STM JOURNALS

Research & Reviews: A Journal of
Microbiology & Virology

ISSN: 2230-9853 (Online)
ISSN: 2349-4360 (Print)
Volume 13, Issue 2, 2023
DOI (Journal): 10.37591/RRJoMV

http://medicaljournals.stmjournals.in/index.php/RRJoMV/index

Research RRJoMV

Assessment of Antibody Titer Distribution Against *Salmonella enterica* Among Suspected Enteric Fever Patients

Guheswari Chataut[1], Suja Maharjan[2], Keshab Bhattarai[3], Pusparaj Bhatt[4,*], Laxman Bhatt[5]

Abstract

Typhoid fever is a global health problem, causing millions of deaths each year. It is an endemic problem in many developing countries including Nepal. Development of exceptional mechanisms for persistence in its host supports its survival and transmission. However, due to a lack of sophisticated labs and adequate funds, difficulties in the estimation of worldwide impact are often encountered. The Widal test is a diagnostic technique extensively employed to identify enteric fever, offering a quicker, easier, and more cost-effective method. Within this research, blood samples were analyzed for enteric fever utilizing the Standard Widal Confirmatory Quantitative Tube test. A total of 160 blood samples were collected to analyze the prevalence of enteric fever in Bheemdutta municipality. Out of which 32 showed positive agglutination test where 18 samples belonged to female patients and 14 belonged to male patients. The statistical analysis showed a negative correlation between test results and the gender of the patients ($p<0.05$). Among 25 individuals showing antibody titer against serotype Typhi, 14 had titers of ≥1:20 for anti-O titer and 11 samples demonstrated anti-H titers of ≥1:20. Anti-H titers equal to or exceeding 1:20 were observed in only 6.25% for S. enterica serotype Paratyphi A and 15.63% for serotype B. This corresponded to an additional seven samples that displayed positive results in the agglutination test among all the samples tested. The study showed significant antibody titer among the studied sample in the study area.

Keywords: Enteric fever, *Salmonella*, Widal test, agglutination, antibody titer

***Author for Correspondence**
Pusparaj Bhatt
E-mail: pusparajbhatt2803@gmail.com

[1]Student, Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal
[2]Student, Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal
[3]Scientist/Executive Director, Sattwik Nutrifood A Diet Therapy and Research Center, Sattwik Group Pvt Ltd, Pokhara Nepal
[4]Teaching Assistant, Department of Microbiology, Siddartha Science Campus, Mahendranagar, Nepal
[5]Student, Department of Microbiology, Siddartha Science Campus, Mahendranagar, Nepal

Received Date: July 31, 2023
Accepted Date: August 30, 2023
Published Date: September 10, 2023

Citation: Guheswari Chataut, Suja Maharjan, Keshab Bhattarai, Pusparaj Bhatt, Laxman Bhatt. Assessment of Antibody Titer Distribution Against *Salmonella enterica* Among Suspected Enteric Fever Patients. Research & Reviews: A Journal of Microbiology & Virology. 2023; 13(2): 26–33p.

INTRODUCTION

Typhoid fever, also known as enteric fever, is a systemic disease caused by gram-negative bacteria known as *Salmonella enterica* subspecies Enterica Serovar Typhi (*S.* Typhi). Strains of *Salmonella enterica* serotypes Paratyphi A, B, or C (*S.* Paratyphi) are associated with paratyphoid cases. The clinical feature tends to be more severe with *S.* Paratyphi [1]. These pathogens exclusively infect humans. Humans can become chronic carriers resulting in food contamination and *S. Typhi* transmission because of poor food handling practices [2]. Based on the latest data derived from population-based statistics, approximately 9.2 million instances of typhoid fever and 110,000 fatalities occur globally annually. Among these, 216,000 deaths have been recorded worldwide. The regions with the most considerable estimated occurrence are the WHO South-East Asian (with

306 cases per 100,000 individuals), Eastern Mediterranean (with 187 cases), and African (with 111 cases) regions. Since 2018, five nations (Liberia, Nepal, Pakistan, Samoa through self-assessment, and Zimbabwe) have implemented the typhoid conjugate vaccine due to their projected elevated incidence of typhoid fever (≥100 cases per 100,000 population per year) [3, 4].

