Original Research Paper

Prevalence and Antibiotic Sensitivity test of *Salmonella* Serovars from Enteric Fever Suspected Patients Visiting Alka Hospital, Lalitpur

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Abstract: Enteric fever is still an important public health problem in developing countries including Nepal. A changing antibiotic susceptibility pattern of *Salmonella* Typhi and *Salmonella* Paratyphi A has increased to a great concern. To determine the prevalence and antibiotic sensitivity pattern of *S.* enteric serovars Typhi and Paratyphi from blood specimen in enteric fever suspected culture positive cases. A cross-sectional study was carried out at Alka hospital, lalitpur. Blood culture samples were collected from suspected enteric fever patient and tested microbiologically by standard procedure. AST was based on Kirby disc diffusion method and results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. Of total 37 (6.10%) *Salmonella* serotype isolated from 604 blood culture samples, 25 (67.57%) were *S.* Typhi and 12 (32.43%) were *S.* Paratyphi A. Among the culture positive cases, the incidence rate was high in male 70.27% and the age group of 21-30 years showed maximum number of growth i.e., 14 (37.8%). Among the tested antibiotics *S.* Typhi was fully susceptible towards Ceftriaxone. In case of *S.* Paratyphi A Ciprofloxacin, Cefriaxone and Cefixime showed 100% susceptibility. Resistant pattern of *Salmonella* serovars was seen in higher number 34 (91.9%) in the case of Nalidixic acid. Among 37 *Salmonella* serovars isolated six were found to be Multidrug Resistance (MDR) where four were *Salmonella* Typhi and two were *Salmonella* Paratyphi A. Ceftriaxone and Cefixime was the most effective drugs as no isolates were resistant. Ceftriaxone and Cefixime can be the better choice of antibiotic for *Salmonella* isolates and remains the last drug of choice for *S.* Typhi and *S.* Paratyphi A which are also resistant to the first line agents.

Keywords: Enteric Fever, Nalidixic Acid Resistant *Salmonella*, Multidrug Resistance

Introduction

Enteric fever is a systemic infection caused by the human adapted pathogens *Salmonella enterica* serotype Typhi (*S.* Typhi) and *S. enterica* serotype Paratyphi (*S.* Paratyphi) A, B and C (Crump and Mintz, 2010). Enteric fever remains one of the major public health issues globally, especially in Asia. According to recently revised global estimate, above 22 million cases of typhoid fever occur each year round the world while 90% of the sufferers are from the South East Asia (Rahman *et al.*, 2011). Its incidence is highest in children and young adults between 5 and 19 years old (WHO, 2007). In context of Nepal, enteric fever caused by *Salmonella enterica* serovar Typhi and Paratyphi A is the most common clinical diagnosis among febrile patients (Maskey *et al.*, 2008).

A changing antibiotic susceptibility pattern of *Salmonella* isolates and emergence of *Salmonella* Paratyphi A as a cause of enteric fever has increased to a great concern. So, this study was undertaken to isolate and identify *S.* Typhi and *S.* Paratyphi and their antibiotic sensitivity pattern which are the major cause of enteric fever in the developing countries like Nepal.

Methods

The study was carried out at Alka hospital, Lalitpur from 15th of March to 15th of September, 2014.

Blood culture samples were collected visiting the hospital. Brain-Heart Infusion (BHI) broth was used as enrichment media which supports the growth of all common pathogens causing enteric fever. Collection of blood, incubation and subcultures onto blood agar and Mac-Conkey agar were done as per the standard methods (Cheesbrough, 2006). Suspected non-lactose-fermenting colonies were further processed and identified by biochemical tests like Catalase test, Citrate test, Triple Sugar iron test, Urease test, Motility test, Methyl-Red and Voges-Proskauer test, Gas production test, Sulfide production test and confirmed (Chakraborty, 2007). Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method (Collee *et al*., 2006). The antibiotic discs used were Cotrimoxazole (25 mcg), Ciprofloxacin (5 mcg), Ceftriaxone (30 mcg), Azithromycin (15 mcg), Nalidixic acid (30 mcg), Amoxycillin (30 mcg), Cefixime (5 mcg) and Ofloxacin (5 mcg). Result was interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. Data were entered in Microsoft Excel and analyzed by SPSS version 16.0.

