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RESEARCH ARTICLE

PREVALENCE OF *SALMONELLA TYPHI* AND *SALMONELLA PARATYPHI* IN STOOL SAMPLES FROM SERO-POSITIVE WIDEL TEST PATIENTS IN DHAMAR CITY, YEMEN

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Abstract

Background and Aims: There are various diagnostic tests for typhoid fever. However, because the majority of healthcare facilities solely employ the Widal test without verifying the results with a second test method, misdiagnosis is frequent. In a number of public and private hospitals in Dhamar, Yemen, the study sought to ascertain the incidence of *Salmonella typhi* and *Salmonella paratyphi* as well as related factors among clinically diagnosed typhoid fever patients.
Methods: The study included 250 patients with clinical signs of typhoid fever, from whom 250 blood samples were collected. The Widal test was then used to detect typhoid fever. Stool samples were also collected from all patients who tested positive, and these samples were cultured on bismuth sulphite agar to isolate *S. typhi* and *S. paratyphi*; then confirmation was done with biochemical reactions. Sociodemographic data were collected from the patients through a structured questionnaire and then analysed using Epi-info 7 software.
Results: Out of 250 blood samples, 194 (77.6%) were positive for seropositivity via the Widal test. Stool samples from these patients were cultured on bismuth sulphide agar, leading to 63 positive cultures (32.5%). Notably, 46 isolates (23%) were from patients recently treated with antibiotics. Among the 63 positives, 9 samples were *S. typhi* and 54 were *S. paratyphi*. Affected patients included 45 females (23.2%) and 18 males (9.3%), with the highest prevalence in the 21-30 age group (42.8%); 55 isolates (87.3%) were susceptible to ciprofloxacin and ofloxacin, while 41 (65.1%) were resistant to cefotaxime.
Conclusion: In conclusion, antibiotic treatment for typhoid fever often fails due to high rates of resistance to antibiotics such as ciprofloxacin, ofloxacin, and cefotaxime, which are considered first-line treatments for typhoid. Furthermore, the reliability of the Widal test in diagnosing typhoid fever is questionable due to a high rate of false-positive results.
Keywords: *Salmonella*, stool culture, typhoid fever, Widal test.

INTRODUCTION

Typhoid fever is still a leading cause of death and morbidity worldwide. According to estimates from the World Health Organisation (WHO), there are around 21 million cases and 222,000 fatalities of typhoid fever worldwide each year. It represents an important foodborne disease for humans. An estimated 93.8 million cases of gastroenteritis caused by *Salmonella* species occur globally each year and of these, nearly 80.3 million cases are foodborne[1-3]. *Salmonella* is a Gram-negative, rod-shaped bacillus, a facultative, intracellular bacterium within the family *Entero-bacteriaceae*. Both humans and animals with the infection have bacteria in their digestive tracts. There are now just two species in the genus *Salmonella*: S. enterica and S. bongori. There are more than 2,659 serotypes in the genus *Salmonella*[3]. Only *S. typhi* and *S. paratyphi* are human-specific pathogenic *Salmonella* serotypes that cause systemic infection[4]. Foods and drinks tainted by the faeces of chronic carriers are usually the source of *S. typhi* and *S. paratyphi* infections in humans. Carriers may occasionally excrete the bacteria in their urine. People can also become infected by consuming tainted shellfish or defective canned meat, or by drinking water tainted with sewage. The classic prolonged fever, persistent bacteremia without endothelial or endocardial involvement, and bacterial invasion and multiplication within mononuclear phagocytic cells in the liver, spleen, lymph nodes, and Peyer patches are the hallmarks of induced typhoid fever, a severe multi-systemic illness.

If left untreated, typhoid illness can be lethal[4]. Laboratory confirmation is typically necessary for a provisional clinical diagnosis. The Widal agglutination test, which has been used for more than a century to diagnose typhoid disease, is one of the many serological methods of diagnosis that have been established. As previously mentioned, the Widal test has a sensitivity and specificity restriction despite being frequently utilised. *Salmonella* can be isolated from clinical specimens more accurately than by serological testing[1,5].

Because antibiotic resistance is more prevalent in underdeveloped nations, *Salmonella* infections are frequently more dangerous there[6,7]. Without a proper diagnosis and efficient antibiotic treatment, the fatality rate from enteric fever is predicted to rise by 30%[5]. The preferred antibiotics for treating enteric fever include ampicillin, chloramphenicol, cotrimoxazole, fluoroquinolones, and third-generation cephalosporins. However, in developing nations, typhoidal *Salmonella* species have grown more resistant to traditional antibiotics[8].

