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RESEARCH ARTICLE

Serological determination of *Salmonella typhi* and Para typhi infection in clinical acute febrile illness in Bangladesh

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ABSTRACT

Enteric fever remains a significant public health concern in Bangladesh and other low- and middle-income countries, largely due to inadequate sanitation and limited access to safe drinking water. Although blood culture is considered the diagnostic gold standard, serological tests such as the Widal test continue to be widely used in resource-limited settings. This cross-sectional study was conducted in the Immunology Laboratory of the Bangladesh Institute of Health Sciences (BIHS) and Bangladesh University of Health Sciences (BUHS), Dhaka, from October to December 2020, to determine the serological prevalence of enteric fever among clinically suspected acute febrile patients and to analyze antigen-specific reactivity patterns. A total of 35 blood samples were collected and analyzed using slide and tube Widal agglutination methods to detect antibodies against *Salmonella Typhi* and *Salmonella Paratyphi* antigens. Of the 35 samples, 16 (45.71%) showed positivity for at least one Widal antigen, while 19 (54.29%) were negative. Seropositivity was slightly higher among males (25.71%) than females (20%). The TH antigen (*Salmonella Typhi* H) demonstrated the highest positivity in both males (20%) and females (27%). Positivity for the BO antigen was observed in both genders, whereas TO antigen positivity was limited and detected only among females (7%). No reactivity was observed for AO or AH antigens in either group. The predominance of TH antigen positivity suggests previous exposure or immune sensitization to *Salmonella Typhi* rather than acute infection in many cases. While the Widal test remains a useful screening tool in resource-poor settings, its results should be interpreted cautiously and correlated with clinical findings and local epidemiological data. Strengthening diagnostic capacity, surveillance systems, and vaccination strategies is essential to reduce the burden of enteric fever in Bangladesh.

Keywords: Enteric fever, Salmonella Typhi, Widal test, Seroprevalence, Bangladesh

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1. Introduction

Enteric fever remains a major public health problem in many low- and middle-income countries, including Bangladesh. It is a systemic infectious disease primarily caused by *Salmonella enterica* serovar Typhi, which results in typhoid fever, and by *Salmonella enterica* serovars Paratyphi A, B, and C, which cause paratyphoid fever[1]. These organisms belong to the family *Enterobacteriaceae* and are Gram-negative, facultative anaerobic bacilli that infect humans exclusively[2].

Typhoid and paratyphoid fevers are transmitted through the ingestion of food or water contaminated with human feces containing the bacteria. The disease spreads mainly via the fecal–oral route, often due to poor sanitation, unsafe drinking water, and inadequate personal hygiene. Infected individuals, including asymptomatic carriers, play a significant role in the transmission of the infection by contaminating food and water sources[3].

After ingestion, the bacteria multiply in the intestinal tract and may invade the bloodstream, leading to systemic infection. The incubation period usually ranges from one to two weeks, and the illness typically lasts three to four weeks if left untreated. Common clinical features include prolonged high-grade fever, headache, anorexia, generalized body aches, abdominal discomfort, diarrhea or constipation, lethargy, and, in some cases, chest congestion. A small proportion of infected individuals may become chronic carriers, harboring the bacteria in the biliary tract even after clinical recovery[4].

Accurate and timely diagnosis of enteric fever is essential for appropriate management and prevention of complications. Culture-based methods, particularly blood culture, are considered the gold standard for diagnosis; however, their use is often limited in resource-constrained settings due to cost, laboratory facilities, and prior antibiotic use. Bone marrow culture, although more sensitive, is invasive and rarely performed in routine clinical practice[5].

In many endemic regions, including Bangladesh, serological methods such as the Widal test are widely used for the diagnosis of enteric fever. The Widal test detects agglutinating antibodies against the somatic (O) and flagellar (H) antigens of *Salmonella* Typhi and Paratyphi in patient serum. Despite limitations related to sensitivity and specificity, the test remains a commonly employed diagnostic tool due to its simplicity, low cost, and availability in peripheral healthcare facilities[6].

