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SEASONAL VARIATION OF ENTERIC FEVER IN KATHMANDU VALLEY AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *SALMONELLA ENTERICA*

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ABSTRACT

Enteric fever is of public health concern globally, more in the developing countries, which has been causing many morbidity and mortality in low- and middle-income countries. It is mostly related to poor sanitation and contaminated drinking water. This study aims to study the seasonal variation of enteric fever seen in Kathmandu valley and antimicrobial susceptibility profiles of the isolated bacteria. Therefore, blood culture of suspected patients of enteric fever was conducted from April 2018 to April 2020 using standard microbiological techniques, antimicrobial susceptibility testing was performed following Clinical and Laboratory Standard Institute -2017 guidelines. During the study, 104 isolates of *Salmonella enterica* were isolated, of which *Salmonella enterica* serovar Typhi were 77.9%, while *Salmonella enterica* serovars Paratyphi A and B were 21.1% and 1% respectively. Although enteric fever was prevalent throughout the year, the highest number of cases was during the summer. With the increase in temperature, there was also an increase in the cases. Antimicrobial susceptibility profile recorded high resistance of isolates towards Nalidixic acid (97.1%) and Ciprofloxacin (91.3%) while 96% sensitivity to both Ampicillin and Cefixime. This study revealed the occurrence of enteric fever throughout the year, but a large number of cases (51%) are concentrated in the monsoon. Similarly, the resistance of fluoroquinolones reached an alarming state making them inappropriate for use. Ampicillin and Cefixime can be the drug of choice for empirical therapy of enteric fever.

Keywords: enteric fever - fluoroquinolones - antimicrobial resistance - seasonal variation - *Salmonella enterica*

INTRODUCTION

Enteric fever is still a major public health issue in developing countries with a considerable burden in South Asia with significant mortality and morbidity (Saad *et al.* 2017). It is a systemic febrile disease of the gastrointestinal tract associated with the infection of two serovars of *Salmonella enterica*, Typhi and Paratyphi (Khanal *et al.* 2017). In 2019, due to enteric fever, there were more than 14.4 million illnesses and 135.9 thousand deaths worldwide (Stanaway *et al.* 2019). Areas with inadequate sanitation and unsafe drinking water are prone to enteric fever infections (Khanal *et al.* 2017). The increase in antimicrobial resistance has created a threat in the proper management of this disease (Bhutta *et al.* 2018).

Enteric fever is associated with ingestion of food and water contaminated with the carrier's feces and urine, and humans are the only reservoir host for enteric fever (Parry & Maskell 2006). The seasonal occurrence of enteric fever is found to be variable in various regions throughout the globe (Saad *et al.* 2018). In Nepal, enteric fever is predominant mostly in the rainy season (Singh *et al*. 2011, Joshi *et al*. 2019). Implementation of strategies of addressing environmental factors like improved public sanitation and provision of safe drinking water at the local level aids to decrease the risk of acquired enteric fever (Bhutta *et al.* 2018). A significant rise in enteric fever cases leading to epidemics was recorded in Nepal over time (Maskey *et al.* 2008, Lewis *et al.* 2005) while Kathmandu city is considered as the capital of enteric fever (Karkey *et al*. 2008). According to the Annual Report by the Department of Health Services, there were 18.8 cases of enteric fever per 1000 during 2009-2014 (DoHS 2020). Similarly, the rate of infection was high among young adults, especially in areas with high population density (Andrews *et al.* 2018). This shows that enteric fever is still endemic in Nepal.

The change in the susceptibility pattern of antibiotics towards *Salmonella* Typhi and *Salmonella* Paratyphi over time has created a serious challenge in the effective treatment of enteric fever. The commonly used antibiotics are showing resistance in increasing trends over a few decades (Zellweger *et al.* 2017). Resistance towards fluoroquinolones is increasing rapidly in recent years, posing challenges in disease management (Shrestha *et al.* 2016, Bhetwal *et al.* 2017, Mutai *et al.* 2018). On the other hand, the

reemergence of the sensitivity of conventional first line drugs has created a ray of hope for treatment options (Bhetwal *et al.* 2017, Khanal *et al.* 2017).

