

ENTERIC FEVER PATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN CHITWAN, NEPAL

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ABSTRACT

Enteric fever is one of the common clinical conditions in patients presenting to the hospitals. The study was carried out to assess the rate of isolation of common serotypes of enteric fever pathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. A retrospective study was carried out in the laboratory of the Department of Microbiology, Chitwan Medical College Teaching Hospital from 15th June 2009 to 14th June 2010. A total of 4355 blood culture samples received in the laboratory were processed by standard microbiological technique to identify the causative agents and determine their antimicrobial susceptibility pattern. Isolation rate of *Salmonella* species was 0.9%. Among a total of 42 *Salmonella* isolates, 24 (57.1%) isolates were obtained as *Salmonella* Paratyphi A and 18 (42.9%) isolates as *Salmonella* Typhi. Male preponderances were seen in infections caused by both the organisms. On performing antimicrobial susceptibility by Kirby Bauer disk diffusion method, *Salmonella* Paratyphi A demonstrated 100% susceptibility to Amikacin, Chloramphenicol and Ofloxacin while it was least susceptible to Ampicillin (21.7%). Similarly, *Salmonella* Typhi was highly susceptible to Ceftriaxone (94.1%) followed by Ofloxacin (90.9%) and Cephalexin (90%). It was also least susceptible to Ampicillin (29.4%). Multidrug resistance was found to be 16.66% among the *Salmonella* Typhi isolates. Enteric fever is common in Bharatpur. *Salmonella* Paratyphi A is the most common agent of enteric fever in this area. Both *Salmonella* Typhi and *Salmonella* Paratyphi A have developed varying frequencies of resistance against almost all commonly used antimicrobials.

Keywords: Antimicrobial susceptibility, Bharatpur, Enteric fever pathogens.

INTRODUCTION

Enteric fever, that is typhoid and paratyphoid fevers, is the common name for infections caused by *Salmonella enterica* serotypes Typhi and Paratyphi (commonly known as *Salmonella* Typhi and *Salmonella* Paratyphi A respectively)¹. The infection is especially prevalent in developing countries like Nepal with substandard water supply and sanitary systems². According to WHO, 16.6 million cases of enteric fever occurs each year accounting for 600,000 deaths³ primarily in developing countries⁴.

Over the past decade, increasing antibiotic resistance in *Salmonella enterica* has led to a shift in the antibiotics used against this organism from Chloramphenicol and Ampicillin to Trimethoprim - Sulfamethoxazole, Fluoroquinolones (Ofloxacin, Ciprofloxacin), and Ceftriaxone⁵. Even with the use of these antibiotics, the positive response to treatment has only been in the range of 16–40% in Nepal⁶.

Multidrug-resistant *Salmonella* Typhi (MDRST) is epidemiologically defined as strains resistant to any two antimicrobials in vitro even if the antimicrobials tested are known to be clinically ineffective⁷. A more useful definition

of MDRST is reserved for strains resistant to all three firstline antityphoidal antimicrobial agents, namely Ampicillin, Chloramphenicol, and Trimethoprim- Sulphamethoxazole (Co-trimoxazole)⁷.

Detection of common serotypes of enteric fever pathogens and their antimicrobial susceptibility pattern is of utmost importance to institute the effective therapy. After an epidemic of enteric fever occurred in 2002 in Bharatpur where multi drug resistant typhoid affected more than 6000 patients in a 4 to 5 weeks period⁸, any evidence thereafter on the status of isolation and susceptibility pattern of these terrible pathogens in this particular area have not been documented. Therefore, the present study was undertaken to assess the rate of isolation and antimicrobial susceptibility pattern of *Salmonella* species isolated from suspected cases of enteric fever coming to Chitwan Medical College Teaching Hospital, Bharatpur, Nepal.

MATERIAL AND METHODS

A retrospective study was carried out in the Laboratory of Department of Microbiology at Chitwan Medical College

Teaching Hospital, Chitwan, Nepal. During one year period (15th June 2009 to 14th June 2010), a total of 4355 blood culture samples were received in the Laboratory. Brain Heart Infusion (BHI) broth was used as primary culture medium for all blood culture samples. Incubation and subcultures on blood agar and MacConkey agar were done as per the standard methods⁹. Suspected colonies were further processed and identified by biochemical reactions⁹ and confirmed by group and type specific Salmonella antisera.