Enteric fever is treatable with appropriate antibiotic therapy, which significantly reduces morbidity and mortality. Before the introduction of antibiotics, the disease was associated with high fatality rates due to severe complications such as intestinal hemorrhage, perforation, and overwhelming systemic infection[7]. With timely diagnosis and effective antimicrobial treatment, patient outcomes have markedly improved. This study aims to determine the serological prevalence of *Salmonella* Typhi and Paratyphi infections among clinically suspected acute febrile patients, thereby contributing to a better understanding of disease patterns in the local healthcare setting.

2. Literature Review

Enteric fever is a systemic infectious disease characterized by prolonged fever and abdominal discomfort, caused by Salmonella enterica serovar Typhi or, less commonly, Salmonella Paratyphi A, B, or C. Typhoid fever refers specifically to infection caused by S. Typhi, while paratyphoid fever generally follows a milder clinical course. Transmission occurs through ingestion of food or water contaminated with feces from infected individuals or chronic carriers, making the disease closely associated with poor sanitation, unsafe water supplies, and inadequate hygiene practices[8].

Despite major reductions in incidence in industrialized nations, typhoid fever remains a significant public health concern in many developing countries. Improvements in sanitation and water treatment have reduced cases in the United States to a few hundred annually, most linked to international travel. In contrast, regions such as South Asia, parts of Africa, and the Middle East continue to experience a high disease burden. Globally, typhoid fever affects millions of people each year and is responsible for considerable morbidity and mortality, particularly in populations with limited access to clean water, healthcare, and preventive services[9,10].

The clinical course of untreated typhoid fever typically progresses over several weeks. Early disease is characterized by gradually rising fever, malaise, headache, cough, and abdominal discomfort, often accompanied by relative bradycardia and leukopenia. As the disease advances, fever becomes sustained and high, and patients may develop profound fatigue, delirium, rose spots on the trunk, hepatosplenomegaly, and gastrointestinal symptoms[11]. In later stages, serious complications such as intestinal hemorrhage, intestinal perforation, encephalitis, pneumonia, thrombocytopenia, and severe dehydration may occur, significantly increasing the risk of death if appropriate treatment is not provided.

The etiological agent, Salmonella enterica serovar Typhi, is a gram-negative bacillus with humans as its only known reservoir. Molecular epidemiological studies have identified globally distributed lineages, including a dominant multidrug-resistant haplotype that originated in South Asia and has since spread internationally[12]. Advances in genotyping have improved understanding of transmission patterns and antimicrobial resistance. Chronic human carriers, who continue to shed bacteria long after recovery, play a crucial role in maintaining endemicity and facilitating ongoing transmission[13].

Diagnosis of typhoid fever is based on microbiological culture and serological testing. Blood, bone marrow, and stool cultures remain the diagnostic gold standard, although their sensitivity varies depending on disease stage and prior antibiotic exposure. Serological tests such as the Widal test are widely used in resource-limited settings because of their simplicity and low cost, despite limited sensitivity and specificity. Rapid diagnostic tests, including Typhidot and Tubex, have been developed to provide quicker results by detecting specific antibodies to S. Typhi. While these tests offer moderate diagnostic accuracy, variability in performance and lack of quantification restrict their reliability as standalone diagnostic tools[14].

Prevention of typhoid fever relies heavily on improved sanitation, access to safe drinking water, and proper food handling practices. Historical reductions in disease incidence in developed countries highlight the effectiveness of water chlorination, waste management, and hygiene promotion. In endemic regions, sustained public health interventions remain essential to interrupt transmission and reduce disease burden[15]. Vaccination has emerged as an important preventive strategy, particularly in high-risk populations. Currently available vaccines include a live oral vaccine and an injectable Vi polysaccharide vaccine, both of which provide moderate protection. Although older whole-cell vaccines are still used in some settings, they are largely discouraged due to higher rates of adverse effects. More recently, typhoid conjugate vaccines have demonstrated high efficacy, especially in children, and represent a major advancement in long-term disease control. Vaccination programs, when combined with improvements in sanitation and health education, have proven effective in reducing outbreaks and overall incidence[16].

Treatment of typhoid fever involves supportive care and antimicrobial therapy. Oral rehydration therapy is essential in preventing complications related to dehydration. Antibiotic treatment markedly reduces disease duration and mortality; however, increasing antimicrobial resistance has complicated management. Fluoroquinolones, once the mainstay of therapy, have lost effectiveness in many regions due to resistance, leading to greater reliance on third-generation cephalosporins and azithromycin[17]. Multidrug-resistant and

fluoroquinolone-resistant strains of S. Typhi are now widely reported, particularly in South and Southeast Asia[18]. This growing resistance highlights the need for continuous surveillance, judicious antibiotic use, and integrated prevention strategies combining vaccination, improved sanitation, and strengthened public health systems.

