preferred Fluoroquinolone group of antibiotics, namely, Ciprofloxacin and Ofloxacin. Association between

Nalidixic acid resistance and reduced susceptibility to Ciprofloxacin among the *Salmonella* isolates has also been reported earlier and Nalidixic acid resistance is thought to be an indication of decreased susceptibility to Ciprofloxacin.[3,23,24] This might be the reason for the increasing number of Ciprofloxacin resistant isolates in Nepal over the past years. Present finding together with previous findings indicated the easy public access to these antibiotics and its irrational use in Nepal. Azithromycin, recommended as an effective alternative drug for the treatment of enteric fever patients was not effective against nearly two-thirds of the isolates in this study. [12]

The higher blood culture positive rate of *S. enterica* serotype Typhi indicates that provision of safe drinking water particularly during the monsoon together with improvement in hygienic and sanitary practices in the community would be effective measures against spread of the disease. Also, the rational use of antibiotics in the treatment of enteric fever must be emphasized. *Salmonella* isolates susceptible to the first line antibiotics (ATCoC) suggested the need for a review of the antibiotic guidelines in the treatment of enteric fever.

ACKNOWLEDGEMENTS

We would like to thank Mr. Saroj Nepal, Tri-Chandra Multiple Campus and the staff of Nepal Police Hospital, especially Mr. Madan Krishna Shrestha and Mr. Rajan Koirala for their kind cooperation and support during the study period.

REFERENCES

1. Crump JA, Luby SP, Mintz ED. The global burden of Typhoid fever. *Bull World Health Organ* 2004; 82: 346-53.
2. Karkey A, Arjyal A, Basnyat B, Stephen B. Still an enteric fever capital of the world. *J Infect Developing Countries* 2008; 2: 461-5.
3. Acharya D, Bhatta DR, Malla S, Dumre SP, Adhikari N, Kandel BP. *Salmonella enterica* Paratyphi A; an emerging cause of febrile illness in Nepal. *Nepal Med Coll J* 2011; 13: 69-73.
4. Maskey AP, Basnyat B, Thwaites GE, Campbell JI, Farrar JJ, Zimmerman MD. Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993-2003. *Trans R Soc Trop Med Hyg* 2008; 102: 91-5.
5. Pokharel P, Rai SK, Karki G, Katuwal A, Vitrakoti R, Shrestha SK. Study of enteric fever and antibiogram of *Salmonella* isolates at a Teaching Hospital in Kathmandu Valley. *Nepal Med Coll J* 2009; 11: 176-78
6. Karki AB, Bhatta DR, Shrestha B *et al*. Higher Nalidixic acid resistance pattern of *Salmonella* isolates from enteric fever patients in Kathmandu Model Hospital, Nepal. *Res J Pharmaceut Biol Chem Sci* 2013; 4: 1687-93.
7. Maskey AP, Day JN, Phung QT *et al*. *Salmonella enterica* serovar Paratyphi A and *S. enterica* serovar Typhi cause indistinguishable clinical syndromes in Kathmandu, Nepal. *Clin Infect Dis* 2006; 9: 1247-53
8. Shakya KN, Baral MR, Shrestha R. A study of atypical manifestation of enteric fever in children. *J Nepal Health Res Council* 2008; 6: 1-4
9. Joshi BG, Keyal K, Pandey R, Shrestha BM. Clinical profile and sensitivity pattern of *Salmonella* serotypes in children: A hospital based study. *J Nepal Paediatr Soc* 2011; 31: 180-3.
10. Rai GK, Karki S, Prajapati B. Is antimicrobial resistance pattern of enteric fever changing in kathmandu valley? *J. Nepal Paediatr Soc* 2012: 32; 221-8.
11. Lewis MD, Serichantalergs O, Pitarangsi C *et al*. Typhoid fever: A massive, single–point source, multidrug-resistant outbreak in Nepal. *Clin Infect Dis* 2005; 40: 554–61.
12. Crump JA, Mintz ED. Global trends in Typhoid and Paratyphoid fever. *Clin Infect Dis* 2010; 50: 241-6.
13. Prajapati B, Rai GK, Rai SK *et al*. Prevalence of *Salmonella typhi* and *paratyphi* infection in children: a hospital based study. *Nepal Med Coll J* 2008; 10: 238-41.
14. Rai SK, Ono K, Yanagida JI, Ishiyama-Imura S, Kurokawa M, Rai CK. A large-scale study of bacterial contamination of drinking water and its public health impact in Nepal. *Nepal Med Coll J* 2012; 3: 234-40.
15. Kelly-Hope LA, Alonso WJ, Thiem VD *et al*. Geographical distribution and risk factors associated with enteric diseases in Vietnam. *Amer J Trop Med Hyg* 2007; 76: 706-12.
16. Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, Bhutta ZA. Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. *Int'l J Infect Dis* 2006; 10: 215-22.
17. Ochiai RL, Wang XY, Seidlein LV *et al*. *Salmonella* Paratyphi A Rates, Asia. *Emerg Infect Dis* 2005; 11: 1764-6.
18. Amatya NM, Shrestha B, Lekhak B. Etiological agents of bacteraemia and antibiotic susceptibility pattern in Kathmandu Model Hospital. *J Nepal Med Assoc* 2007; 46: 112-8.
19. Adhikari D, Acharya D, Shrestha P, Amatya R. Ciprofloxacin susceptibility of *Salmonella enterica* serovar Typhi and Paratyphi A from blood samples of suspected enteric fever patients. *Int'l J Infect Microbiol* 2012; 1: 9-13
20. World Health Organization. Background document: The diagnosis, treatment and prevention of typhoid fever. WHO/V and B/03.07. Geneva: *World Health Organ*; 2003.
21. Sharma N, Koju R, Karmacharya B *et al*. Typhoid fever in Dhulikhel Hospital, Nepal. *Kathmandu Univ Med J* 2003; 2: 188-92.
22. Chau TT, Campbell JI, Galindo CM *et al*. Antimicrobial drug resistance of *Salmonella enterica* serovar Typhi in Asia and molecular mechanism of reduced susceptibility to fluoroquinolones. *J clin Microbiol* 2007; 51: 4315-23.
23. Renuka K, Kapil A, Kabra SK *et al*. Reduced susceptibility to ciprofloxacin and gyrA gene mutation in North Indian strains of *Salmonella enterica* serotype Typhi and serotype Paratyphi A. *Microbiol Drug Resist* 2004; 10: 146-53.
24. Mandal S, DebMandal M, Pal NK. Nalidixic acid susceptibility status of *Salmonella enterica* serovar Typhi isolates from Kolkata, India. *Jundishapur J Microbiol* 2011; 4: 55-60.