Results

A total of 604 patients suspected of enteric fever were studied, among them 6.10% were culture positive. Out of 37 *Salmonella* isolates, 25 (67.57%) were *S*. Typhi and 12 (32.43%) were *S*. Paratyphi A. Among the culture positive cases, the incidence rate was high in male 70.27% than female 29.73% and the age group of 21-30 years showed maximum number of growth i.e., 14. However, the association of organism isolated with respect to different age groups and gender was found to be statistically insignificant (p>0.05). Most of the isolates (27.0%) were isolated in the month August and September and the association was statistically insignificant (p>0.05). Ceftriaxone was found to be 100% effective in all the *S*. Typhi isolates. It was followed by Cefixime (96%) and Cotrimoxazole with 88% susceptibility (Table 1-3.2).

Table 1. Age wise distribution of *Salmonella* serovars

Age in years	Organisms		Total no
	S. Typhi	*S*. Paratyphi A	
0-10	4	4	8
11-20	10	3	13
21-30	10	4	14
31-40	1	0	1
41-50	0	1	1

Table 1.1. Gender wise distribution of *Salmonella* serovars

Sex of the patient	Organism		Total (%)
	S. Typhi	*S*. Paratyphi A	
Male	19	7	26(70.2)
Female	6	5	11(29.7)

Table 1.2. Month wise distribution of isolates

Months	Organism isolated		Total no (%)
	S. Typhi	*S*. Paratyphi A	
March/April	3	2	5 (13.5)
April/May	3	1	4 (10.8)
May/June	4	2	6 (16.21)
June/July	4	1	5 (13.56)
July/August	5	2	7 (18.91)
August/Sep.	6	4	10 (27.0)

Table 2. Antibiotic susceptibility pattern of *Salmonella* isolates

Antibiotic used	Antibiotic susceptibility Pattern	
	S. Typhi	*S*. Paratyphi A
Cotrimoxazole	22(88%)	10(83.33%)
Cefixime	24(96%)	12(100)
Amoxycillin	19(76%)	10(83.33%)
Ciprofloxacin	15(60%)	12(100)
Azithromycin	18(72%)	8(66.67%)
Nalidixic acid	1(4%)	0
Ceftriaxone	25(100%)	25(100%)
Ofloxacin	17(68%)	9(75%)

Table 3. Multidrug resistant isolates among *Salmonella*

Organism	Number	MDR isolates	Total (%)
S. Typhi	25	4	16.0
S. Paratyphi A	12	2	16.7
Total	37	6	16.2

Table 3.1. Antibiotic susceptibility pattern of multidrug resistant *Salmonella* Typhi

Antibiotic used	Antibiotic susceptibility pattern of multidrug resistant *Salmonella* Typhi (n = 4)			
	Multidrug resistant *S*. Typhi$_1$	Multidrug resistant *S*. Typhi$_2$	Multidrug resistant *S*. Typhi$_3$	Multidrug resistant *S*. Typhi$_4$
Cotrimoxazole	Resistant	Resistant	Resistant	Sensitive
Cefixime	Sensitive	Sensitive	Sensitive	Sensitive
Amoxycillin	Resistant	Resiatant	Resistant	Resistant
Ciprofloxacin	Sensitive	Resistant	Resistant	Sensitive
Azithromycin	Sensitive	Sensitive	Resistant	Resistant
Nalidixic acid	Resistant	Resiatant	Resistant	Resistant
Ceftriaxone	Sensitive	Sensitive	Sensitive	Sensitive
Ofloxacin	Resistant	Resistant	Sensitive	Resistant

Table 3.2. Antibiotic susceptibility pattern of multidrug resistant *Salmonella* Paratyphi A

Antibiotic used	Antibiotic susceptibility pattern of multidrug resistant *Salmonella* Paratyphi A	
	Multidrug resistant Paratyphi A_1	Multidrug resistant Paratyphi A_2
Cotrimoxazole	Sensitive	Sensitive
Cefixime	Sensitive	Sensitive
Amoxycillin	Resistant	Resistant
Ciprofloxacin	Sensitive	Sensitive
Azithromycin	Sensitive	Resistant
Nalidixic acid	Resistant	Resistant
Ceftriaxone	Sensitive	Sensitive
Ofloxacin	Resistant	Resistant

In case of *S*. Paratyphi A all the isolates were sensitive to Ceftriaxone, Ciprofloxacin and Cefixime. Among 37 isolates six isolates were found to be multi drug resistance where 4 were *Salmonella* Typhi and 2 isolates were *Salmonella* Paratyphi A.