Knowledge of variation in disease with season helps in maintaining preventive measures and avoid from being infected. Although few studies were carried out previously, this study will provide the variation of enteric fever with season in the present scenario. Likewise, the current susceptibility pattern helps in the proper selection of drugs. Thus, this study was conducted to study the variation of enteric fever with the season in Kathmandu valley and study the current antibiotic sensitivity profile.

MATERIALS AND METHODS

Study design

This was a hospital based cross sectional study conducted for two years from April 2018 to April 2020 at Manmohan Memorial Medical College and Teaching Hospital, Kathmandu.

Bacterial isolation

Blood samples were collected aseptically following standard microbiological techniques from patients suspected of enteric fever. Both inpatients and outpatients were enrolled in the study, while those with prior administration of antibiotics and samples other than blood were excluded. Samples were immediately cultured in Brain Heart Infusion broth at 37^0C for 24 hours. Subculture was done at 37^0C for 24 hours on Blood agar and MacConkey agar regardless of the turbidity every 24 hours until 96 hours of culture. If the growth was observed, identification of the isolated strains was done by observing Gram staining results and colony morphology followed by biochemical testing (Cheesbrough 2006). Culture media and Biochemical media manufactured by HiMedia Laboratories; India were used. Out of the total samples, only the samples positive with *Salmonella enterica* were subjected to the study of seasonal variation of enteric fever.

Antibiotic susceptibility testing

The antibiotic susceptibility test of the isolates of *Salmonella enterica* was done on Mueller Hinton Agar (HiMedia, India) by modified Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI 2017) guidelines. Commonly used antibiotics Ampicillin (10μg), Azithromycin (15μg), Cefixime (30μg), Cefotaxime (30μg), Nalidixic acid (30μg) and Ciprofloxacin (5μg) were used (HiMedia, India).

Results of susceptibility testing were interpreted on the basis of standard interpretative zone diameters as suggested in CLSI (2017) guidelines.

RESULTS

In a total of 11,224 blood samples processed, 104 (0.92%) were culture positive with *Salmonella enterica*. *Salmonella* Typhi was predominant with 81 (77.9%) while *Salmonella* Paratyphi A were 22 (21.1%). Only one (1%) *Salmonella* Paratyphi B was isolated.

Demographic scenario

Patients between the ages of 2 to 77 years have participated in this study. The mean age was 22 years while the median age was 21 years. Out of all patients, 63 (60.6%) were males while 41 (39.5%) were females. Most of the patients were below the age of 30 years with most of the patients falling in the age group 21-30 years. Males were predominant in all age groups except 51-60 years (Figure 1).

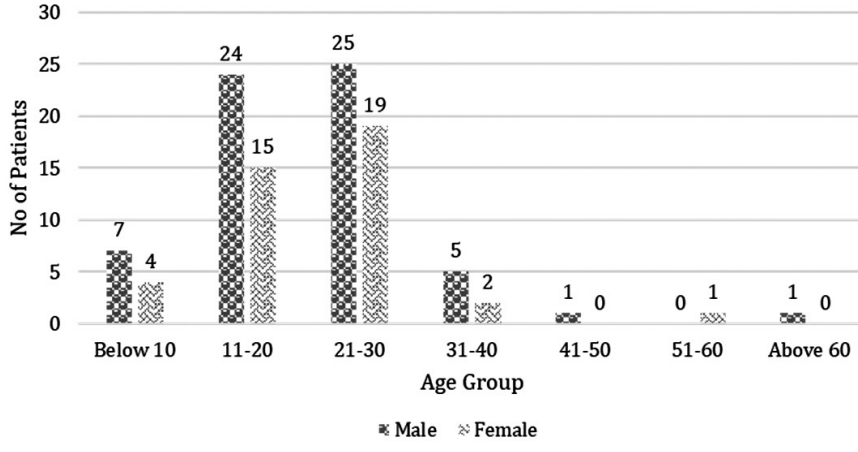

Figure 1: Age and gender distribution of patients

Source: Demographic data

Seasonal variation of enteric fever

Figure 2 shows that enteric fever was prevalent throughout the year. The highest number of enteric fever cases was in summer in both years. However, in other seasons, the proportion of cases varies with year. Overall, most cases, i.e. 41 (40%) and 31 (30%) cases were seen in summer and spring seasons respectively. The lowest number of cases (n=13) (12%)