Typhoid fever was first described accurately by French workers mainly Bretonneau who called the disease Dothienterite. In 1829, Louis described typhoid separated it from other causes of fever, and related the clinical features from lesions in intestinal mesenteric lymph nodes and spleen [5]. Bud provided evidence that bowel discharge was the main waterborne, mechanism of infection [6].

In 1896, Gaffky achieved the initial cultivation and isolation of *Salmonella* Typhi in a pure culture, deriving it from the spleen of an afflicted patient [7]. The disease may be complicated by relapse and carriage in the gall bladder, resulting in long-term chronic fecal shedding along with protracted illness lasting several weeks. The disease is seldom fatal, but some patients can develop life-threatening complications, including hypotensive shock and perforation of the intestine. Between 1% and 5% of acutely infected patients may go on to chronically carry and shed the organisms into the local environment [8]. The disease is presumably maintained in human populations through carriage; *S.* Typhi and *S.* Paratyphi A can survive for protracted periods in the gallbladder of apparently healthy people [9].

Along with Africa, South and South-East Asia is among the most prevalent geographical areas enteric fever remains one of the endemic problems in Nepal as in other developing countries due to variable efficacies of currently available vaccine preparation, unplanned urbanization lack of safe water supply and sanitation facilities and increased regional movements of large number of migrant workers [8].

S. Typhi and *S.* Paratyphi have been reported as the most common isolates from patients in major hospitals in Nepal enteric fever is also known as '*bisham joauro*' meaning fever with poison. The fever is endemic from May to August [10] which coincides with the peak of the wet season in tropical and subtropical locations. The occurrence of typhoid outbreaks led to the fatalities of numerous Nepalese individuals once it was identified as a contributing factor to fever [11]. Virtually in all the endemic areas, enteric fever is most prevalent in children 15–19 years of age [12].

A study from Kathmandu, Nepal showed the burden of disease to be greatest in school-age children and young adults, possibly because of a large transient workforce traveling from locations outside the city where exposure to infecting organisms may be less common [13]. There have been reports of seasonal typhoid outbreaks with a recent one in 2002 in Bharatpur, a central town of Nepal. The multidrug-resistant typhoid epidemic affected more than 6000 patients in a 4 to 5-week period and was from a single source of the municipality water supply [11].

Diagnostic methods currently in use are broadly classified into two: conventional methods and rapid methods. Conventional methods involve the isolation and identification of causative organisms by culturing in non-selective, selective enrichment, and differential methods (culture methods) followed by serological confirmation. Culture methods are highly time-consuming generally presumptive result takes about 3–4 days while definitive positive result is obtained only after 5–6 days. The serological test which examines the patient's serum for *Salmonella* antibody is a rapid tool in the diagnosis of enteric fever [14, 15].

Bacterial culture facilities are often unavailable, expensive, and time-consuming, especially in developing countries like Nepal. Therefore, a simple and rapid serological diagnostic test for typhoid fever would be of great benefit in circumstances where more sophisticated laboratory support has not been practiced. Several agglutination assays have been formulated, with the Widal technique being the earliest and still the most commonly employed. This method relies on a visible agglutination reaction facilitated by serum, occurring at a macroscopic level, between the somatic lipopolysaccharide O

antigens (TO) and flagellar H antigens (TH) of *S.* Typhi. The diagnosis involves correlating clinical symptoms indicative of typhoid with a notable level of agglutination antibodies against the H and/or O antigen of *Salmonella* Typhi in the bloodstream [16].

The Widal test yields a positive result after the tenth day of illness and could be erroneously positive in individuals who have previously been vaccinated with TAB. Levels are assessed through sequential dilutions of sera. The preferred approach for conducting the Widal test is the tube agglutination method, involving stepwise two-fold dilutions of the subject's serum, ranging from 1:20 to 1:1280 [17].

MATERIALS AND METHODS

Study Design, Site and Duration

This study was carried out on a cross-sectional basis in Bhimdutta Municipality of Kanchanpur. It is located in southern-west part of Nepal and is situated at an elevation of 228 meters above sea level. The weather gets extremely hot during summer, surging the cases of many seasonal diseases, especially those transmitted from the faeco-oral route as underground water is still the main source of drinking water. The unmanaged housing, improper sewage system, and proximity of the shallow borehole to pit latrines cause pollution of underground water. Enteric fever cases are diagnosed every day in clinics and hospitals in Kanchanpur. The study was conducted from October 2016 to December 2016.