3. Methodology

This study was conducted as a cross-sectional investigation to evaluate enteric fever among febrile patients. The study was carried out in the Immunology Laboratory of the Bangladesh Institute of Health Sciences (BIHS) in collaboration with the Bangladesh University of Health Sciences (BUHS), Dhaka, over a three-month period from October to December 2020. The study population consisted of patients attending BIHS General Hospital with a history of febrile illness. Participation was voluntary, and only patients who provided informed written consent were included in the study, while those unwilling to participate were excluded.

A total of 35 blood samples were collected under aseptic conditions using sterile syringes and needles. Approximately 8–10 ml of blood was collected from adult patients and 3–5 ml from pediatric patients. Samples were promptly transported to the immunology laboratory for analysis. For bacteriological investigation, a portion of each blood sample was inoculated into Tryptic Soy Broth and incubated at 37°C. After incubation, subculturing was performed on Xylose Lysine Deoxycholate (XLD) agar. Suspected bacterial colonies were further identified by standard biochemical procedures, including Triple Sugar Iron (TSI) agar test, citrate utilization test, motility test, urease test, and lysine decarboxylation test.

Serological analysis was performed using the Widal agglutination test to detect antibodies against *Salmonella Typhi* and *Salmonella Paratyphi*. The Widal test was carried out by both the slide agglutination method for screening and the standard tube agglutination method for quantitative estimation of antibody titres. Commercially available Widal antigen kits containing O, H, AH, and BH antigens were used. Agglutination reactions were observed macroscopically, and antibody titres were interpreted according to standard diagnostic criteria for enteric fever.

All laboratory investigations were conducted in accordance with established biosafety and quality control guidelines. Ethical approval for the study was obtained from the relevant institutional authority prior to initiation, and strict confidentiality of patient information was maintained throughout the study.

4. Results

The distribution of Widal test results among male and female patients is presented in Figure 1. Out of the total 35 samples analyzed, 16 (45.71%) were positive and 19 (54.29%) were negative. Among male patients, 9 (25.71%) tested positive and 11 (31.43%) tested negative, comprising 57.14% of the total study population. Among female patients, 7 (20%) were positive and 8 (22.86%) were negative, accounting for 42.86% of the samples. Overall, Widal test positivity was slightly higher among males than females.