were in winter. Similarly, the highest number of cases was observed in June with 21 (20.2%) patients, which were followed by May with 20 (19.2%) patients. January and October constitute the least occurrence of patients with 2 (1.92%) patients each.

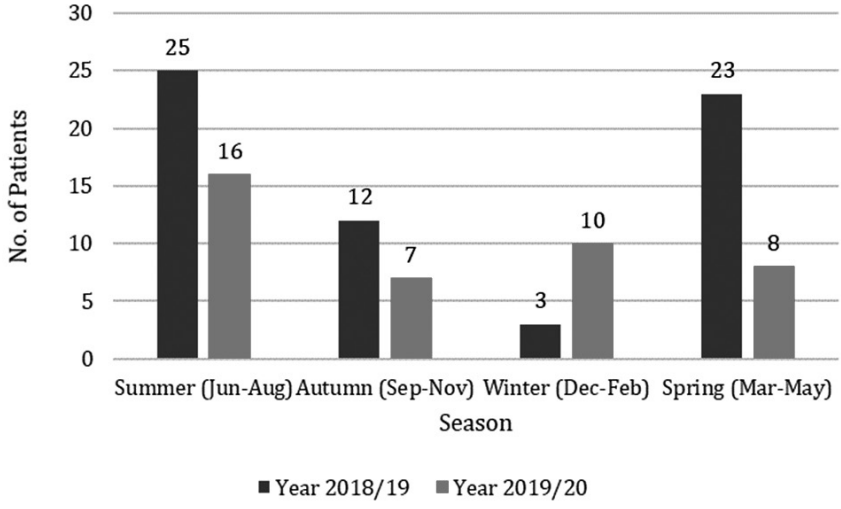

Figure 2: Incidence of enteric fever with season

Source: Experimental results

With an increase in temperature and rainfall, the number of cases was also found to be in the increasing trend as shown in Figure 3. Seasonal variation is compared with the average temperature and rainfall of Kathmandu valley from 1991-2020 (DoHM 2020). Of the total, 53 (51%) cases were between June to September in which the temperature was found to reach the peak and most rainfall occured in Nepal.

Antibiotic susceptibility profile

The most commonly used antibiotics for typhoid patients were used for antibiotic susceptibility testing. Ampicillin and Cefixime were found to be the most appropriate drugs with a sensitivity of 96 (92.3%) each. Azithromycin was effective against 81 (77.9%) isolates that is followed by Cefotaxime with 77 (74%) susceptible isolates. The isolates were highly resistant towards Nalidixic acid and Ciprofloxacin, with only 3 (2.9%) and 9 (8.7%) sensitive isolates respectively (Table 1).