Sample and Selection Criteria

For research purposes, blood samples were collected from patients suspected of enteric fever in Mahakali Zonal Hospital, Life Care, and Kanchan Laboratories of Bhimdutta Municipality, Kanchanpur.

Inclusion Criteria

Both male and female patients suspected of enteric fever who visited the above-mentioned hospital and clinics during the study period were included in the study.

Exclusion Criteria

Patients who had already taken antibiotics and the sample from the patients that were not sufficient as per requirement according to research criteria were excluded during the study.

Sample Size

A total of 160 samples were collected for study purposes during the study period, which was further processed using the Widal test.

Sample Collection and Processing Data Collection

A set of rules was established to collect the information of each patient regarding their name, age sex address, and clinical history.

Sample Collection

Blood samples were collected by using aseptic techniques in culture tubes. The specimens were conveyed to the microbiology laboratory at Siddhanath Science Campus (SNSC) within 2 hours and subjected to subsequent processing.

Widal Test

A Widal test was performed using 2 ml of collected blood sample. The blood sample that was gathered underwent centrifugation to isolate the serum. Identification of positive and negative samples was done using sterilized plastic microtiter plates.

Thorough scrutiny was conducted to detect agglutination following the introduction of the serum sample, followed by the addition of an equivalent volume of antigens ('O', 'H', 'AH', 'BH') and quality control into the plate wells using a micropipette. This process was carried out to determine the

conclusive positive or negative agglutination outcomes for each sample. The examination was executed in precise accordance with the guidelines furnished in the instructional materials from the manufacturer (Span Diagnostics).

Agglutination Test for Antibody Titer Determination

Antibody titers were determined by semi-quantitative slide agglutination and quantitative tube agglutination Widal blood test to establish the presence of the level of antibodies in a blood sample and predict the acuteness of enteric fever.

Statistical Analysis

The information derived from the study was subjected to analysis using statistical software tools, namely SPSS version 19.0 and Excel.

RESULT AND DISCUSSION

A total of 160 blood samples were collected from enteric fever suspected persons visiting Mahakali Zonal Hospital, Life Care, and Kanchan Laboratories from October to December. Samples were collected from individuals of age groups from 1 to 70 years. The antibody levels against different *Salmonella enterica* serotypes were assessed using the Standard Widal Confirmatory Tube technique on the isolated serum.

Agglutination Result Analysis

Among the total 160 samples tested, 32 samples showed positive agglutination for the Widal test. Of which 25 (81.25%) showed agglutination for *Salmonella enterica* serotype Typhi and 7 (18.75%) for *Salmonella enterica* Paratyphi. Among the paratyphoid samples, 2 demonstrated positive agglutination test for *S.* Paratyphi A and 5 for *S.* Paratyphi B. Involvement of *Salmonella* Paratyphi C was absent in studied samples.

Gender-wise Distribution of Samples Showing Positive Agglutination Test

Out of 160 blood samples that were received for study purposes, 97 (60.64%) were received from female and 63 (39.38%) were received from male patients. Among 63 male patients, 14 (43.75%) patients, and among 97 enteric fever suspected females, 18 (56.25%) patients showed positive agglutination results on the microtiter plate agglutination test. The gender was found to be statistically insignificant ($p<0.05$) to the enteric fever-positive blood samples. Most of the positive samples showed *S.* Typhi infection among the total positive samples processed. Thus, *S.* Typhi is associated with more patients with enteric fever cases in this study. Similar results were reported in studies from Far-Western Nepal by Bhatt, 2015 [18] (Figures 1–3).