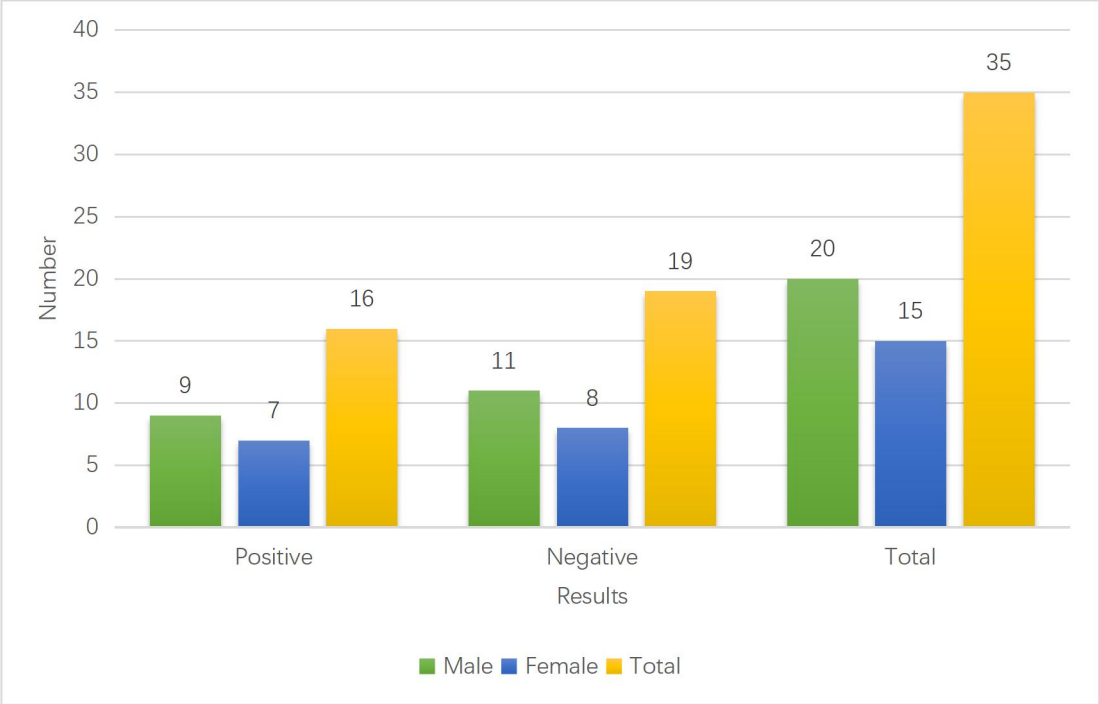

Figure 1. Distribution of Widal test results according to gender

Table 1 presents the distribution of Widal test results among male participants aged 19 to 65 years. Overall, the majority of male subjects showed negative results for all Widal antigens (TO, TH, AO, AH, BO, and BH), indicating a low serological positivity rate in this group. Positivity was observed mainly for the TH antigen, with positive reactions detected in males aged 25, 38, 49, and 60 years, suggesting previous exposure or possible infection with *Salmonella typhi*. Additionally, BO antigen positivity was identified in males aged 27, 40, and 44 years, while BH antigen positivity was observed in two older participants aged 53 and 65 years. No positive results were recorded for TO, AO, or AH antigens among male participants.

Table 1. Widal Test Results among Male Participants

No.	Age (Years)	TO	TH	AO	AH	BO	BH
1	19	Negative	Negative	Negative	Negative	Negative	Negative
2	21	Negative	Negative	Negative	Negative	Negative	Negative
3	23	Negative	Negative	Negative	Negative	Negative	Negative
4	25	Negative	Positive	Negative	Negative	Negative	Negative
5	27	Negative	Negative	Negative	Negative	Positive	Negative
6	28	Negative	Negative	Negative	Negative	Negative	Negative
7	31	Negative	Negative	Negative	Negative	Negative	Negative
8	34	Negative	Negative	Negative	Negative	Negative	Negative
9	35	Negative	Negative	Negative	Negative	Negative	Negative
10	38	Negative	Positive	Negative	Negative	Negative	Negative
11	40	Negative	Negative	Negative	Negative	Positive	Negative
12	44	Negative	Negative	Negative	Negative	Positive	Negative
13	45	Negative	Negative	Negative	Negative	Negative	Negative
14	49	Negative	Positive	Negative	Negative	Negative	Negative

No.	Age (Years)	TO	TH	AO	AH	BO	BH
15	50	Negative	Negative	Negative	Negative	Negative	Negative
16	53	Negative	Negative	Negative	Negative	Negative	Positive
17	55	Negative	Negative	Negative	Negative	Negative	Negative
18	56	Negative	Negative	Negative	Negative	Negative	Negative
19	60	Negative	Positive	Negative	Negative	Negative	Negative
20	65	Negative	Negative	Negative	Negative	Negative	Positive

Table 1. (*Continued*)

Table 2 summarizes the Widal test findings among female participants aged 18 to 62 years. Similar to males, most female participants demonstrated negative serological results across all tested antigens, reflecting a generally low prevalence of Widal positivity. Positive reactions were predominantly noted for the TH antigen, observed in females aged 31, 34, 50, and 57 years. Notably, one female participant aged 34 years showed dual positivity for TO and TH antigens, which may indicate recent or active exposure to *Salmonella typhi*. Positivity for the BO antigen was detected in females aged 41 and 55 years. No positive results were found for AO, AH, or BH antigens among female participants.

