Antibiotic Susceptibility Pattern of *Salmonella enterica* serovars Typhi and Paratyphi A Isolated from Patients Suspected of Enteric Fever

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ABSTRACT

Objectives: The study aimed to assess the antibiotic susceptibility profile of *Salmonella* spp isolated from patients suspected of enteric fever.

Methods: This cross-sectional prospective study was carried out from April to June, 2014among 484 patients clinically suspected of enteric fever visiting Bir Hospital, Kathmandu, Nepal. Blood sample collected from each patient was processed for culture in bile broth. Identification of *Salmonella* spp was done by conventional microbiological techniques including colony characteristics, Gram's staining and biochemical tests. Antibiotic susceptibility testing of identified isolates was done by Kirby-Bauer disk diffusion method following the 2014 CLSI guideline.

Results: Out of 484 blood samples, 36 (7.43%) cases showed the growth of *Salmonella* spp. of which 27 (75%) were *Salmonella enterica* serovar Typhi (ST) and 9 (25%) were *Salmonella enterica* Paratyphi A (SPA). Among the *Salmonella* isolates, 5.55% were multidrug resistant and 41.66% were fluoroquinolone resistant. More than 80% of isolates were sensitive to chloramphenicol, amoxicillin, and cotrimoxazole whereas 58%, 50% and 6% of isolates were sensitive to fluoroquinolone antibiotics i.e. ciprofloxacin, ofloxacin and nalidixic acid respectively. All the isolates were susceptible to ceftazidime. All SPA and 89% of ST were sensitive to azithromycin.

Conclusion: Higher percentage of susceptible isolates to chloramphenicol, cotrimoxazole, and amoxicillin suggests the reconsideration of these antibiotics for the treatment of enteric fever. Azithromycin can be considered as drug of choice for the treatment of enteric fever.

Keywords: Enteric fever, *Salmonella* isolates, MDR, antibiotic susceptibility

INTRODUCTION

Enteric fever, a febrile disease caused by the *Salmonella enterica* serovars Typhi and Paratyphi A, B, and C, causes 21 million new infections and claims 161,000 lives each year worldwide (WHO 2019). It is a systemic disease, endemic in developing countries like Nepal (Crump and Mintz 2010). Definitive diagnosis of enteric fever is done by the isolation of organisms from the blood and bone marrow (Gasem et al. 1995). Serological tests (Widal test) can also be done, however, they are not reliable due to false-positive results. DNA probes and PCR can be used to detect organism from the blood, however, their use in the developing countries is not feasible due to high cost (Parry et al. 2002).

Initially, ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole (cotrimoxazole) had been used as a first-line drug for the treatment of enteric fever. *Salmonella enterica* serovars Typhi and Paratyphi resistant to these three first-line antibiotics are called as multidrug-resistant (MDR) strains (Crump et al. 2015). With the increase in MDR strains, fluoroquinolones

(ofloxacin, nalidixic acid, ciprofloxacin etc.) became the drug of choice for the treatment of enteric fever. However, the dramatic increase in fluoroquinolone-resistant strains has been observed after 2000 (Mirza and Khan 2008). Therefore, third-generation cephalosporins (cefixime, cefalexin, ceftazidime etc.), and azithromycin have been used recently for the treatment of MDR, and fluoroquinolone-resistant *Salmonella* strains (Effa et al. 2011) though sporadic cases of resistance have been observed for ceftriaxone and azithromycin (Kobayashi et al. 2014, Parry et al. 2015).

In Kathmandu, the burden of the enteric fever is high and is the leading cause of febrile illness (Karkey et al. 2008). Lack of proper diagnosis of disease, on one hand, and continuous development of antimicrobial resistance, on the other hand, are the issues of major concern in the countries of low economic settings like Nepal (Parry et al. 2011). Therefore, this study aims to assess the antibiotic resistance among *Salmonella* spp isolated from febrile cases in a tertiary care hospital of Kathmandu, Nepal.