The validity of the Widal test has not been evaluated in Dhamar. Also, there are few studies conducted in Yemen on the prevalence of *Salmonella*. Therefore, our study aimed to determine the prevalence of *S. typhi* and *S. paratyphi* in stool samples from patients who had positive Widal test results, to validate the Widal test by comparing it with stool culture, and to develop an antibiotic regimen for *S. typhi* and *S. paratyphi* isolates in Dhamar for the purpose of empirical treatment of this infection.

MATERIALS AND METHODS

Study design: This is a cross-sectional study carried out during the period from 6 Dec 2025 to 1 Feb 2026 (Time allocated for study by the department).

Specimens: The patients were venipunctured to get about 3-4 ml of blood, which was then promptly transferred into plain tubes, allowed to clot, and then centrifuged to prepare it for the Widal test. Additionally, stool samples were taken from the same patient and stored in the refrigerator for culture in universal (plastic) disposable vials with screw tops[9].

Data collection: A pre-made questionnaire was used to gather individual data, such as antibiotic therapy and demographic information.

Ethical consideration: The institution's ethical review committee approved data collection before it began. Prior to participation, the objectives and benefits of the study were explained to each participant, and their verbal informed consent was acquired. Additionally, participants were informed that participation was entirely voluntary and that they might withdraw without providing an explanation.

Fields and laboratory work: From six December 2025 to one February 2026, a cross-sectional study centred on selected patients who were suspected of having typhoid fever according to presumptive diagnosis by a medical practitioner attending hospitals and clinics in Dhamar city. The serological test and bacterial culture of stool were done by standard laboratory methods.

Statistical Analysis: Collected data were analysed using Epi Info 7, in which data were expressed as frequencies and percentages (the result was considered significant with $p \leq 0.05$). Also associated risk factors of *S. typhi* and *S. paratyphi* infection were calculated.

RESULTS

This study was conducted to determine the prevalence of *S. typhi* and *S. paratyphi* infections and associated factors among patients in hospitals in Dhamar City, Yemen. 194 stool samples were collected from patients with confirmed infections using the Widal tube test. Bacteria were isolated from 63 stool samples (32.5%). Two *Salmonella* serotypes (*S. typhi* and *S. paratyphi*) were isolated from stool samples. *S. typhi* was detected in 9 samples (14.3%), while *S. paratyphi* was detected in 54 samples (85.7%) of the tested stool samples. The prevalence of *S. paratyphi* was significantly higher than that of *S. typhi* (p=0.01). Targeted *Salmonella* serotypes were isolated from 45 (23.2%) and 18 (9.3%) female and male participants, respectively, as shown in Table 1. There was no significant difference in prevalence between males and females. Regarding the association between *Salmonella* isolates and participant age, the highest isolate rate was recorded in the 21–30 age group, with 29 isolates (14.9%). The lowest isolate rate was recorded in the 11–20 age group, with 5 isolates (7.94%). However, no significant difference in odds ratios was observed between the different age groups (Table 2).

Table 1: The relationship between typhoid infection (positive culture) and patient gender.

Gender	Total N (%)	Positive N (%)	Negative N (%)	*OR(CI)*	*p* value
Male	54 (27.8)	18 (9.3)	36 (18.6)	1.1(0.5-2.2)	0.87
Female	140 (72.2)	45 (23.2)	95 (49)	0.9 (0.5-1.9)	
Total	194 (100)	63 (32.5)	131 (67.5)		

Table 2: The Relationship between typhoid infections and age of participants.

Age group	Total no. (%)	Positive (%)	Negative (%)	OR	CI	X^2	*p*
11-20 years	13 (6.7)	5 (2.6)	8 (4.1)	1.4	0.36-4.8	0.25	0.61
21-30 years	83 (42.8)	29 (14.9)	54 (27.8)	1.2	0.63-2.3	0.4	0.52
31-40 years	44 (22.7)	16 (8.2)	28 (14.4)	1.2	0.58-2.6	0.39	0.53
41-50 years	29 (14.9)	6 (3.1)	23 (11.9)	0.49	0.17-1.4	2.2	0.41
>50 years	25	7 (3.6)	18 ()	0.8	0.3-2.1	0.26	0.63

Table 3: The association between typhoid infection residence areas of the patients.

