Antimicrobial Susceptibility Profile of *Salmonella enterica* Serovars and Determination of Minimum Inhibitory Concentration of Ciprofloxacin and Cefotaxime

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ABSTRACT

Objectives: To study the antibiotic susceptibility profile of *Salmonella enterica* serovar Typhi and Paratyphi A and determine the minimum inhibitory concentration of Cefotaxime and Ciprofloxacin.

Methods: Blood samples from patients suspected of enteric fever were cultured following standard microbiological techniques. Antibiotic susceptibility tests for commonly used drugs were performed by modified Kirby Bauer disc diffusion technique following the guidelines of Clinical and Laboratory Standard Institute (CLSI). Determination of minimum inhibitory concentration (MIC) was done by agar dilution method.

Results: A total of 1,328 blood samples were processed out of which 64 were culture positive. Among the 33 isolates of *Salmonella enterica*, 22 were *Salmonella* Typhi while the rest 11 were *Salmonella* Paratyphi A. This study showed that 96.96% of isolates were resistant to Nalidixic acid and 87.87% were resistant to Ciprofloxacin. In contrast, 93.93% isolates were sensitive to Ampicillin and 90.90% were sensitive to Cefixime. 65.62% isolates were resistant with MIC ≥1 µg/ml against Ciprofloxacin while against Cefotaxime, 53.12% isolates were sensitive with MIC ≤1 µg/ml.

Conclusion: Fluoroquinolones have shown the reduced susceptibility towards *Salmonella* Typhi and *Salmonella* Paratyphi A making them inappropriate to use for treating enteric fever. Ampicillin and Cefixime were found to be highly susceptible suggesting them for possible treatment. However, susceptibility testing must be done before the administration of any antibiotics.

Keywords: Enteric fever, antibiotic resistance, *Salmonella* spp, MIC.

INTRODUCTION

Enteric fever is a serious public health issue in developing countries of tropical regions where there is a lack of safe drinking water, sanitation, and personal hygiene practices (Bhetwal et al. 2017; Nagshetty et al. 2010). Enteric fever is a collective term for typhoid fever and paratyphoid fever caused by *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A, B, and C respectively. It is the leading cause of morbidity and mortality in the South Asian region causing approximately 21.7 million illnesses by typhoid and 5.4 million cases of paratyphoid fever per year with 217,000 deaths worldwide (Harish and Menezes 2011). In Nepal, enteric fever is still a leading cause of inpatient morbidities (DoHS 2020) and is

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often associated with fecal contamination of food and drinking water (Pokharel et al. 2009).

Enteric fever on appropriate antimicrobial therapy diminishes within a few days with a fatality rate below 1%, however, without treatment illness persists more than a month with mortality rate upto 30% (Mohanty et al. 2006). Chloramphenicol was considered as the gold standard therapy for enteric fever (Bhatia et al. 2007), however, the first line of drugs Ampicillin, Cotrimoxazole including Chloramphenicol began to develop resistance making them inappropriate for treatment of enteric fever. Afterwards, fluoroquinolones started to emerge as treatment of choices for the disease (Shrestha et al. 2016). After the introduction of fluoroquinolones as a regimen for enteric fever, Nalidixic acid resistant isolates associated with decreased susceptibility to fluoroquinolones have been increasingly reported in Nepal (Bhetwal et al. 2017; Khanal et al. 2017). Some high fluoroquinolones resistant and third-generation cephalosporin resistant *Salmonella* Typhi and *Salmonella* Paratyphi A have been reported sporadically in Nepal (Chau et al. 2007; Shirakawa et al. 2006). The prescription of third generation cephalosporins and Azithromycin has added treatment options (Chand *et al*., 2014). However, the re-emergence of susceptibility towards conventional first line of drugs has been published which has raised expectations in the antimicrobial armamentarium (Bhetwal et al. 2017; Chand et al. 2014; Khanal et al. 2017). But the irrational use of these antibiotics may again lead to the resistance in near future (Patil and Mule, 2019).