The antimicrobial susceptibility pattern of the Salmonella isolates was determined by Kirby-Bauer disc diffusion method in compliance with CLSI guidelines¹⁰ on Muller- Hinton agar plates using Amikacin (30µg), Ampicillin (10µg), Ceftriaxone (30µg), Cephalexin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25µg), Gentamicin (10µg) and Ofloxacin (5µg) (Hi Media Laboratory Ltd., Mumbai, India).

RESULTS

The isolation rate of Salmonella species in this study during one year period was found to be 0.9% in total blood culture specimens. Of 191 culture positive bacterial isolates in total, 42 isolates were identified as Salmonella species. Among them, 24 isolates (57.1%) were confirmed as Salmonella Paratyphi A and 18 isolates (42.9%) as Salmonella Typhi. Other serotypes were not isolated.

The following table (Table 1) displays the age wise distribution of the isolates. The highest number of isolates was obtained from the age group 21-30 years and the least from the group aged more than 50 years.

Table 1: Age wise distribution of Salmonella isolates

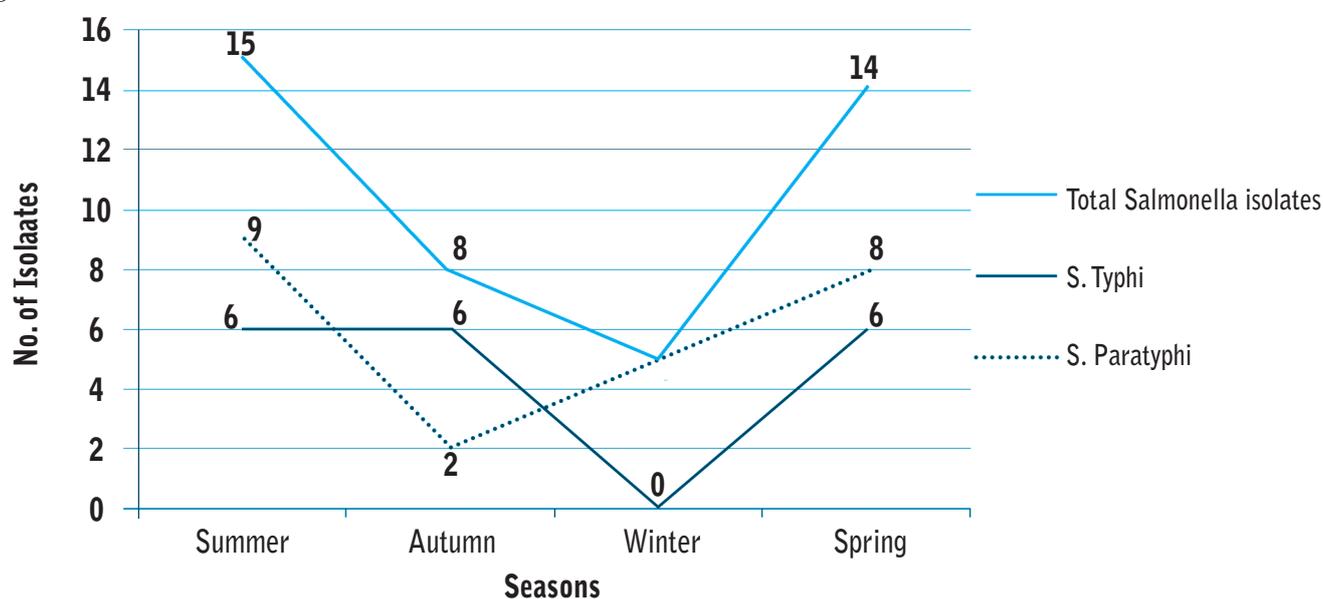
S.N.	Age group (years)	Total enteric fever pathogens		
		Isolated	S. Typhi isolates	S. Paratyphi A isolates
1.	Upto 10 years	4	3	1
2.	11-20 years	7	2	5
3.	21-30 years	21	8	13
4.	31-40 years	7	3	4
5.	41-50 Years	-	-	-
6.	>50 years	3	2	1
	Total	42	18	24

(-) no isolates obtained.

Male preponderances were seen in infections caused by both the organisms (male: female ratio = 2:1 for Salmonella Paratyphi A and 11: 7 for Salmonella Typhi i.e., 27:15 for all Salmonella isolates).

