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Antimicrobial Resistance in Typhoidal *Salmonella* in Nepal: Surveillance for Enteric Fever, 2016-2019

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ABSTRACT

Background: Enteric fever (caused by *Salmonella enterica*) has been associated with poor hygiene and is endemic in the South-Asian countries. The increase in resistance to first line antimicrobials has been observed, while the emergence of multi/extremely drug resistance cases have been identified in several countries. The objective of this study is to analyze the current trend of antimicrobial resistance in *Salmonella* isolates in Nepal, and to identify the status of multi- and extremely- drug resistant isolates.

Methods: We recruited individuals at study hospitals with suspected enteric fever between September 2016 and August 2019 and performed blood cultures. The *Salmonella* isolates were tested for antimicrobial susceptibility and the antimicrobial resistance trend was evaluated.

Results: 1438 positive blood culture isolates were studied for antimicrobial resistance. 88% were culture positive for *Salmonella* Typhi and 12% for *Salmonella* Paratyphi. Multidrug resistant *S.* Typhi cases appeared mostly in December 2018 and January 2019, while there were no multidrug resistant *S.* Paratyphi cases. Also, extremely drug resistant *S.* Typhi cases were not observed during the study period.

Conclusions: The *Salmonella* isolates were mostly susceptible to first-line antimicrobials, cephalosporins and others. Many fluoroquinolones non-susceptible *Salmonella* were obtained, nevertheless their overall trend seems to be declining. In addition, the *S.* Paratyphi total cases are reducing since September 2017. Among *S.* Typhi isolates, only few were multidrug resistant and there were no extremely drug resistant isolates.

Keywords: Antimicrobial resistance; enteric fever; multi-drug resistant; Nepal; *Salmonella*; typhoid

INTRODUCTION

Enteric fever is one of leading cause of morbidity and mortality in developing countries.[1] Like other tropical developing countries, typhoid fever is endemic to Nepal and has been a public health concern throughout history.[2,3]

In Nepal, self-medication or the dependence on pharmacies for antibiotics, rather than physician's prescription, is common. This haphazard use of antimicrobials has contributed to the emergence of resistance strains of *Salmonella* against commonly used antibiotics, which has imposed threat and challenge to the world.[4-6] Earlier, the multidrug resistant isolates emerged and have lately developed resistance against fluoroquinolones and third generation cephalosporins as well.[7-9]

Typhoid immunization programs seem promising, but have not been effectively implemented.[10,11] Thus, we have to rely on an effective antibiotic therapy. To address this, we intend to study the antimicrobial resistance (AMR) pattern in enteric fever isolates in Nepal, and identify the multidrug resistance (MDR) and extremely drug resistance (XDR) isolates.

METHODS

This prospective surveillance study was conducted at two hospitals and five laboratory networks in Nepal from

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September 2016 to August 2019. The study recruited cases from Dhulikhel Hospital in Kavre district, a peri-urban setting, and Kathmandu Medical College and Teaching Hospital in Kathmandu district, an urban setting. Cases were also recruited from the laboratory networks in Kathmandu, the Alka Hospital, Nepal Medical College, Kathmandu Model Hospital, Bir Hospital and Helping Hands Clinic.

The study was approved by Nepal Health Research Council Ethical Review Board. The consents were taken from the patients for the enrollment in the study. Written and/or verbal consents were taken for patients above 16 years. Similarly, guardian consent was taken for children less than 16 years, and assent was taken from children between 14 to 16 years as well. All study participants were interviewed using a standardized questionnaire to ascertain demographic and clinical history. A unique identifier, with no personal identifying details, was assigned to maintain confidentiality. All consent/assent forms, signatures, and personal details were kept in locked cabinets accessible by the Principal Investigator. All tablets and computers were password protected with a secured central server system to archive data.

The participants of all age groups who visited outpatient, inpatient, hospital laboratory and emergency department of prospective sites with febrile illness were included. Outpatients from catchment area with fever for ≥3 days in the last 7 days and advised blood culture were enrolled into the study. For inpatients, all suspected or culture confirmed cases were enrolled. Patients from the hospital laboratories and laboratory network sites were enrolled, if their blood culture was positive for *Salmonella*.