Table 2. Widal Test Results among Female Participants

No.	Age (Years)	TO	TH	AO	AH	BO	BH
1	18	Negative	Negative	Negative	Negative	Negative	Negative
2	22	Negative	Negative	Negative	Negative	Negative	Negative
3	25	Negative	Negative	Negative	Negative	Negative	Negative
4	29	Negative	Negative	Negative	Negative	Negative	Negative
5	31	Negative	Positive	Negative	Negative	Negative	Negative
6	32	Negative	Negative	Negative	Negative	Negative	Negative
7	34	Positive	Positive	Negative	Negative	Negative	Negative
8	36	Negative	Negative	Negative	Negative	Negative	Negative
9	41	Negative	Negative	Negative	Negative	Positive	Negative
10	44	Negative	Negative	Negative	Negative	Negative	Negative
11	50	Negative	Positive	Negative	Negative	Negative	Negative
12	55	Negative	Negative	Negative	Negative	Positive	Negative
13	57	Negative	Positive	Negative	Negative	Negative	Negative
14	60	Negative	Negative	Negative	Negative	Negative	Negative
15	62	Negative	Negative	Negative	Negative	Negative	Negative

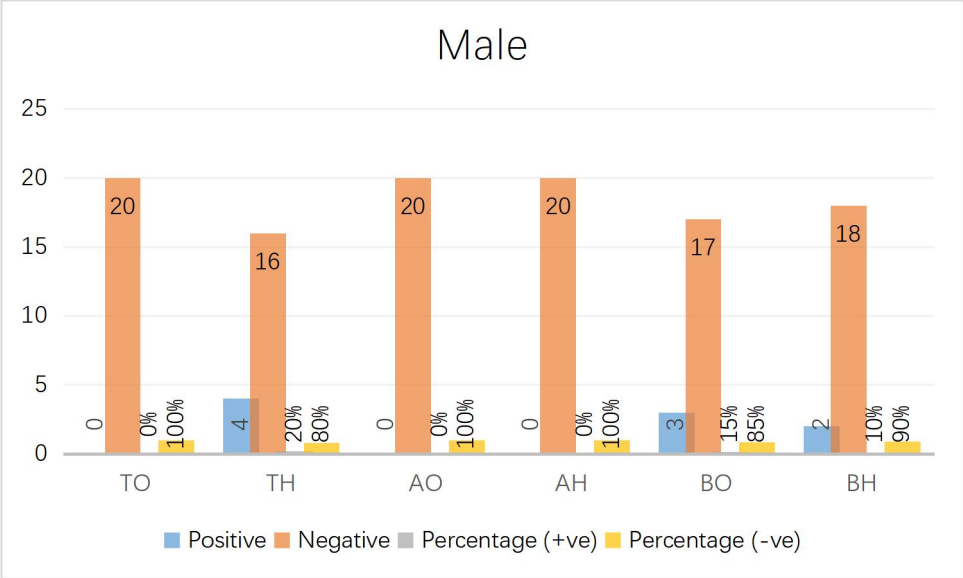

Figure 2. Distribution of Widal Test Antigen Positivity among Male Patients

Figure 2 illustrates the distribution of Widal test results among male patients (n = 20). No positive reactions were observed for TO, AO, or AH antigens, with 100% of samples remaining negative for these parameters. Positivity was mainly observed for the TH antigen, with 4 cases (20%) showing positive results. Additionally, BO and BH antigens showed positivity in 3 (15%) and 2 (10%) cases, respectively. Overall, TH antigen demonstrated the highest rate of positivity among male patients, indicating a greater serological response to *Salmonella Typhi* H antigen.