Residents	Total no. (%)	Positive (%)	Negative (%)	OR	CI	X^2	*p*
Urban	95 (49)	31(16)	64 (33)	1.1	0.5-1.9	0.0	0.96
Rural	99 (51)	32 (16.5)	67 (34.5)	0.99	0.7-1.8	0.0	0.96

Table 4: Relationship between typhoid infection, educational level, and antibiotics previously used prior to diagnosis.

Education	Total no. (%)	Positive (%)	Negative (%)	OR	CI	X^2	*p*
Illiterate	131 (67.5)	37(19.1)	94 (48.5)	0.56	0.28-1.1	3.3	0.06
Primary	26 (13.4)	13(6.7)	13 (6.7)	2.4	1.0 -5.8	4.2	0.04
Secondary	6 (3.1)	3(1.5)	3 (1.5)	2.13	0.33-13.7	0.87	0.35
University	9 (2.6)	3(1.5)	6 (3.1)	7.1	1.7-34.6	10.5	0.001
Higher education	22 (11.3)	7(3.6)	15 (7.7)	0.97	0.33-2.7	0.0	0.94
Receive antibiotics recently	150 (77.3)	46 (23.7)	104 (53.6)	0.7	0.3-1.5	0.99	0.32

The relationship between *Salmonella* isolation and residential area. *Salmonella* serotypes were isolated from urban and rural areas at rates of 31 (16.0%) and 32 (16.5%), respectively, as shown in Table 3. There was no statistically significant relationship between *Salmonella* isolation and residential area, whether urban or rural. This study categorized the population into five groups based on educational level: illiterate individuals (37 isolates, 19.1%), followed by primary school students (13 isolates, 6.7%), then secondary school students (7 isolates, 3.6%), while the lowest rates were recorded in middle school, high school, and university (3 isolates, 1.5% each). The odds ratio was statistically significant among university students, reaching 7.1 (p=0.001). Regarding the relationship between *Salmonella* isolates and recent antibiotic treatment, the results showed that patients with a recent infection history who received antibiotic treatment did not exhibit a statistically significant rate (46 isolates, 23.7%) of *Salmonella* serotype isolates compared to patients without a recent infection history who received antibiotic treatment (17 isolates, 8.8%). *Salmonella* isolates exhibited varying susceptibility to antibiotics, with 55 isolates (87.3%) resistant to ciprofloxacin and ofloxacin, 53 isolates (84.1%) resistant to chloramphenicol, 32 isolates (50.8%) resistant to azithromycin, 29 isolates (46.0%) resistant to ceftriaxone, and 18 isolates (28.6%) resistant to cefotaxime. More than half of the *Salmonella* isolates, 41 isolates (65.1%), were resistant to cefotaxime (Table 5).

DISCUSSION

Typhoid fever remains one of the most common infectious diseases in tropical and subtropical countries. Therefore, this study was conducted to determine the prevalence of *S. typhi* and *S. paratyphi* using stool cultures from patients suspected of having typhoid fever who visited various healthcare centres in Dhamar, Yemen. The study also aimed to determine the effectiveness of the Widal test compared to stool cultures, given the increasing use of the Widal test for diagnosing typhoid and paratyphoid fever in our city's hospitals and clinics. The current study sample included individuals with a Widal test positivity rate of 77.6% (194/250) among patients suspected of having typhoid fever.

Table 5: Antimicrobial sensitivity of isolated *S. typhi* and *S. para-typhi* isolated from typhoid patients.

Antimicrobial agents	Sensitive N (%)	Resistance N (%)	Moderate N (%)	Total N (%)
Ciprofloxacin	50 (87.3)	8 (12.7)	0 (0.0)	63 (100)
Cefotaxime	18 (28.6)	41(65.1)	4 (6.3)	63 (100)
Azithromycin	32 (50.8)	17 (27)	14 (22.2)	63 (100)
Chloramphenicol	53 (84.1)	9 (14.3)	1 (1.6)	63 (100)
Ceftriaxone	26 (46.0)	24 (38.1)	10 (15.9)	63 (100)
Ofloxacin	55 (87.3)	3 (4.8)	5(7.9)	63 (100)