Blood sample of enrolled participants were collected before antibiotic administration by a trained phlebotomist at prospective sites. Sample was inoculated into Bactec Ped Plus or Aerobic bottle, and processed on BD Bactec automated blood culture system. Gram stain and subsequent subcultures were performed on Sheep Blood Agar and MacConkey's Agar from samples positive by Bactec. After incubation, colony resembling *Salmonella* was confirmed by biochemical testing.

Antibiotic susceptibility testing of the organism was performed using disc diffusion method. The antibiotics used for *Salmonella* enlist first-line antibiotics - ampicillin, chloramphenicol, cotrimoxazole; fluoroquinolones - moxifloxacin, pefloxacin, ciprofloxacin; cephalosporins - cefepime, cefixime, ceftriaxone and cefotaxime; and other antimicrobials - gentamicin, piperacillin, imipenem and azithromycin. All isolates were tested for their susceptibility as per Clinical and Laboratory Standards Institute Guidelines M100-ED-29, 2019.[12] Isolates were multidrug resistant (MDR) if they were resistant to ampicillin, chloramphenicol, and cotrimoxazole. Isolates were fluoroquinolone (FQ) non-susceptible if they had resistance to ciprofloxacin and/or moxifloxacin and/or pefloxacin and extensively drug resistant (XDR) if they were MDR and were also non-susceptible to fluoroquinolone and any 3rd generation cephalosporins.

Trained research associates interviewed the patients or their caretakers to collect socio-demographic and other information related to the illness. Data were entered on password secured tablets using electronic case report forms. Descriptive analyses such as age, gender, emergence of cases by months and antimicrobial resistance were performed.

RESULTS

During the study period, 1438 patients with enteric fever were included in the epidemiologic study based upon their positive culture test for typhoidal *Salmonella* organisms. Among the *Salmonella enterica* isolates, 88% (1265/1438) were serovar Typhi, while 12% (173/1438) were serovar Paratyphi (Table 1).

Table 1. Socio-demographic and Microbiological Characteristics of Culture-confirmed Enteric Fever cases in Nepal, Surveillance for Enteric Fever in Asia Project (SEAP).

Characteristics	S. Typhi		S. Paratyphi	
	n=1265	%	n=173	%
Sex				
Male	745	58.9	106	61.3
Female	520	41.1	67	38.7
Age (in years)				
≤ 2	27	2.1	2	1.2
3 to ≤ 5	45	3.6	6	3.5
6 to ≤ 15	254	20.1	26	15
16 to ≤ 25	677	53.5	91	52.6
26 to ≤ 35	187	14.8	35	20.2
≥ 36	75	5.9	13	7.5
MDR[a]	23/1102	2.1	0/137	-
XDR[b]	-		-	-
MDR+Cipro^R	23/23	100		
MDR+Peflo^R	22/23	95.7		
MDR+Moxif^R	11/23	47.8		

[a]Multidrug Resistance (MDR) - Resistance to Ampicillin, Chloramphenicol and Cotrimoxazole
[b]Extremely Drug Resistance (XDR) - MDR with fluoroquinolone (FQ) and a third-generation cephalosporin Cipro^R, Peflo^R, Moxif^R means non-susceptibility to ciprofloxacin, pefloxacin and moxifloxacin respectively.

Among S. Typhi isolates, 58.9% were males while 61.3% of S. Paratyphi isolates were males. The median age of the cases was 20 years (interquartile range IQR, 15.25 to 25 years). The highest number of enteric fever cases occurred among the age group of 16 to 25 years among both the serovars, followed by the age group of 6 to 15 years and/or 26 to 35 years. The number cases among children below 5 years and older adults above 35 years lower (5% to 10%).

Antibiotic susceptibilities of the *Salmonella* isolates were determined by disc-diffusion method. Among the 173 S. Paratyphi isolates, AST against first-line drugs were available for 137 isolates, of which none (zero) were MDR-multidrug resistance (Table 1). On similar susceptibility testing, among the 1102 S. Typhi isolates, 23 (2.1%) were MDR with resistance to first-line drugs ampicillin, chloramphenicol and cotrimoxazole. Among those MDR isolates of S. Typhi, none (zero) were resistant to the tested cephalosporin (here ceftriaxone and cefixime). Thus, no extremely drug resistant (XDR) isolates were attained. However, all MDR were non-susceptible to, at least, one of the fluoroquinolones, 23 (100%) MDR isolates were non-susceptible to ciprofloxacin, 22 (95.7%) to pefloxacin and 11 (47.8%) to moxifloxacin (Table 1).