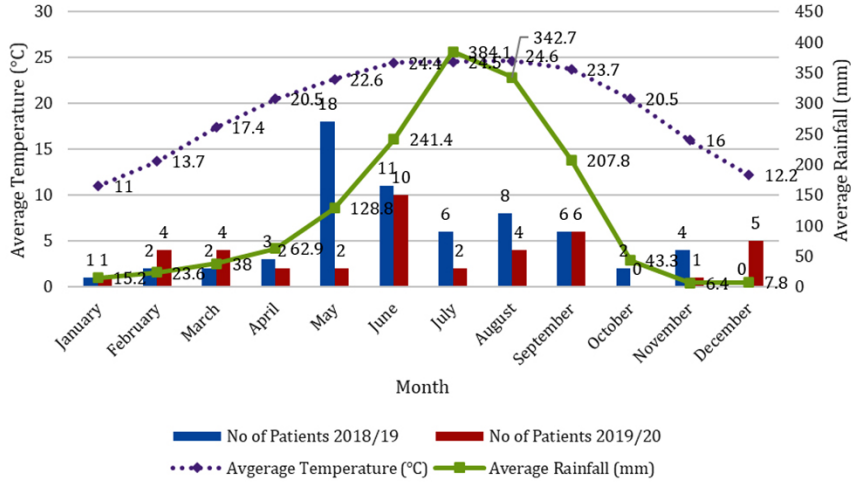

Figure 3: Variation of enteric fever cases with temperature and rainfall

Source: Department of hydrology and meteorology (2020), and experimental results

Table 1: Antibiotic susceptibility profile

Antibiotics	**Sensitive**		**Resistant**	
	(n)	**(%)**	**(n)**	**(%)**
Ampicillin	96	92.3	8	7.7
Azithromycin	81	77.9	23	22.1
Cefixime	96	92.3	8	7.7
Cefotaxime	77	74	27	26
Nalidixic acid	3	2.9	101	97.1
Ciprofloxacin	9	8.7	95	91.3

Source: Experimental results

DISCUSSION

This study shows that enteric fever is prevalent throughout the year and a high proportion of infection is observed in summer. Fluoroquinolones are seen to be highly ineffective against enteric fever. Kathmandu city, the centre of administration of Nepal, has been termed as the capital of enteric fever of the world. Both population and enteric fever cases are increasing in this area with a gradual shifting of the cases towards *Salmonella* Paratyphi

from *Salmonella* Typhi (Karkey *et al.* 2008). This study reveals only 0.9% culture positivity towards *Salmonella enterica* which is lower than other studies. This might be due to the increase in the flow of patients with fever in the hospital because of the dengue fever epidemic in Nepal during the study period (Pandey & Costello, 2019), all of whom were requested for blood culture to rule out the possibility of enteric fever as per the hospital protocol. In our study, there were 77.9% *Salmonella* Typhi, 21.1% *Salmonella* Paratyphi A, and 1% *Salmonella* Paratyphi B. This was similar to the findings of Maskey *et al.* (2008) where 71% were *Salmonella* Typhi and 29% were *Salmonella* Paratyphi A. Similarly, 75% of *Salmonella* Typhi were isolated in a study by Niroula *et al.* (2020). However, greater percentage of *Salmonella* Paratyphi A were recorded by Karkey *et al.* (2013) (52%) and Vidyalakshmi *et al.* (2008) (53.8%) with 0.6% *Salmonella* Paratyphi B. Likewise greater number of *Salmonella* Paratyphi were reported by Shrestha *et al.* (2016) (42.26%) and Petersiel *et al.* (2018) (44.3%).

In this study, males were predominant with 60.6% of total cases. This was similar to the study by various investigators (Bhetwal *et al.* 2017, Petersiel *et al.* 2018, Niroula *et al.* 2020, Karkey *et al.* 2013). Likewise, most of the patients (90.38%) in our study were below the age of 30 years, predominantly in the age group 21-30 years. Similar to this, 91.95% of patients were below 30 years as reported by Niroula *et al.* (2020) with most cases in 21-30 years. In a study by Bhetwal *et al.* (2017), 72.6% of patients were between the age of 15 to 45 years while Andrews *et al.* (2018) documented 45.9% of patients in the age group 16-24 years. This reveals the high incidence of enteric fever in patients with active age groups. This might be due to the consumption of untreated drinking water and consumption of unsafe foods such as street food which is popular among these age groups.