Figure 1: Illustrates the season wise distribution of the Salmonella isolates. Highest number of isolates was observed in Summer followed by Spring season and the lowest was observed in Winter season.

Figure 1: Season wise distribution of the Salmonella isolates



Antimicrobial susceptibility pattern of the Salmonella isolates has been shown in the Tables 2 and 3. Salmonella Paratyphi A demonstrated 100% susceptibility to Amikacin, Chloramphenicol and Ofloxacin while it was least susceptible to Ampicillin (21.7%). Similarly, Salmonella Typhi was highly susceptible to Ceftriaxone (94.1%) followed by Ofloxacin (90.9%) and Cephalexin (90%). Least susceptibility was observed for Ampicillin (29.4%). Moreover, 3 isolates (16.66%) were found to be multidrug resistant among the Salmonella Typhi isolates including one isolate which was resistant to all the antimicrobials tested.

Table 2: Antimicrobial susceptibility pattern of Salmonella Paratyphi A

Antimicrobials used	Antimicrobial susceptibility pattern of Salmonella Paratyphi A			
	No. of isolates tested	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	17	17 (100)	-	-
Ampicillin	23	5 (21.7)	-	18 (78.3)
Ceftriaxone	24	23 (95.8)	-	1 (4.2)
Cephalexin	20	19 (95)	-	1 (5)
Chloramphenicol	20	20 (100)	-	-
Ciprofloxacin	20	19 (95)	-	1 (5)
Co-trimoxazole	19	14 (73.7)	1 (5.2)	4 (21.1)
Gentamicin	23	22 (95.7)	-	1(4.3)
Ofloxacin	9	9 (100)	-	-

(-) no isolates obtained

Table 3: Antimicrobial susceptibility pattern of Salmonella Typhi

Antimicrobials used	Antimicrobial susceptibility pattern of Salmonella Typhi			
	No. of isolates tested	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	13	11 (84.6)	1 (7.7)	1 (7.7)
Ampicillin	17	5 (29.4)	-	12 (70.6)
Ceftriaxone	17	16 (94.1)	-	1 (5.9)
Cephalexin	10	9 (90)	-	1 (10)
Chloramphenicol	15	12 (80)	-	3 (20)
Ciprofloxacin	16	13 (81.3)	-	3 (18.7)
Co-trimoxazole	16	11 (68.8)	-	5 (31.2)
Gentamicin	18	16 (88.9)	-	2 (11.1)
Ofloxacin	11	10 (90.9)	-	1 (9.1)

(-) no isolates obtained

DISCUSSION

Enteric fever is a major health problem in developing countries attributed to poor sanitary and hygienic conditions including lack of potable water¹¹. The disease has remained endemic in different areas of Nepal with outbreaks occurring time and again^{8,12,13}.

Growth positive rate of Salmonella species in our study was 0.9%. Almost similar positive rates have also been reported by Prajapati et al¹³ (2.3%) from Kathmandu and Gupta et al¹⁴ (2.7%) from India. In contrast, high positive rate (23.1%) has been reported by Amatya et al¹⁵ from Kathmandu. Relatively low positive rate observed in this study might be due to the use of antibiotics prior to sample collection.

In contrast to previous reports^{2,12,13}, Salmonella Paratyphi A was more commonly isolated than Salmonella Typhi in the present study. The higher isolation rate of Salmonella Paratyphi A observed in this study indicates an increasing trend of this organism in the recent years. Similar finding was also reported by Maskey et al¹⁶. In their observation, Salmonella Paratyphi A as a proportion of

all Salmonella isolates rose significantly from 23.0% during 1993-1998 to 34.0% in 1999-2003. An increase in enteric fever cases due to Salmonella Paratyphi A has also been reported from neighboring country India¹⁴.

Salmonella infection has been found predominantly in males (64.3%) in this study. Previous reports^{13,17,18} from Nepal have also shown higher prevalence of Salmonella infection in males than in females. More outdoor exposure of males has been given the possible reason for higher positive rate among them¹¹.

In the current study, Salmonella isolates were obtained in the highest number from the age group 21-30 years. Our findings were comparable to that seen by K.C. et al¹⁹ who had observed an average age of presentation of typhoid fever to be 26.1 year. The particular age group seems to be more vulnerable to exposure as they may not be having enough care for their foods and potable drinking water due to their busier schedule than that of other age groups.