The change in susceptibility pattern over a time has led to difficulties in effective treatment of enteric fever. This has made it mandatory for determining the sensitivity of a drug before initiating the treatment. Thus, this study is intended to study the antimicrobial susceptibility profile of *Salmonella enterica* isolated from the patients suspected with enteric fever and determine the minimum inhibitory concentration (MIC) of Cefotaxime and Ciprofloxacin at a tertiary care hospital in Kathmandu.

METHODS

A hospital-based cross-sectional study was carried out at Manmohan Memorial Medical College and Teaching Hospital, Kathmandu from February 2020 to October 2020.

Bacterial culture and identification:

The blood of the patients suspected of enteric fever was collected following standard methods (Isenberg 2004). Both inpatients and outpatients were included in the study regardless of age and gender. Duplicate samples and samples other than blood were disregarded. The blood sample was collected aseptically and immediately cultured in Brain Heart Infusion broth at 37^0C for 24 hours followed by subculture on MacConkey agar and Blood agar. Isolation and identification of *Salmonella enterica* were done based on colony morphology, gram staining, and biochemical tests following standard microbiological techniques (Isenberg 2004). All culture media and biochemical media used were from Hi Media Laboratories, India.

Antibiotic susceptibility testing:

The antibiotic susceptibility profile of the isolates was determined by the modified Kirby-Bauer disc diffusion technique on Mueller-Hinton Agar, (HiMedia Laboratories, India) following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2019). The commonly prescribed antibiotics were used which includes, Ampicillin (10µg), Azithromycin (15µg), Cefotaxime (30µg), Cefixime (5µg) Nalidixic acid (30µg), and Ciprofloxacin (5µg). Multidrug-resistant isolates were further tested with Imipenem (10µg), Meropenem (10µg), Amikacin (30µg), Piperacillin/Tazobactam (100/10µg), Polymyxin B (10µg), Colistin Sulphate (10µg) and Chloramphenicol (30µg). The interpretation of the results was done based on the interpretative chart suggested by CLSI guidelines (CLSI 2019).

Minimum inhibitory concentration (MIC)

The MIC of Cefotaxime and Ciprofloxacin was carried out by agar dilution method as suggested by Andrews (2001) based on CLSI guidelines (CLSI 2019). *Escherichia coli* ATCC 25922 was used as a standard strain in each batch of the experiment. The 0.5 McFarland standard was used for the standardization of inoculum before antibiotic susceptibility test in Mueller Hinton Agar.

Data analysis:

Data obtained throughout the study were entered in Microsoft Excel 2019 and analysis were done by using both Microsoft excel and Statistical Package for the Social Sciences (SPSS) version 23.0.

Ethical Approval and Consent:

Ethical approval Ref:170/077/078 was taken from Manmohan Memorial Teaching Hospital after submission of the research proposal and informed consent was taken from the patients before enrolling them in this study.

RESULTS

A total of 1,328 blood samples were processed, of which, 33 (2.48%) were culture positive for *S. enterica*. Of the total *Salmonella enterica*, 22 (66.67%) isolates were *S. enterica* serovar Typhi and 11 (33.33%) were *S. enterica* serovar Paratyphi A. Out of total patients with positive results, 21 (63.63%) were males as shown in Figure 1.

The patients between age 11-30 years were affected in high proportion with 25 (75.8%) individuals. Similarly, the number of males is greater in most of the age groups. The age gender distribution is illustrated in Figure 2.

In the antibiotic susceptibility profile (Table 1), the highest number of isolates i.e., 32 (96.96%) isolates showed resistance towards Nalidixic Acid followed by Ciprofloxacin in which 29 (87.87%) isolates were resistant. Ampicillin and Cefixime were highly effective, 31 (93.93%) isolates were susceptible to Ampicillin and 30 (90.90%) isolates were sensitive to Cefixime. Moderate sensitivity of *Salmonella enterica* against Azithromycin and Cefotaxime was observed with 19 (57.57%) isolates sensitive to Azithromycin and 18 (54.54%) isolates sensitive to Cefotaxime.