Discussion

In this study, out of 604 blood culture samples, 37 (6.10%) were culture positive. Similar results have been reported by Acharya *et al*. (2012; Adhikari *et al*., 2012) who found 7.6 and 8.99% culture positive result respectively. However, the low isolation of the pathogen in blood culture may be due to the partial treatment received elsewhere before coming to the hospital Shakva *et al*. (2008) and our reliance upon a single blood culture for diagnosis.

Out of 37 isolates 25 (67.57%) were *S*. Typhi and 12 (32.43%) were *S*. Paratyphi A. The most diagnosed enteric fever cases were in the month of August and September. The association was statically insignificant (p>0.05). This may be possibly due to the sewage-mediated contamination of water sample during the rainy seasons (WHO, 2003).

Our study showed 100% susceptibility of Ceftriaxone for both *S*. Typhi and *S*. Paratyphi A strains Raza *et al*. (2012; Khatiwada, 2006) revealed that Ceftriaxone is found to be one the most effective (100%) antibiotics against *Salmonella* Paratyphi A Ceftriaxone remains the last line drug against infections with Ciprofloxacin resistant *Salmonella* when it is resistant to other first line drug.

Salmonella Typhi and *Salmonella* Paratyphi A were found to be 72 and 66.67% susceptible towards Azithromycin respectively. The emerging resistant of Azithromycin has also been reported in India by Choudhary *et al*. (2013) This may be the result of misuse of Azithromycin that has been propelled due to its oral route of administration, as well as broad-spectrum antimicrobial activity with minimal side effects and interactions (Capoor and Nair, 2010).

The susceptibility pattern of Ofloxacin was 68% for *S*. Typhi and 75% for *S*. Paratyphi A respectively. Out of 37 isolates 34 (91.9%) were Nalidixic acid resistant. *S*.

Typhi strains showed higher rate of resistance (92%) towards Nalidixic acid than *S*. Paratyphi A (91.67%). The study shows the emergence of Nalidixic acid resistant strains. It is a serious matter related to the health as most of the hospital at remote areas of Nepal don't rely on the sensitivity test due to unavailability and prescribe Ciprofloxacin and Ofloxacin blindly. Other factors for the emergence of Nalidixic acid include self prescription by patients and incomplete course of treatment Pokharel *et al*. (2006).

The present study showed that only 2 *S*. Paratyphi MDR isolate was found resistant to Amoxycillin, Ofloxacin, Azithromycin and Nalidixic acid. Similarly, 4 MDR isolate was found in *S*. Typhi. Besides being resistant to Amoxycillin, Ofloxacin, Azithromycin and Nalidixic acid, it was found to be resistant to Ciprofloxacin and Cotrimoxazole. In a study carried out in Nepal, all the *S*. Typhi and *S*. Paratyphi A isolates were reported as susceptible until 1998 but during 1999 to 2003 Ciprofloxacin resistance increase 5% in the *S*. Typhi and 13% in *S*. Paratyphi A Maskey *et al*. (2008). This increased resistance reflects the overuse of Ciprofloxacin in the treatment of typhoid, as well as in other unrelated infections. Incomplete treatment may also be a factor contributing to development of resistance.

Conclusion

Cotrimoxazole and Amoxycillin resistant by *Salmonella* were found to be minimal, so these drugs may also be useful option for treatment of enteric fever. However, Cefixime and Ceftriaxone was cent percent susceptible towards *Salmonella*. Hence Cefixime remain as oral drug of choice and Ceftriaxone remain intravenous option for treatment of typhoid fever.

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Author's Contributions

Kalpana Pandey: Conducted all laboratory experiments, co-ordinated the data analysis and interpretation of the data and contributed to the manuscript writing.

Vijay K. Sharma: Designed the research conception, organized the study and critically reviewed the manuscript.

Roshani Maharjan: Contributed in the data analysis and interpretation of the data and also assisted in manuscript writing.

Ethics

The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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