This study reported summer as the season with the highest number of cases, followed by spring and autumn. Most enteric fever cases (39.4%) were observed in summer and the least number of cases were observed in winter (12.5%). One of the largest outbreaks of enteric fever in Nepal, which was reported in Bharatpur occurred in summer in which 5,963 cases were recorded within 7 weeks (Lewis *et al.* 2005). The intermittent supply of water in urban areas like Kathmandu and Bharatpur causes cross-contamination of water distribution channels and sewage systems posing a threat to public health (Cairncross & Feachem 2019). Joshi *et al*. (2019) reported enteric fever cases throughout the year with 43.06% of cases in the rainy and summer season between May to August. In this study, on a monthly basis, we observed that most cases (20.2%) were in June and the

least was in both January and October with 1.9% of cases in each. In the rainy (monsoon) season, 51% of cases were observed. The similarity was also noted in a study by Petersiel *et al.* (2018) in which 56% of cases were recorded in the monsoon season and 44% cases in the dry season. Singh *et al.* (2011) documented a seasonal trend similar to our study with a high number of cases (78.72%) in the rainy season. Our study also shows an increase in cases with an increase in temperature. This was supported by a three-year study in Kathmandu which demonstrated an association between average monthly rainfall, seasonal temperatures, and cases between June and August (Karkey *et al.* 2008). In New Delhi, India, a study by Sinha *et al*. (1999) demonstrated the highest number of cases in monsoon and the lowest number of cases in winter which is similar to our study. This high number of morbidities could be attributable to contaminated water, as only 30% of urban populations and 12% of the rural population follow proper water treatment practices prior to drinking. Besides, unhygienic meal preparation practices and improper disposal of excreta, which leads to water and food material contamination are also of concern as 15% of households still practice open fields for defecation (MOH 2017).

This study reflected the inappropriateness of fluoroquinolones for enteric fever treatment. *Salmonella enterica* isolates were highly resistant towards Nalidixic acid and Ciprofloxacin (97.1% and 91.3% respectively). This result resembles the Annual Report of the Department of Health Services, Nepal, which has shown increasing fluoroquinolone resistance in the past three years (DoHS 2020). Shrestha *et al.* (2016) reported decreased susceptibility of Ciprofloxacin with 3.6% resistant isolates and 79.5% intermediate isolates whereas Nalidixic acid resistant *Salmonella* were 83.1%. Similar results were obtained from various studies conducted at different hospitals in Kathmandu (Khanal *et al.* 2017, Bhetwal *et al.* 2017 & Niroula *et al.* 2020). Most of the isolates in our study were sensitive to Ampicillin and Cefixime, which indicates that these drugs are appropriate for the empirical therapy of enteric fever. Both Ampicillin and Cefixime were effective towards 92.3% of isolates. This shows the reemergence of the conventional drug, Ampicillin, against *Salmonella enterica*. A similar result was obtained in a study conducted in 2017 by Khanal *et al.* (95.5%) and Bhetwal *et al.* (97.9%) with Ampicillin while 100% sensitivity of the isolates towards Cefixime was recorded in both studies. The Annual Report by the Department of Health Services also shows the increased susceptibility of *Salmonella enterica* to Ampicillin (DoHS 2020). The isolates showed moderate sensitivity towards Azithromycin and Cefotaxime which suggests their use only after susceptibility testing. None of the drugs used in this

study were completely effective. Increasing resistance may be due to the indiscriminate use of antibiotics without a physician's prescription. Therefore, clinicians should prescribe antibiotics only after studying antibiotic susceptibility profile. It is equally essential that the patients use only the antibiotics prescribed after studying the resistance pattern.

The study of antimicrobial susceptibility patterns of *Salmonella enterica* along with seasonal variation is the focus of this study so that it would be helpful to assess the appropriateness of particular antibiotics as a treatment regimen. However, since the study site is a single hospital, it has limited findings to reflect the scenario of the whole country. Studies at different geographical areas within Nepal might have been more effective to know about the overall seasonal variation and burden of the disease. Such results would be a significant tool for the government to design better health programs in the country.

CONCLUSION

Enteric fever cases are high in summer and it rises with the increase in rainfall and temperature. However, there is a chance of infection in all seasons. Therefore, it is crucial to be aware of water quality, food hygiene, sanitation, and vaccination. One should be highly attentive towards the risk of infection, particularly during the summer and rainy seasons. Moreover, effective treatment of enteric fever with appropriate medication is needed for the cure of infection. An effective antibiotic should be selected by studying the antibiotic susceptibility profile to reduce treatment failure and burden of antibiotic resistance.

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