Out of 33 isolates, 10 (30.30%) of them were found to be multidrug resistant (MDR), i.e., resistant to any one drug of at least three different antibiotics categories. Their susceptibility was tested further against various antibiotics (Table 2). All 10 (100%) MDR isolates were sensitive to Chloramphenicol. Imipenem, Meropenem, Polymyxin B and Colistin Sulphate were sensitive for 9 (90%) isolates. Likewise, Amikacin was sensitive with 8 (80%) of the isolates followed by Piperacillin+Tazobactam, with 7 (70%) isolates. None of the isolates showed intermediate sensitivity on disc diffusion technique.

The antibiotic susceptibility pattern of the isolates against Cefotaxime and Ciprofloxacin was also determined by minimum inhibitory concentration (MIC) method. using two antibiotics Cefotaxime and Ciprofloxacin (Table 3). All the isolates that were sensitive with disc diffusion technique were also sensitive through agar dilution method. However, 2 (6.06%) and 7 (21.21%) isolates that were shown to be resistant by disc diffusion technique were found to demonstrate intermediate sensitivity against Cefotaxime and Ciprofloxacin respectively through agar dilution method. All the remaining isolates were resistant through both techniques. The mean MIC against Cefotaxime was 2.24 µg/ml with standard deviation of 1.45 while that of Ciprofloxacin was 0.78 µg/ml with standard deviation 0.33. Eighteen (54.54%) of isolates were sensitive with Cefotaxime with MIC ≤1 µg/ml and only 4 (12.12%) isolates were sensitive with Ciprofloxacin with MIC ≤0.06 µg/ml.