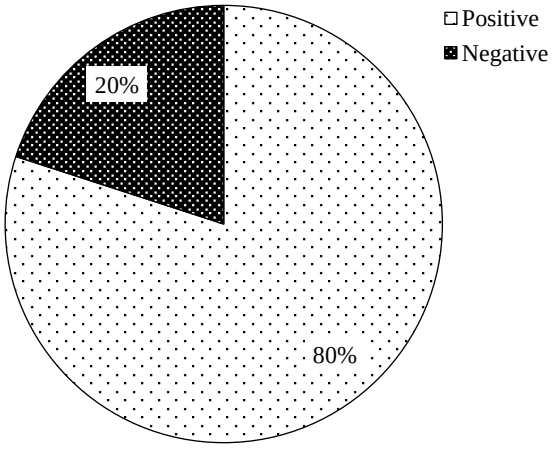

Figure 1. Percentage distribution of agglutination results.

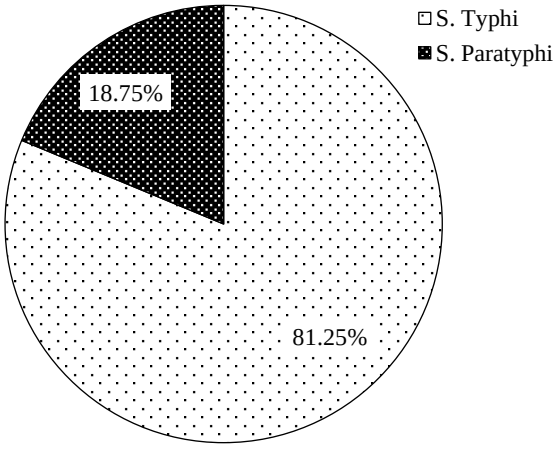

Figure 2. Percentage distribution of *S.* Typhi, *S.* Paratyphi.

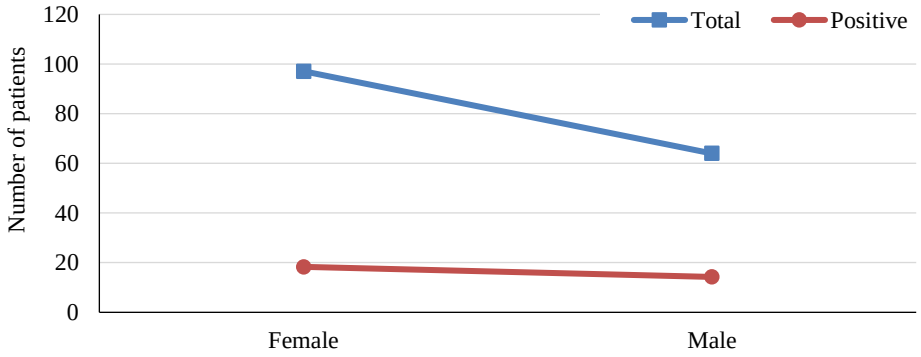

Figure 3. Variation of positive agglutination samples among males and females.

Age-wise Distribution of Positive Samples

The total blood samples studied and analyzed during the study period included patients whose ages ranged from 1 to above 70 years. The largest proportion of patients fell within the age bracket of under 30. In this age group, 9 patients' blood groups were found to be positive. In this age group, 5 (28.13%) were female and 4 (27.78%) were male. The age was found to be statistically significant to the positive cases. The study showed that the highest number of patients (28.13%) belonged to the age group 21–30, of which 5 belonged to female patients and 4 belonged to male patients. Bhatt (2015) also reported the similar results. A possible reason behind this observation is the more exposure of the people of this age to the environment. They drink and eat foods from outside more often, especially street food where the chances of contamination are highest. *S.* Typhi was predominantly found in all age groups and showed the highest rate than *S.* Paratyphi. This observation does agree with the results reported by Khanal et al. [19].

Distribution of Positive Samples by Month

The duration of the study was from October 2016 to December 2016. Blood samples were collected except on holidays. Although the peak season lies from June to August, significant positive samples were observed during this study period. 15 (30%) positive samples were observed in the first month of the study, i.e., in October. 10 (20%) samples were found positive among the total blood samples that were collected and analyzed by Widal test in November. Similarly, 7 (11.67%) positive samples were observed in December among total blood samples that were collected from the patients suspected of enteric fever. Several positive samples gradually decreased from October to December as per expectations. This result agrees with some other studies conducted during the same months. A study done in Spain also showed a higher rate of enteric fever in colder months (October–December) [20]. Seasonal trends of non-typhoidal Salmonellosis (Enteritidis) in humans in East Asia and (Typhimurium) in India were found to be highest in colder months (November–December) [21] (Figure 4 and Table 1).