Resistance of *Salmonella* isolates to first-line antibiotics (ampicillin, chloramphenicol and cotrimoxazole) ranged from zero to 10% (Table 2). However, fluoroquinolone (ciprofloxacin, pefloxacin and moxifloxacin) non-susceptibility in S. Typhi isolates ranged from 30 to 90%, with increased pattern (80 to 90%) in S. Paratyphi as well. Resistance to antimicrobials gentamicin, piperacillin and imipenem was not seen among *Salmonella* Typhi serovar. Surprisingly, 4.6% (7/151) of the S. Paratyphi isolates showed resistance to azithromycin, a commonly used antibiotic, while that of S. Typhi was minimal (0.7%). All the *Salmonella* isolates were susceptible (98-100%) to cephalosporins cefepime and cefixime, and partially non-susceptible to ceftriaxone (2 to 13%).

Geographically, more than two-third of cases (66.5%) were from Kathmandu followed by Lalitpur (15.3%), Kavrepalanchok (10.2%) and Bhaktapur (5.4%) (Table 3). The neighboring districts of Kathmandu valley and Kavrepalanchok contributed only ~3% of the cases (Table 3). The cases in Kathmandu appeared to be distributed around the human settlements near to the major rivers. The places along the Dhobikhola river (Kapan, Chabahil, Kalopul, Ratopul, Maitidevi / Ghattekulo and Anamnagar) had 23.3% of the total cases in Kathmandu while Bagmati river surroundings (Jorpati, Sinamangal and Baneswor) contributed 8.6%. The cases in Lalitpur district were concentrated in the major towns of Pulchowk, Jawalakhel, Patan, Bhaisepati, Hattiban / Harisiddhi, Imadol and Satdobato, contributing 38.1% of the cases.

Table 2. Distribution of Antimicrobial Susceptibility Among Culture Confirmed Enteric Fever Patients.

	S. Typhi				S. Paratyphi			
	Susceptible	%	Non-Susceptible	%	Susceptible	%	Non-Susceptible	%
First-line antibiotics								
Ampicillin	1078/1130	95.4	52/1130	4.6	132/147	89.8	15/147	10.2
Chloramphenicol	1107/1131	97.9	24/1131	2.1	147/149	98.7	2/149	1.3
Cotrimoxazole	1092/1117	97.8	25/1117	2.2	147/147	100	-	-
Fluoroquinolones								
Ciprofloxacin	117/1131	10.3	1014/1131	89.6	14/155	9.0	141/155	91.0
Pefloxacin	114/1009	11.3	895/1009	88.6	14/94	14.9	80/94	85.1
Moxifloxacin	621/938	66.2	317/938	33.8	15/85	17.6	70/85	82.4
Cephalosporins								
Ceftriaxone	821/839	97.9	18/839	2.1	112/129	86.8	17/129	13.2
Cefixime	520/522	99.6	2/522	0.4	108/109	99.1	1/109	0.9
Cefepime	788/788	100	-	-	71/71	100	-	-
Other antimicrobials in use								
Azithromycin	1125/1133	99.3	8/1133	0.7	144/151	95.4	7/151	4.6
Gentamicin	1059/1059	100	-	-	136/136	100	-	-
Imipenem	955/955	100	-	-	91/92	98.9	0.01	1.1
Piperacillin	789/789	100	-	-	75/75	100	-	-

Table 3. Geographic Distribution of Enteric Fever Cases.		
A. District-wise distribution	n=1438	%
Kathmandu	956	66.5
Lalitpur	220	15.3
Kavrepalanchok	147	10.2
Bhaktapur	78	5.4
Others	37	2.6
B. Concentrated cases along Dhobikhola river in Kathmandu (23.3%)		
Kapan	29	
Chabahil	64	
Kalopul	36	
Ratopul	17	
Maitidevi / Ghattekulo	63	
Anamnagar	14	
C. Concentrated cases along Bagmati river in Kathmandu (8.6%)		
Jorpati	18	
Sinamangal	35	
Baneshwor	29	
D. Concentrated cases in Lalitpur (38.1%)		
Pulchowk	16	
Jawalakhel	10	
Patan	14	
Bhaisepati	20	
Hattiban / Harisiddhi	8	
Imadol	9	
Satdobato	7	

Trends in S. Typhi occurrence varied over time. During the study period, the proportion of S. Typhi cases occurred more from March – September 2018 (Figure 1). The proportion of S. Typhi multidrug resistance (MDR) isolates were almost negligible, except for two months during December 2018 – January 2019 (Figure 1). Trends in S. Paratyphi occurrence reduced over time (Figure 2). The proportion of S. Paratyphi cases occurred more from January 2017 – July 2017, after which the percentage of cases reduced over time with few fluctuations. The fluoroquinolones (ciprofloxacin, pefloxacin and moxifloxacin) non-susceptibility among the enteric fever isolates was high, nevertheless the proportion appears to be reducing across the study period (Figure 3).