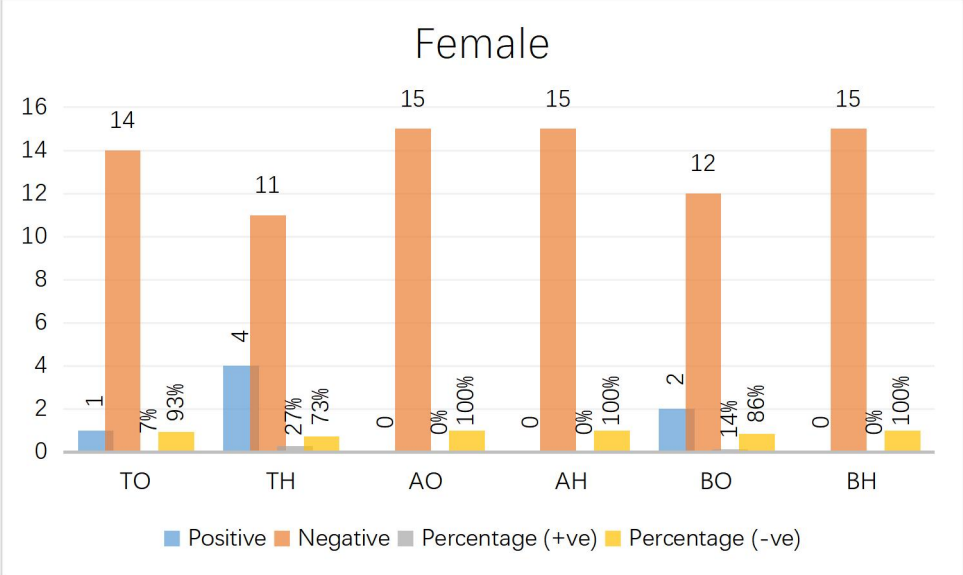

Figure 3. Distribution of Widal Test Antigen Positivity among Female Patients

Figure 3 shows the distribution of Widal test results among female patients (n = 15). A small proportion of samples showed positivity for the TO antigen, with 1 case (7%) testing positive. The highest positivity was observed for the TH antigen, with 4 cases (27%) showing positive results. No positive reactions were detected for AO, AH, or BH antigens. Positivity for the BO antigen was observed in 2 cases (14%). Similar to males, the TH antigen showed the highest positivity among female patients, suggesting a predominant immune response to *Salmonella Typhi* H antigen in the study population.

5. Discussion

In the present study, a total of 35 blood samples (100%) were analyzed using the simple Widal agglutination test to evaluate serological evidence of enteric fever. Of the total study population, 20 samples (57.14%) were obtained from male patients aged 19–65 years, while 15 samples (42.86%) were collected from female patients aged 18–62 years. Overall, 16 samples (45.71%) showed positivity for at least one Widal antigen, whereas 19 samples (54.29%) were non-reactive, indicating a moderate level of serological response among the study participants.

A gender-based comparison demonstrated a slightly higher Widal positivity among male patients compared to females. Among males, 9 samples (25.71%) were positive and 11 (31.43%) were negative, whereas among females, 7 samples (20%) were positive and 8 (22.86%) were negative. This higher seropositivity observed among males may be attributed to greater occupational and environmental exposure, increased outdoor activities, consumption of food from unhygienic sources, and differences in healthcare-seeking behavior, factors commonly reported in enteric fever-endemic regions.

Analysis of individual Widal antigens revealed that TH (*Salmonella Typhi* H) antigen exhibited the highest positivity rate in both sexes. Among male patients, TH positivity was detected in 4 cases (20%), followed by BO (*Salmonella Paratyphi* B O) antigen positivity in 3 cases (15%) and BH antigen positivity in 2 cases (10%). In contrast, TO, AO, and AH antigens were non-reactive in all male samples. Similarly, among female patients, TH antigen showed the highest positivity (4 cases, 27%), followed by BO positivity in 2 cases (14%) and TO positivity in 1 case (7%), while AO, AH, and BH antigens remained negative.

The predominance of TH antigen positivity in this study suggests prior exposure or immune sensitization to *Salmonella Typhi*, as H antibodies are known to persist for longer periods compared to O antibodies. Consequently, TH positivity alone may not reliably distinguish between acute, recent, or past infections. The comparatively low TO antigen positivity, particularly among female patients, further highlights the limited sensitivity of single-sample Widal testing for diagnosing acute enteric fever, as O antibodies tend to rise early in infection and decline rapidly.

The complete absence of AO and AH antigen positivity across both male and female participants indicates a low circulation of *Salmonella Paratyphi* A in the study population. This finding is epidemiologically significant, as it suggests that *S. Typhi* may remain the predominant etiological agent of enteric fever in the study area, with limited contribution from paratyphoid strains.

6. Conclusion

Accurate diagnosis is essential for estimating the true burden of typhoid and guiding public health planning. In resource-limited settings, the Widal test remains a simple, affordable, and widely available diagnostic tool, but proper interpretation is crucial, considering possible cross-reactions with other infections. Local surveillance data can further aid clinical decision-making. The recent WHO approval of the Tybar–TCV vaccine, effective from six months of age with a single dose, offers a valuable opportunity to reduce typhoid incidence in Bangladesh.

Conflict of interest

The authors declare no conflict of interest

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