MATERIALS AND METHODS

This cross-sectional prospective study was carried out from April to June, 2014 among 484 clinically suspected enteric fever patients visiting Bir Hospital, a tertiary hospital of Nepal. From each patient, 5-10 ml of blood sample was collected aseptically by vein puncture and inoculated directly into a bottle containing bile broth. Each sample was incubated at 37°C for 48 hours and

subcultured into Blood Agar (BA) and Mac-Conkey Agar (MA). Each sample was cultured till 7 days to consider as negative. Identification of *Salmonella* spp was done based on colony characteristics, Gram's staining, catalase, oxidase and other biochemical tests (Cheesbrough 2009). The antibiotic susceptibility pattern of isolates was done in Muller Hinton Agar by Kirby-Bauer disk diffusion method following CLSI guidelines (CLSI 2014). The antimicrobial susceptibility of 10 antimicrobial agents- amoxicillin (amx) (30μg), chloramphenicol (cpl, 30μg), cotrimoxazole (TMP-SMX, 25μg), nalidixic acid (nal, 30μg), ciprofloxacin (cip, 5μg), ofloxacin (ofx, 5μg), cefixime (cfm, 5μg), azithromycin (azm, 15μg), cefalexin (cfx, 30μg), and ceftazidime (caz, 30μg) (Hi-Media Laboratory Ltd, Mumbai, India) were performed. The results were interpreted as sensitive, intermediate, or resistant based on the size of zone of inhibition and comparing it with the standard chart provided by the manufacturing company. (CLSI 2014).

RESULTS

Out of 484 blood samples, 36 (7.43%) were positive for *Salmonella* spp of which 27 (75%) were *Salmonella enterica* serovar Typhi (ST) and 9 (25%) were *Salmonella enterica* serovarParatyphi A (SPA).

Most of the isolates were from patients in the age group 21-30 years followed by the age group 11-20 years. Similarly, male patients (8.27%) were found to be more susceptible to *Salmonella* infection than female patients (6.18%) but was not statistically significant (Table 1).

Table 1: Age and gender wise distribution of total and positive cases for *Salmonella* spp

Age group in years	Number of cases tested		Number of positive cases			
			Salmonella spp		*Salmonella* Typhi	*Salmonella* Paratyphi A
	Male	Female	Male	Female		
<10	1	3	0	0	0	0
11-20	51	39	7	4	8	3
21-30	79	41	15	6	16	5
31-40	49	27	0	1	1	0
41-50	33	20	1	0	0	1
51-60	27	32	0	0	0	0
61-70	29	19	1	1	2	0
>70	21	13	0	0	0	0
Total	290	194	24	12	27	9

More than 80% of isolates were sensitive to chloramphenicol, amoxicillin, and cotrimoxazole whereas 58%, 50% and 6% of isolates were sensitive to fluoroquinolone antibiotics i.e ciprofloxacin, ofloxacin and nalidixic acid respectively. All the isolates

were sensitive to third-generation cephalosporin, ceftazidime. To azithromycin, 89% of ST were sensitive and 11% were intermediately sensitive whereas 100% of SPA were sensitive (Table 2).

32

Table 2: Antibiotic susceptibility pattern of *Salmonella* isolates

Antibiotics	*Salmonella* Typhi (n=27)			*Salmonella* Paratyphi A (n=9)		
	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Amoxycillin	22 (81)	-	5 (19)	7 (78)	-	2 (22)
Cotrimoxazole	26 (96)	-	1 (4)	8 (89)	-	1 (11)
Chloramphenicol	26 (96)	-	1 (4)	8 (89)	-	1 (11)
Ciprofloxacin	12 (44.5)	3 (11)	12 (44.5)	6 (67)	-	3 (33)
Ofloxacin	11 (41)	2 (7)	14 (52)	5 (56)	-	4 (44)
Nalidixic acid	2 (7)	-	25 (93)	-	-	9 (100)
Cefixime	25 (93)	-	2 (7)	9 (100)	-	-
Cefalexin	24 (89)	3 (11)	-	6 (67)	-	3 (33)
Ceftazidime	27 (100)	-	-	9 (100)	-	-
Azithromycin	24 (89)	3 (11)	-	9 (100)	-	-

Among the *Salmonella* isolates, 5.55% were multidrug resistant and 41.66% were fluoroquinolone resistant (Table 2).