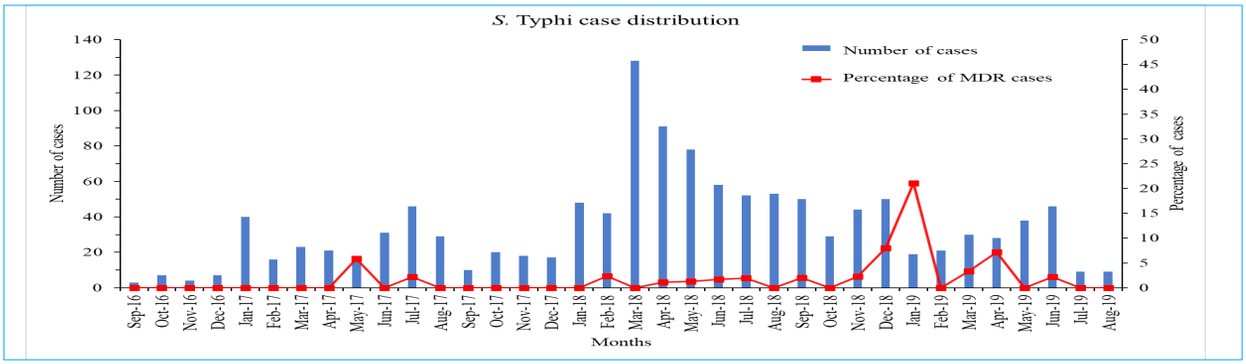

Figure 1. Trend of S. Typhi cases over time, September 2017 – August 2019. The number of total S. Typhi cases (blue bars) and percentage of MDR S. Typhi cases (red line) are plotted in the graph. FQR here means resistance to either ciprofloxacin, pefloxacin or moxifloxacin.

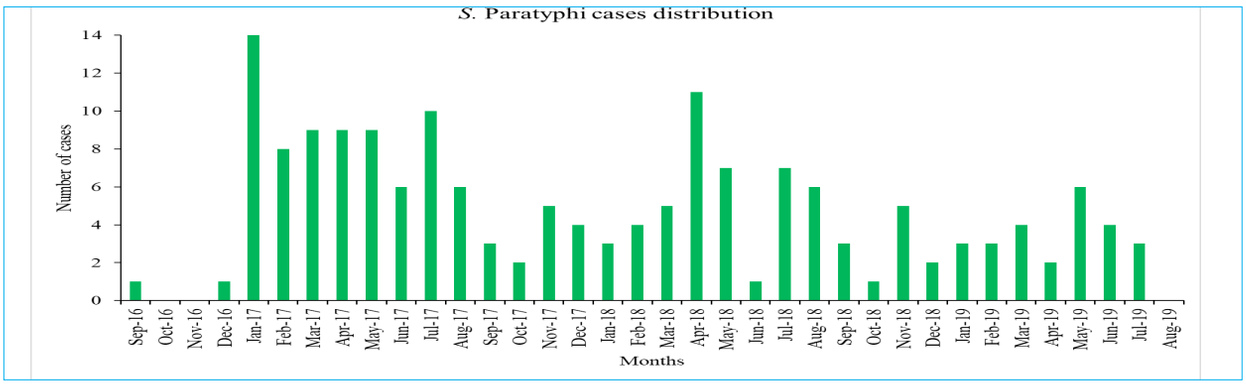

Figure 2. Trend of S. Paratyphi cases over time, September 2017 – August 2019. The number of total S. Parayphi cases (green bar) is plotted in the graph. The number of S. Paratyphi cases looks declining with time.