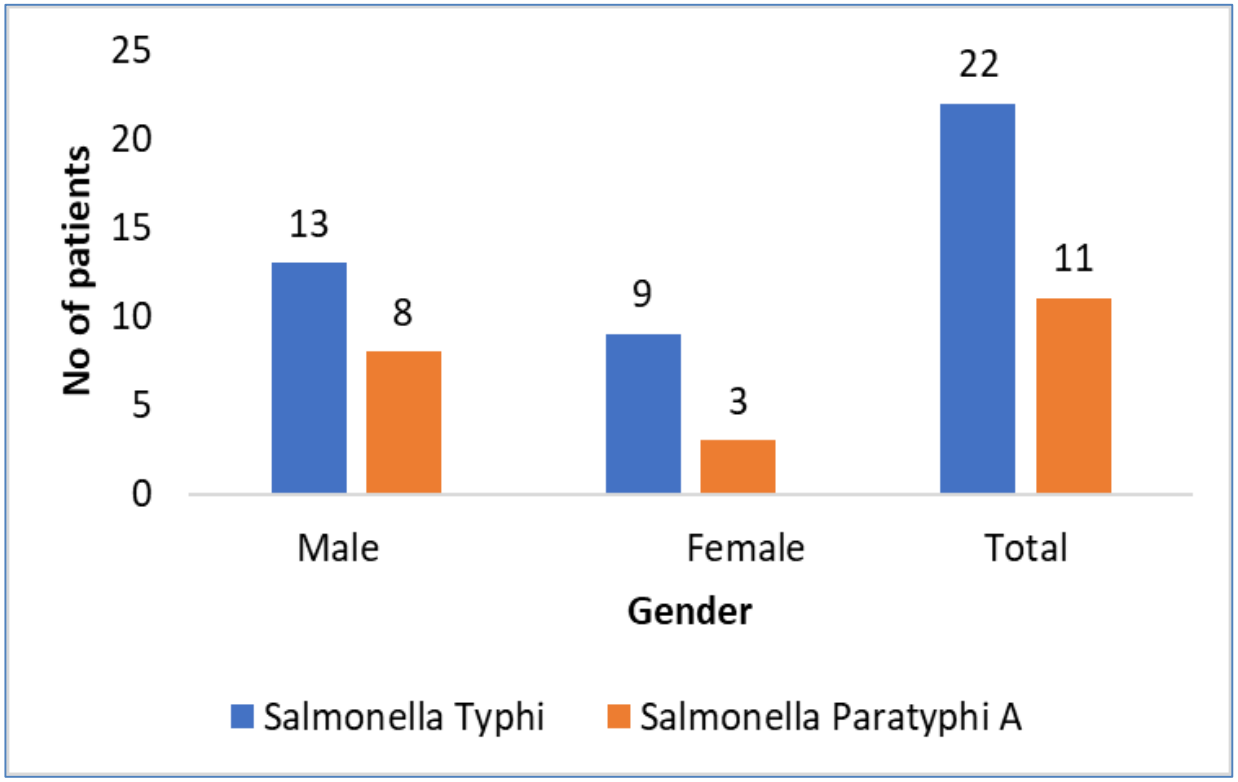

Figure 1: Gender-wise distribution of *Salmonella enterica*

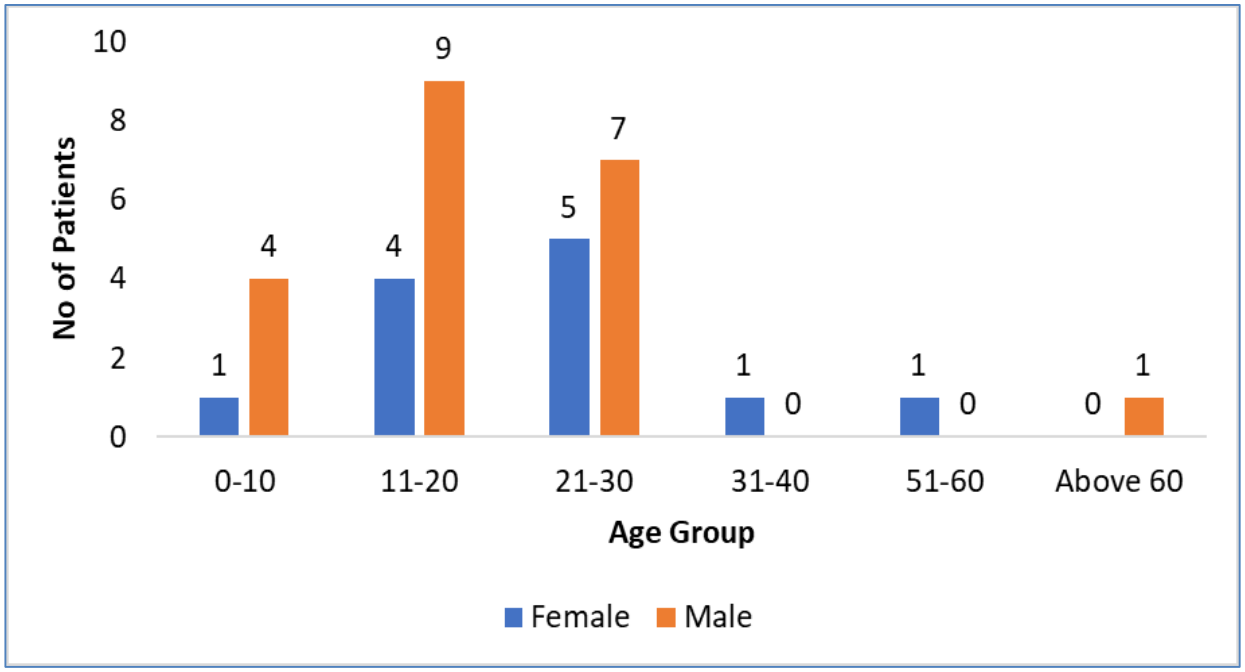

Figure 1. Age gender distribution of patients

Table 1: Antimicrobial susceptibility profile of *Salmonella enterica*

Antibiotics	*S.* Typhi		*S.* Paratyphi A	
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
Ampicillin	22 (66.67%)	0 (0.00%)	9 (27.27%)	2 (6.06%)
Azithromycin	17 (51.52%)	5 (15.15%)	2 (6.06%)	9 (27.27%)
Cefixime	21 (63.64%)	1 (3.03%)	9 (27.27%)	2 (6.06%)
Cefotaxime	13 (39.39%)	9 (27.27%)	5 (15.15%)	6 (18.18%)
Nalidixic acid	1 (3.03%)	21 (63.64%)	0 (0.00%)	11 (33.33%)
Ciprofloxacin	3 (9.09%)	19 (57.58%)	1 (3.03%)	10 (30.30%)