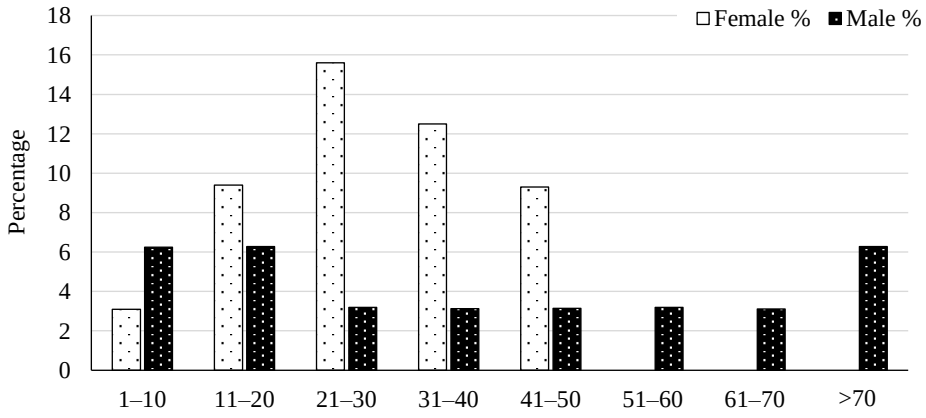

Figure 4. Distribution of positive samples according to age.

Table 1. Distribution of positive samples by months.

Months	Positive samples	Positive (%)
October	15 out of 50	30
November	10 out of 50	20
December	7 out of 60	11.67

Distribution of the Samples According to Antibody Titer

The serum samples that showed positive agglutination tests were further subjected to determination of antibody titer. Among total positive cases, 6 (3.8%) positive samples with *S.* Typhi O had an end titer of 1:20. 1 positive case (0.6%) had an end titer of 1:40. Same results were noticed for an end titer of 1:320. About 11 (34.4%) of total positive samples showed *S.* Typhi H. Of which 4 positive had end titer of 1:20. 3 (1.9%) of the positive samples with *S.* Typhi H had an end titer of 1:80. One positive case had an end titer of 1:160 and accounted for 0.6% and 2 positive samples had an end titer of 1:320 and accounted for 1.3%.

Among the total positive samples with *S.* Paratyphi, 2 positive samples were found to show positive agglutination test against *S.* Paratyphi A. Of which 1 (0.6%) positive case was found to have an end titer of 1:20. No positive samples were found to have an end titer of 1:40, 1:160, and 1:320. 1 positive case had end titer of 1:80 and accounted for 0.65% of total positive samples against *S.* Paratyphi A. 5 positive agglutination results were found against *S.* Paratyphi B. Out of which 3 positive samples had end titer of 1:20, 1 showed end titer of 1:40. No positive samples exhibited end titers of 1:80 or 1:160. Only a single sample displayed an end titer of 320.

In the tube agglutination test, the highest dilution to which patients' serum showed agglutination was found to be 1:1280 indicating the presence of a higher concentration of antibodies against *Salmonella*. Two positive samples were found to have end titer 1:1280; one of them was against *S.* Typhi O and another against Typhi H. Both of them accounted for 0.6% separately of total positive cases. The antibody titer observed from the slide agglutination method and tube agglutination method was different for *Salmonella* Typhi O, Typhi H, and Paratyphi A and B. The possible reason behind this might be the time duration between the slide agglutination test and the tube agglutination test. The slide agglutination test was done immediately to find out end titers but sera obtained after centrifugation was stored to carry out tube agglutination test of 3 samples together for convenience. Another reason might be the difference in technique in both methods. These results show similarities with the findings of Ibegbulam et al. [22] (Figure 5).