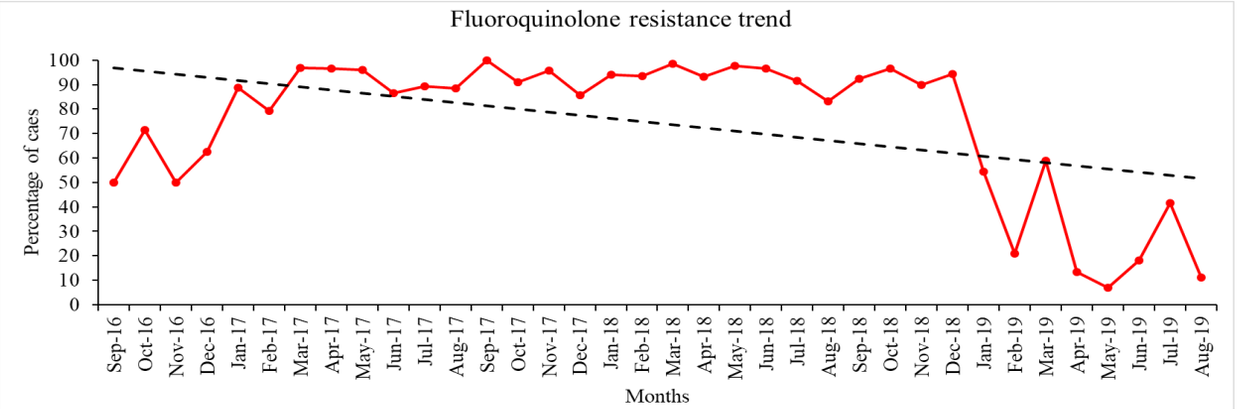

Figure 3. Time trend of proportion of *Salmonella* isolates (Typhi and Paratyphi) with fluoroquinolone non-susceptibility (red). The trendline (black dashed) indicates the reduction in FQR isolates. FQR here means non-susceptibility to either ciprofloxacin, pefloxacin or moxifloxacin.

DISCUSSION

Enteric fever has been a leading cause of morbidity and mortality in urbans of South and South-east Asia.[9,13] Here we have studied the epidemiology of bacteremic enteric fever cases of Kathmandu valley (districts Kathmandu, Lalitpur and Bhaktapur), Kavrepalanchok and few neighboring districts.

Among the serovars, *Salmonella enterica* Typhi accounted for almost 90% of the cases, while only 10% was constituted by *Salmonella enterica* Paratyphi. This is in contrast to the earlier similar reports in Nepal, where a decrease in the ratio of *S.* Typhi to *S.* Paratyphi was observed between 1992 and 2014.[14-17] The ratio of Typhi to Paratyphi serovar seem to be high in 90s, gradually reduced over time with increase in Paratyphi in 2000s, and again our data shows the increase in ratio with more Typhi cases since 2015. The reason behind this serovar switching over time is not understood, however may have been affected by various epidemiological factors and global trend.

Similar to our earlier report,[18] culture confirmed enteric fever cases were highest among the young adults aged 16 to 25 years for both serovars *S.* Typhi and *S.* Paratyphi. Cases among the children (below five years) and older adults (above 35 years) were comparably less, possibly because these age groups have home-cooked foods rather than outdoors. The children are also being taken care by the parents, thus might have contributed to the lesser cases.[19] The young adults aged 16 to 25 are the college-going population and are more exposed to the food and drinks outside of home, suggesting the outdoor foods to be responsible for enteric fever cases.[20]

Antimicrobial resistance is a major public health problem around the globe including Nepal. We found the increase in proportion of MDR cases among *S.* Typhi, as reported by previous studies.[14-17] Among the patients with *S.* Paratyphi, there were no MDR cases in Nepal. After the emergence of MDR cases in Nepal, the fluoroquinolones have been the drug of choice, and have been used extensively.[15] However, in the past few years, *S. enterica* has acquired resistance to fluoroquinolones and such non-susceptibilities have been reported in other South and South-East Asian countries as well.[21-23] Here, our data shows the decreasing trend of fluoroquinolone non-susceptibility over past two years, however this might be due to the shift from fluoroquinolone to azithromycin as the drug of choice. Thus, there remains a possibility of increase in FQR isolates, after its use is prioritized again. These non-susceptible stains could have acquired the mutations in DNA gyrase and topoisomerase genes[24] and/or the resistance plasmids containing the *qnr*S fluoroquinolone resistance gene.[8] The fluoroquinolones non-susceptibility seems to be a threat for typhoid treatment in endemic country like Nepal and dual antibiotic therapy could significantly improve the condition.[25]

After the emergence of fluoroquinolone non-susceptibility, azithromycin has been the drug of choice in many countries.[13,26] In low and middle-income countries, patient seek treatment from pharmacies rather than health care centers, and the sale of antibiotics is not regulated, making it available over the counter.[27,28] Thus, the consumption of antibiotic without prescription has increased, which could have led to the tolerance and resistance to even azithromycin.