Table 2: Antimicrobial susceptibility profile of MDR isolates

Antibiotics	*Salmonella* Typhi		*Salmonella* Paratyphi A	
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
Imipenem	4 (40%)	0 (0%)	5 (50%)	1 (10%)
Meropenem	4 (40%)	0 (0%)	5 (50%)	1 (10%)
Amikacin	3 (30%)	1 (10%)	5 (50%)	1 (10%)
Piperacillin+Tazobactam	2 (20%)	2 (20%)	5 (50%)	1 (10%)
Polymyxin B	4 (40%)	0 (0%)	5 (50%)	1 (10%)
Colistin Sulphate	4 (40%)	0 (0%)	5 (50%)	1 (10%)
Chloramphenicol	4 (40%)	0 (0%)	6 (60%)	0 (0%)

Table 3. Minimum inhibitory concentration of *Salmonella enterica*

Cefotaxime		**Ciprofloxacin**	
MIC Value (µg/ml)	**No of isolates (%)**	**MIC Value (µg/ml)**	**No of isolates (%)**
≤1	18 (54.54%)	≤0.06	4 (12.12%)
2	2 (6.06%)	0.12-0.5	7 (21.21%)
≥4	13 (39.39%)	≥1	22 (66.67%)
Total	**33 (100%)**	**Total**	**33 (100%)**

DISCUSSION

In our study, 2.48% of the blood samples were culture positive for *Salmonella enterica.* This was similar to the study by Andrews et al. (2018) (2.5%) and Adhikari et al. (2012) (7.6%). Other studies conducted at different hospitals in Kathmandu had slightly more culture positivity rate with 13.5%, 10.63% and 9.2% *Salmonella enterica* (Acharya et al. 2012; Bhetwal et al. 2017; Shrestha et al. 2016). In this study, higher distribution of *Salmonella enterica* serovar Typhi to Paratyphi (2:1) was observed with 66.67% and 33.33% respectively. Similar to this study, Andrews et al. (2018) found the distribution of *Salmonella enterica* serovar Typhi and Paratyphi to be 79.8% and 20.2% respectively. Similarly, in a study conducted at KIST Medical College Teaching Hospital there were 64.1% *Salmonella* Typhi and 35.9% *Salmonella* Paratyphi A (Adhikari et al. 2012). Likewise, Acharya et al. (2012) found *Salmonella enterica* serovars Typhi and Paratyphi to be 58.5% and 41.5% respectively. Furthermore, a study by Bhetwal et al. (2017) isolated 66.1% *Salmonella enterica* serovar Typhi and 33.9% *Salmonella enterica* serovar Paratyphi which is almost similar to our study. Likewise, a study conducted at a tertiary care hospital in Kathmandu revealed 57.8% *Salmonella enterica* serovar Typhi and 42.2% *Salmonella enterica* serovar Paratyphi (Shrestha et al. 2016). In contrast, a study in 2013 at Kathmandu had *Salmonella enterica* serovar Paratyphi predominant with 52% isolates which is less frequent in this area (Karkey et al. 2013). The reason might prior administration of the drug.