Table 3: Multidrug resistant (MDR) and Fluroquinolone resistant (FQR) *Salmonella* isolates

Salmonella serovar	Total isolates	MDR n (%)	FQR n (%)
Salmonella Typhi (n=27)	27	1 (3.71%)	12 (44.44%)
Salmonella Paratyphi A (n=9)	9	1 (11.11%)	3 (33.33%)
Total	36	2 (5.55%)	15 (41.66%)

DISCUSSION

Higher prevalence of *Salmonella* Typhi and *Salmonella* Paratyphi A in our study was similar to that reported by Petersiel et al. (Petersiel, Shresta et al. 2018).. In contrast, Pokhrel et al. found that the prevalence of ST (47%) infection was lower than SPA (53%) (Pokharel et al. 2006). A similar study reported that the paratyphoid fever was associated with flood and contaminated street vendor's food, whereas, typhoid fever was associated with household contamination (Woods et al. 2006). So, we expect most of the patients in our study were associated with household contamination. The proportion of enteric fever positive male patients (66.67%) was higher than female patients (33.33%) . A similar study had the proportion of enteric fever positive male patients higher than female patients (Amatya et al. 2007). Males have greater chances of acquiring enteric fever than females, probably due to the indiscriminate eating habits of the male in the roadside locations. The age group 21-30 years had the highest culture positivity (58.33%) similar to previous studies in Nepal (Adhikari et al. 2012).

Amoxicillin,cotrimoxazole, and chloramphenicol had been used previously as the first-line drug against the *Salmonella* infection. However, resistance to these first-line drugs has been reported(Ochiai et al. 2008). We found only 5.55% of the MDR *Salmonella* isolates (ST-3.71%, SPA-11.11%). Chloramphenicol was used previously (since the 1940s) as a gold standard for the treatment of enteric fever, however, due to the emergence of resistance, it is no longer a drug of choice for the treatment of enteric fever (Mandal et al. 2004). Our study shows that both the serovars of *Salmonella* were highly susceptible to chloramphenicol (ST-96%, SPA-89%) which is in agreement to another similar study conducted in Nepal (Amatya et al. 2007). The sensitivity of ST and SPA toward cotrimoxazole was found to be 96% and 89% respectively; similar findings were obtained by two different studies (Murdoch et al. 2004, Amatya et al. 2007). Both the MDR isolates were resistant to cotrimoxazole which is in agreement with the finding of Amatya et al. (100% resistant) (Amatya et al. 2007). Antibiotic susceptibility pattern of ST and SPA for amoxicillin were 81% and 78% respectively, this pattern corresponds with the finding by Amatya et al. (ST- 75% and SPA- 49%) (Amatya et al. 2007). Hence, increased susceptibility of ST and SPA towards amoxicillin, cotrimoxazole, and chloramphenicol over the period in Nepal (Karki et al. 2013) mandates the reconsideration of these antibiotics for the treatment of enteric fever.