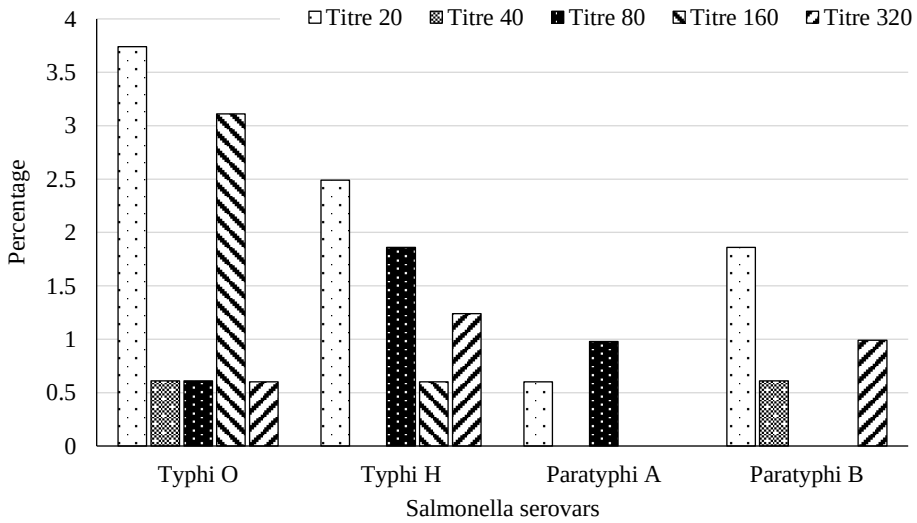

Figure 5. Distribution of antibody titer among different serovars.

Statistical Analysis of Association of Test Results with Gender

Statistical analysis of the association of test results with gender was done using the Chi-square test and $\chi 2$ was found to be 0.32. However, $\chi 2$ tab at (2-1) (2-1) degree of freedom =1 and 95% confidence level, is equal to 3.841. Hence, there is no significant difference in the rate of occurrence of enteric fever among males and females (Table 2).

Table 2. Positive cases in males and females.

Cases	Male patients	Female patients	Total
Positive	14	18	32
Negative	49	79	128
Total	63	97	160

CONCLUSION

In the present study, *S.* Typhi was found to be more prevalent than *S.* Paratyphi and is the main cause of enteric fever in the study site which correlates with the general trend of typhoid incidences. Among suspected patients studied 25 (78.13%) and 7 (21.87%) cases were confirmed to have the presence of significant antibody titer against *S.* Typhi and *S.* Paratyphi respectively. Incidence of enteric fever was found to be highest in the age group 21–30 years with 9 (28.13%) cases, so this age group can be considered to be at greater risk than other age groups. Most of the positive samples were observed among female patients although the difference was not much large. Out of a total of 32 positive cases 15 positive samples were observed in October, 10 in November, and 7 in December, this indicates that enteric fever is common in the study area and prevails regardless of the change in seasons and weather conditions. Thus, the results suggest that enteric fever is the most common systemic illness that is often diagnosed in the study area, throughout the year in the patients visiting hospitals and clinics. Therefore, necessary steps need to be taken to break the chain of transmission to reduce the impact of this systemic illness.

REFERENCES

1. Hawkey PM. Medical Microbiology. A Guide to Microbial Infections: Pathogenesis, Immunity. Laboratory Diagnosis and ControlD. Greenwood, R. Slack & John Peutherer, eds. Pp. S50 + 278 ills. Edinburgh: Churchill Livingstone; 1902. £24.95. Epidemiol Infect. 1993;110(2):425. doi: 10.1017/S0950268800068369.
2. Crump JA, Youssef FG, Luby SP, Wasfy MO, Rangel JM, Taalat M et al. Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. Emerg Infect Dis. 2003 May;9(5):539–44. doi: 10.3201/eid0905.020428.
3. Institute for Health Metrics and Evaluation. (2019). Typhoid fever — Level 4 cause. The Institute for Health Metrics and Evaluation [online]. Seattle, WA: The Institute for Health Metrics and Evaluation. Available from: https://www.healthdata.org/results/gbd_summaries/2019/typhoid-fever-level-4-cause.
4. Antillón M, Warren JL, Crawford FW, Weinberger DM, Kürüm E, Pak GD, et al. The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. PLOS Negl Trop Dis. 2017 Feb 27;11(2):e0005376. doi: 10.1371/journal.pntd.0005376.
5. Louis PCA, Bowditch HI. Anatomical Pathological and Therapeutic Researches Upon the Disease Known Under the Name of Gastro-Enterite Putrid Adynamic Ataxic or Typhoid Fever etc.: Compared with the Most Common Acute Diseases. Boston: I.R. Butts; 1836.
6. Budd W. Typhoid fever its nature, mode of spreading, and prevention. Am J Public Health (N Y). 1918 Aug;8(8):610–2. doi: 10.2105/ajph.8.8.610.
7. Barnett R. Typhoid fever. Lancet. 2016;388(10059):2467. doi: 10.1016/S0140–6736(16)32178-X.
8. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347(22):1770–82. doi: 10.1056/NEJMra020201.
9. Raffatellu M, Wilson RP, Winter SE, Bäumler AJ. Clinical pathogenesis of typhoid fever. J Infect Dev Ctries. 2008 Aug 1;2(4):260–6. doi: 10.3855/jidc.219.