The result demonstrated 31 (93.93%) isolates sensitive towards Ampicillin in our study. This is quite similar to the study conducted by Adhikari et al. (2012) where 95.3% isolates were sensitive and Khanal et al. (2017) where 95.5% isolates were sensitive. Likewise, a higher rate of the sensitivity towards Ampicillin was observed in the study conducted by Bhetwal et al. (2017) (97.95%) and Shrestha et al. (2016) (97.6%). However, in a study conducted by Andrews et al. (2018), only 52% of the isolates were sensitive to Ampicillin. Similar increase in susceptibility towards Ampicillin was observed in various studies in India (Gupta et al. 2013; Nagshetty et al. 2010; Sania et al. 2016; Singhal et al. 2014). In contrast to our study, only 29.47% sensitivity was observed in Kenya (Mutai et al. 2018). The sensitivity of Cefixime was 30 (90.90%) in our study. This was quite similar to a study by Andrews et al. (2018) where 89% of isolates were sensitive to Cefixime. All of the isolates were found to be sensitive in the research carried out at different tertiary hospitals in the year 2016 and 2017 (Bhetwal et al. 2017; Khanal et al. 2017; Shrestha et al. 2016). Similarly, high sensitivity was recorded in India and Pakistan (Patil and Mule 2019; Qamar et al. 2018; Sania et al. 2016). Thus, Ampicillin and Cefixime can be used for appropriate treatment of enteric fever.

Azithromycin is found to be moderately sensitive with only 19 (57.57%) isolates sensitive. This was similar to the study carried out by Sania et al. (2016) in India where only 43.5% susceptibility was recorded. Unlike our study, high sensitivity was observed in various studies conducted at different hospitals in Kathmandu valley (Andrews et al. 2018; Bhetwal et al. 2017). However, complete sensitivity of *Salmonella enterica* against Azithromycin was reported in a study conducted by Khanal et al. (2017) and Shrestha et al. (2016). In India, almost complete susceptibility was recorded in different years (Gupta et al. 2013; Patil and Mule 2019). Eighteen (54.54%) isolates were sensitive towards Cefotaxime in our study. In contrast to this finding, there was 100% sensitivity in the studies carried out in

different hospitals in 2016 and 2017 (Bhetwal et al. 2017; Shrestha et al. 2016) in Nepal and 94% sensitivity in a research carried out at Kenya (Mutai et al. 2018). Although these antibiotics are moderately susceptible, they can be administered after performing the susceptibility testing to reduce treatment failure.

Nalidixic acid was found to be the most inappropriate antibiotic against *Salmonella enterica* in our study. Only 1 (3.03%) isolate was sensitive to Nalidixic acid. Similarly, resistance was found to be high in a study carried out in 2017 where 7.7% were sensitive (Bhetwal et al. 2017). Likewise, Adhikari et al. (2012) conducted a study where only 14.1% of the isolates were sensitive to Nalidixic acid which was similar to other study by Shrestha et al. (2016) where sensitivity was 16.9%. A study conducted at National Public Health Laboratory revealed that only 19.51% of isolates were susceptible to Nalidixic acid (Acharya et al. 2012). Similar result was observed with 72.7% of isolates of *Salmonella enterica* resistant to Nalidixic acid (Khanal et al. 2017). Different studies in India have shown the complete resistance of the pathogen against Nalidixic acid (Gupta et al. 2013; Sania et al. 2016; Singhal et al. 2014) while 69% and 71% resistance were observed in the United States and Kenya respectively (Date et al. 2016; Mutai et al. 2018).

In our study, 4 (12.12%) isolates were sensitive to Ciprofloxacin. However, only 1% sensitive isolates were reported in a research conducted in 2018 (Andrews et al. 2018). Similarly, less sensitivity was observed in different studies carried out by Khanal et al. (2017) and Shrestha et al. (2016) with a sensitivity of 27.27% and 16.9% respectively. However, moderate sensitivity with 41.6% sensitive isolates was observed in the study at a tertiary hospital in 2017 (Bhetwal et al. 2017). However, in contrast to our study, various studies conducted previously have shown a very high to complete sensitivity towards Ciprofloxacin (Acharya et al. 2012; Adhikari et al. 2012). The rise from high to complete resistance was recorded in studies from various countries from Africa, Asia and America (Date et al. 2016; Mutai et al. 2018; Qamar et al. 2018; Sania et al. 2016; Singhal et al. 2014).