The resistance to chloramphenicol and amoxicillin during the 1990s led to the extensive use of fluoroquinolones, such as ciprofloxacin, ofloxacin, and nalidixic acid, effective against ST and SPA (Parry et al. 2002). Our study showed that 44.44% of ST and

33.33% of SPA were resistant to fluoroquinolone antibiotics. Various studies in a different part of the world showed the higher effectiveness of the above-mentioned fluoroquinolone antibiotics against ST and SPA (Gales et al. 2002, Wain et al. 2003, Maskey et al. 2008), contradicting our study. However, in agreement with our findings, Pokharel et al. found the decreased susceptibility of ST and SPA against fluoroquinolone(Pokharel et al. 2006). Similarly, Karki et al. in a review article reported the decreased susceptibility of ST and SPA against a range of fluoroquinolone antibiotics (Karki et al. 2013). The susceptibility towards ciprofloxacin (ST- 44.5%, SPA-67%) in our study corresponds to the study by Pokharel et al. (ST- 57%, SPA- 0%), while incongruent to a study by Amatya et al. (ST-93.59%, SPA-79.54%) (Pokharel et al. 2006, Amatya et al. 2007). The development of resistance towards ciprofloxacin in Nepal is due to the easy availability of ciprofloxacin in the drug store, self-prescription by the patients, and incomplete course of treatment (Pokharel et al. 2006).

Nalidixic acid resistance is considered as the phenotypic marker for the reduced susceptibility to fluoroquinolone. Our study shows that the resistance against nalidixic acid was the highest (ST-93%, SPA-100%) among all the tested antibiotics. In agreement with our study, a recent study was done by Adhikari et al. in the year 2011, found the increased resistance of nalidixic acid antibiotic (ST-82.9%, SPA-91.33%) (Adhikari et al. 2012). Similarly, Maskey et al. in 2008 (ST-49%, SPA-86%), and Neopane et al. in 2007 (ST-73.3%, SPA-94.9%) reported the growing resistance of ST and SPA to nalidixic acid antibiotic (Neopane et al. 2007, Maskey et al. 2008). Nalidixic acid had been used as anindicator of decreased ciprofloxacin susceptibility. Our study also shows a similar trend, however, it is suggested that the determination of minimum inhibitory concentration (MIC) as a reliable indicator (Crump et al. 2003).

The third-generation cephalosporins (cefixime, cefalexin, and ceftazidime) were effective against ST and SPA, as the susceptibility of both *Salmonella enterica* serovars were quite higher. Susceptibility of ST was 89% and the SPA was 100% to cefixime.Astudy reported the susceptibility of ST and SPA to cefixime was found to be 75% and 100% respectively (Amatya et al. 2007). Cefixime, an oral antibiotic, is widely used for the treatment of enteric fever as a first-line drug (Pandit

et al. 2007). As we found two ST isolates resistant against cefixime, which is a worrisome finding, similar to anotherstudy(Qamar et al. 2014). Therefore, further research should be done before blindly prescribing third-generation cephalosporins for the treatment of MDR and FQR *Salmonella* isolates. Ceftazidime was most effective as both ST and SPA were found to be 100% susceptible to it. In a similar study done in ST, Hasan et al. reported that ceftriaxone and ceftazidime were the most effective antibiotics (100% susceptibility) (Hasan et al. 2011). However, mode of administration (intravenous and intramuscular) make ceftazidime a less famous antibiotic, with regards to difficulty in administering to the outpatients.

Furthermore, both ST (89% susceptible, 11% intermediate susceptible) and SPA (100% susceptible) are found to be highly susceptible to azithromycin antibiotic. In agreement with our study, Kumar et al. found the susceptibility of ST and SPA to azithromycin to be 93.6% and 100% respectively (Kumar et al. 2008). Similarly, another study shows that the antibiotic susceptibility of STwas found to be 81.25% to azithromycin(Hasan et al. 2011). Another study also highlighted that azithromycin is better in terms of fever clearance, and relapse rate for the treatment of enteric fever caused by MDR and FQR *Salmonella* isolates (Shah 2009). Hence, our study revealed that azithromycin can be an alternative solution for the MDR and FQR *Salmonella* isolates.

CONCLUSION

Higher percentage of susceptible isolates to chloramphenicol, cotrimoxazole, and amoxicillin suggests the reconsideration of these antibiotics for the treatment of enteric fever. Azithromycin can be considered as drug of choice for the treatment of enteric fever.

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