In this scenario, cephalosporins such as ceftriaxone, cefixime and cefepime may be used for the management of enteric fever.[29] We have shown the increase in resistance to one of the third generation cephalosporins

as well, i.e. ceftriaxone, this raises questions on the selection of effective antimicrobials. Nevertheless, the non-susceptible isolates look 100% sensitive to the fourth-generation cephalosporin, cefepime, suggesting the need to start using the latter. Thus, it seems better to perform the antimicrobial susceptibility test of the *Salmonella* isolated from the individual patient to determine the most effective antibiotic in treating the infection.

XDR typhoid cases have been reported in a nearby country Pakistan,[30] and is a potential threat to Nepal as well. Effective screening of travelers from neighboring countries should be monitored, before the XDR bacteria enters to the community level. As XDR will be resistant to cephalosporins as well, the treatment option for typhoid would be very limited. Azithromycin is one of the hopes, assuming the resistance against it would not occur. But we cannot completely count on azithromycin alone, as our data shows 0.7% of *S.* Typhi and 4.6% of *S.* Paratyphi to be resistant. In this case, carbapenems could also help us fight against AMR, but only to some extent. Eventually, typhoid conjugate vaccine (TCV) remains to be an optimistic alternative to fight *Salmonella*, irrespective of its AMR status. Thus, there seem to be an urgent need of TCV efficacy testing and implementation for early management of enteric fever.

Geographically, Kathmandu had the highest (66.5%) burden of which the human settlements around Dhobikhola river contributed 23.3% and Bagmati river contributed 8.6%. The places around Dhobikhola river were Kapan, Chabahil, Kalopul, Ratopul, Maitidevi/Ghattekulo and Anamnagar. In addition, Jorpati, Sinamangal and Baneshwor were the places around Bagmati river where cases occurred. This suggests the existence of typhoid hotspots around the river, and might have significant public health concerns. The contaminated food and water are the major source of the causative bacteria. Moreover, the waste-water and fecal waste are dumped in these rivers in Kathmandu. Thus, these rivers could be the reservoir of enteric fever causing bacteria, *S.* Typhi and *S.* Paratyphi. However, the links seem to be missing on the route of bacteria transmission from river water to human, as the river-water is not used by the people living in the area. Lalitpur district had the second highest (15.3%) burden, among which 38.1% cases were distributed unevenly among Pulchowk, Jawalakhel, Patan, Bhaisepati, Hattiban, Harisiddhi and Imadol. As there is absence of major river in Lalitpur, we could not confirm the similar distribution of cases as seen in Kathmandu. The geographic data suggests the requirement of population survey considering the mentioned hotspots in Kathmandu and Lalitpur districts.

The limitation of this study is the selection of study sites. As Nepal shares open border with India, the data would have been more representative if the research included data from all over the country.

CONCLUSIONS

The results of this study provide an important insight into the antimicrobial resistance trend among *S.* Typhi and *S.* Paratyphi isolates in Nepal. The existence of MDR *S.* Typhi, and the emergence of azithromycin resistance in *S.* Typhi and more among *S.* Paratyphi impose serious threat on the community level. However, rise in fluoroquinolone susceptibility over time shows its potential use in future again. Cephalosporins hold a potential as a treatment strategy, however should be cautiously prescribed as we report increased resistance to a third-generation cephalosporin cefotaxime at an alarming rate. This also implies the urgency to take advance efforts for the control of enteric fever. Nevertheless, it is encouraging to report the occurrence of no XDR cases in Nepal during the study period, and this could be used as an important reference line for the screening requirement of immigrating nationals of neighboring countries, mainly Pakistan, to prevent the XDR emergence in Nepal. In addition, as the antimicrobial resistance may revert back its susceptibility along with time, it is also suggested to monitor the susceptibility to antibiotics which were resistant earlier.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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