The MIC of Ciprofloxacin showed 12.12% isolates sensitive with MIC ≤0.06 μg/ml, 21.21% intermediate with MIC 0.12-0.5 μg/ml and 66.67% isolates resistant with MIC ≥1 μg/ml. Similarly, against Cefotaxime, 54.54% isolates were sensitive with MIC ≤1 μg/ml, 6.06% isolates were intermediate with MIC 2 μg/ml and 39.39% isolates were resistant with MIC ≥4 μg/ml. A study of Khanal et al. (2017) reported only 4.54% isolates resistant to Ciprofloxacin with 2.27% intermediate isolates. Likewise, 21.4% of isolates were resistant and 59% isolates in intermediate range were reported by Shrestha et al. (2016). In Kenya, 22% isolates with reduced susceptibility with Ciprofloxacin were recorded by Al-Emran et al. (2016) and 13% resistivity was reported by Mutai et al. (2018) with 70% intermediate isolates, while full susceptibility was recorded in Korea (Kim et al. 2010). Ciprofloxacin was effective towards only 8.25% *Salmonella enterica* in India (Sharma et al. 2017). It reflects the increasing pattern of resistance of the pathogen towards Ciprofloxacin in various countries including Nepal.

There is paucity of study on MIC of Cefotaxime in Nepal. However, in a study done by Soe and Overturf in 1987 all of the *Salmonella enterica* were reported to be in susceptible range to Cefotaxime by MIC method (Soe and Overturf 1987). Ekinci et al. (2002) reported the effectiveness of Cefotaxime against *Salmonella enterica* serovar Typhi. Capoor et al. (2006) reported 2% resistant isolates towards Cefotaxime. Various other studies conducted at different years have reported no resistant isolates of *Salmonella enterica* against Cefotaxime (Capoor et al. 2007; Kim et al. 2010; Manchanda et al. 2006; Neupane et al. 2010; Threlfall et al. 2008). Ramachandran (2017) reported 2.12% isolates resistant to Cefotaxime. Likewise, 17% resistant and 6% intermediate isolates were recorded in Kenya in 2018 (Mutai et al. 2018). Very less proportion of resistance was seen in previous studies in contrast to our present study. This might be due to rampant use of antibiotics without prescription in the study area. Similarly, in this study, some of the isolates that were resistant through disc diffusion method were intermediate in agar dilution method of determination of MIC. It gives a ray of possibility for treating the enteric fever by performing the MIC when none of the antibiotics seem to be effective through disc diffusion technique.

Culture and antimicrobial susceptibility profile aids in finding the current pattern of antibiotic resistivity and proper therapeutic choice. Likewise, determination of MIC

of antibiotics through agar dilution method is a gold standard method that helps in tracking the current situation of effectiveness of antibiotics. It also gives ideas on determination of suitable dose and route of administration of antibiotics during treatment. However, study in a single tertiary hospital limits this study as the data from this study does not represent the scenario of the whole country. Further confirmation of the result from molecular analysis would have been better, though is not feasible in routine blood culture and antibiotic sensitivity processes as it is more expensive and time consuming.

Conclusion

Limitations in anti-typhoidal drugs due to elevated resistance has increased the risk of treatment failure of enteric fever. Fluoroquinolones are inappropriate for therapeutic use and moderate sensitivity is observed against Azithromycin and Cefotaxime making them the regimen of choice only after testing their susceptibility. Ampicillin and Cefixime can be used as the drug of choice. However, it is essential to determine the sensitivity pattern of drugs before starting the treatment against enteric fever in order to delay the emerging resistance and minimize the treatment failures. MIC can be used as an additional method to determine the susceptibility of *Salmonella enterica* isolates which have shown resistance against antibiotics by disc diffusion method in order to get a better therapeutic alternative in the current scenario of increasing antibiotic resistance.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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