We have observed the maximum occurrence of Salmonella infection in the summer followed by spring. An outbreak that had occurred in Bharatpur in 2002 was also reported to occur in the summer⁸. The peak occurrence of enteric fever in summer and rainy season has also been reported by Malla et al¹² and Sharma², though it occurs year round in Nepal.

Drug resistance among enteric fever pathogens is considered to be an important factor in the morbidity and mortality of the disease. It has remained a major challenge for the clinicians as well. The emergence of antibiotic resistant strains of bacteria is closely linked to the irrational use of antibiotics in treating human infections².

Since its introduction in 1948, Chloramphenicol has been the gold standard drug in the treatment of typhoid fever in most parts of the world¹⁹. However, the indiscriminate use of the drug and acquisition of plasmid mediated R factor has led to the development of resistance of Salmonella Typhi against the drug²⁰. Alternative drugs suggested were Co-trimoxazole, Ampicillin and Amoxicillin. During 1990, resistance against Chloramphenicol, Ampicillin and Co-trimoxazole appeared, in 50 to 52.9%²¹. In our study, the incidence of Chloramphenicol resistance was found to be 20% for Salmonella Typhi. The resistance observed by us is much lower than the findings of K.C. et al (63%)¹⁹. However, all the isolates of Salmonella Paratyphi A exhibited susceptibility to Chloramphenicol.

Very high percentage of isolates (70.6% of Salmonella Typhi and 78.3% of Salmonella Paratyphi A) in this study demonstrated resistance to Ampicillin. Our findings are much higher than the resistance observed by Khanal et al from Dharan. It indicates that Ampicillin will have very limited value in treating enteric fever. However, Co-trimoxazole resistance was recorded as 31.2% for Salmonella Typhi and 21.1% for Salmonella Paratyphi A; the observation being

comparable to that of Khanal et al²² (27%) but higher than the finding of K.C. et al (17.4%)¹⁹.

The increase in multi-drug resistant Salmonella is a huge problem in developing countries². In India 64.5% of Salmonella Typhi was reported as being MDR in 1993²³. MDR Salmonella Typhi strains were first reported in Nepal in 2002 during an outbreak of enteric fever in Bharatpur, Nepal⁸. This outbreak of enteric fever was reported to be a large single source outbreak due to multidrug resistant Salmonella Typhi⁸. In the Present study, we have also detected three isolates (16.66%) of MDR Salmonella Typhi including one isolate which was resistant to all antimicrobials tested. The finding clearly highlights a fact that the MDR isolates are still prevalent in the Bharatpur region.

With the emergence of MDR Salmonella Typhi, Quinolone, particularly Fluoroquinolone, has been widely used and recommended as an alternative drug for typhoid fever where the first-line drug is no longer in use²². Its increasing resistance was recorded in both Salmonella Typhi and Salmonella Paratyphi A since the nineties.¹² We have detected 5% resistance against Ciprofloxacin in Salmonella Paratyphi A and 18.7% resistance in Salmonella Typhi. The resistance seen in Salmonella Typhi in our study is much higher than 4.3% resistance recorded by K.C. et al¹⁹. However, none of the Salmonella Paratyphi A and only 9.1% of the Salmonella Typhi isolates in the present study demonstrated resistance against Ofloxacin. The salmonella isolates also exhibited relatively lower resistance against 3rd generation Cephalosporins (Ceftriaxone and Cephalexime) and Aminoglycosides (Amikacin and Gentamicin) than that against the other drugs tested. Detection of the relatively lower resistance indicates that these drugs along with Ofloxacin may still be considered as better options for treatment of enteric fever.

CONCLUSION

Enteric fever remains common in Bharatpur area. Occurrence of Salmonella Paratyphi A infection is more common than that of Salmonella Typhi infection in this region. The infections occur throughout the year with peak incidence in summer. These pathogens have developed varying frequencies of resistance against almost all commonly used antimicrobials. Detection of even multidrug resistant isolates in the present study emphasizes on the need of continuous surveillance on their isolation and susceptibility pattern, prudent use of antimicrobials and institution of proper infection control practices to limit their resistance and chances of any outbreak that may occur in the future.

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