10. Lewis MD, Serichantalergs O, Pitarangsi C, Chuanak N, Mason CJ, Regmi LR, et al. Typhoid fever: a massive, single-point source, multidrug-resistant outbreak in Nepal. Clin Infect Dis. 2005;40(4):554–61. doi: 10.1086/427503.
11. Rauniar GP, Das BP, Baral DD, Naga Rani MA. Treatment pattern of typhoid fever at a tertiary care teaching hospital in eastern Nepal. J Nepal Asso. 2000;39:218–21.
12. Who.int. World. Typhoid. World Health Organization: WHO; 2023. [Online] Available from: https://www.who.int/news-room/fact-sheets/detail/typhoid.
13. Karkey A, Arjyal A, Anders KL, Boni MF, Dongol S, Koirala S et al. The burden and characteristics of enteric fever at a healthcare facility in a densely populated area of Kathmandu. PLOS ONE. 2010 Nov 15;5(11):e13988. doi: 10.1371/journal.pone.0013988.
14. Cudjoe KS, Hagtvedt T, Dainty R. Immunomagnetic separation of Salmonella from foods and their detection using immunomagnetic particle (IMP)-ELISA. International Journal of Food Microbiology. 1995; 27(1): 11–25. [Online] Available from: https://doi.org/10.1016/0168-1605(94)00134-r.
15. Gonzatez I, Garca T. Fernadez a, Sanche ZB, Hemandez P.E. and martin R.M. Rapid Enumeration Quant PCR-ELIZ J Appl Microbiol. 1990;86:231–6.
16. Abraham G, Teklu B, Gedebu M, Selassie GH, Azene G. Diagnostic value of the Widal test. Trop Geogr Med. 1981;33(4):329–33.
17. Washington JA, Henry JB. Medical Microbiology in Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: W B Saunders; 1984. p. 1102.
18. Bhatt P. Comparative study of blood culture and Widal test from the patients suspected of enteric fever in Far-Western region of Nepal. [printed thesis] (M Sc. Thesis) Tribhuvan University; 2015, pp-33–44.
19. Khanal B, Sharma SK, Bhattacharya SK, Bhattarai NR, Deb M, Kanungo R. Antimicrobial susceptibility patterns of Salmonella enterica serotype typhi in eastern Nepal. J Health Popul Nutr. 2007 Mar;25(1):82–7.
20. Martinez-Urtaza J, Liebana E, Garcia-Migura L, Perez-Piñeiro P, Saco M. Characterization of Salmonella enterica serovar Typhimurium from marine environments in coastal waters of Galicia (Spain). Appl Environ Microbiol. 2004 Jul;70(7):4030–4. doi: 10.1128/AEM.70.7.4030–4034.2004.
21. Ekdahl K, De Jong B, Wollin R, Andersson Y. Travel-associated non-typhoidal salmonellosis: geographical and seasonal differences and serotype distribution. Clin Microbiol Infect. 2005 Feb 1;11(2):138–44. doi: 10.1111/j.1469–0691.2004.01045.x.
22. Ibegbulam Njoku PN, Chijioke Osuji CC, Duru FC. Prevalence of antibody titre in healthy individual and enteric fever patients in Owerri, Nigeria. J Public Health Epidemiol. 2014 Jun 30;8(6):192–6. doi: 10.5897/JPHE2013.0612.