Based on stool culture, the percentage of positive samples was 32.5% (63/194), as shown in Table 1. Our percentage of positive stool culture results (32.5%) was higher than reported in other studies, where the percentage of positive stool culture results for *S. typhi* and *S. paratyphi* was generally low in patients with serological and clinical confirmation of infection, typically ranging from 2.7% to 11%, despite the high percentage of patients with confirmed infection using the Widal test. Although the Widal test has high sensitivity (often exceeding 70%), it suffers from low specificity and high false-positive rates. This makes stool culture, which has near-100% specificity in confirming the presence of bacteria or infection, a primary, albeit less sensitive, diagnostic tool[10, 11]. The differences in this per cent may be due to cross-reactivity with other infectious agents and antibodies mounted to last infection, which remain for years and consequently contribute to increasing false positives[1, 9]. Because the Widal test can produce false-positive results due to cross-reactivity with other infections (such as malaria and non-typhoidal *Salmonella*), it is not considered highly reliable for diagnosing active infection on its own. The timing of stool culture should also be considered; stool culture is most effective in the second or third week of illness, while blood culture is best performed in the first week[9,11]. In the current study, the most common serotype was *S. paratyphi*

(85.7%), compared to *S. typhi* (14.3%). This contrasts with previous findings suggesting that *S. typhi* was more prevalent than *S. paratyphi*. Typhoid fever cases are estimated at approximately 9.2 million annually worldwide, compared to 3.8 million cases of paratyphoid fever. While *S. typhi* generally poses a greater burden, cases of *S. paratyphi* A are on the rise, particularly in South Asia. Furthermore, both types are major causes of enteric fever in similar communities, with typhoid fever generally being more common, with incidence rates several times higher than paratyphoid fever (e.g., 216/100,000 vs 27/100,000 person-year in selected studies)[13]. However, our findings are consistent with those of Oluwege *et al*.[14], in Nigeria, where *S. paratyphi* accounted for 22.1% and *S. typhi* for 8.1% of patients with fever.

In the current study, specific *Salmonella* serotypes were isolated from 45 males (23.2%) compared to 18 females (9.3%), but there was no significant difference in the probability of infection between males and females (p=0.87). The infection rate in this study differs from that previously reported by Taha[15] in Dhamar, where he found a *Salmonella* infection rate of 54.8% among females, while the rate among males was similar (45.2%), based on blood culture results. Furthermore, Muthoni's study in Kenya indicated that the prevalence of *Salmonella* was higher in females than in males (48.7% and 34.0%), respectively[16]. This difference may be attributed to females having closer access to food sources than males. A sex-related factor may also be responsible for the higher incidence of typhoid fever in females compared to males across all age groups in our study. In addition, women exposed to sources of infection, such as those caring for other family members who may be chronic carriers, may be at higher risk. A mother caring for a child with diarrhoea, which may be caused by *Salmonella*, may also have poor personal hygiene, potentially leading to transmission through food preparation for other family members or the use of contaminated water.

The current study showed that all age groups are susceptible to *Salmonella* infection. These findings are consistent with those of Muthoni[16]. However, the 21-30 age group was the most susceptible (14.9%). This result is similar to that of Olweji *et al*.[14], where the rate was 13.59% in Nigeria. In this study, *Salmonella* serotypes were isolated from urban and rural areas at equal rates of 16.0% and 16.5%, respectively, as shown in Table 5. These results differ from those of a study by Nahab *et al*.[14], in Iraq, where they found that *Salmonella* isolation from rural populations was more common than from urban populations, at 66% and 34%, respectively. These results may be attributed to the lifestyle of rural populations, who drink unsterilised water, which facilitates the direct transmission of bacterial pathogens through water[17]. This study also contradicts a study by Hohmann in Kenya[18], which found that urban slums have a higher incidence of typhoid fever compared to rural areas. This can be linked to overcrowding and limited access to safe drinking water and adequate sanitation facilities, as demonstrated by Muthoni's study[16].

In this study, the population was classified into five groups based on educational level: illiterate individuals (37 cases, 19.1%), followed by primary school students (13 cases, 6.7%), then middle school students (7 cases, 3.6%), while the lowest rates were recorded in middle school, high school, and university (3 cases, 1.5% each). The odds ratio was statistically significant among university students, reaching 7.1 (p=0.001), indicating that all educational levels are susceptible to typhoid fever. This suggests a lack of public health awareness about the modes of transmission of the disease and the inability of people to follow healthy lifestyles, such as washing hands with soap and proper wastewater disposal, as described by Muthoni[16].

In the current study, *Salmonella* isolates exhibited varying antibiotic susceptibility, with 55 isolates (87.3%) resistant to ciprofloxacin and ofloxacin, 53 isolates (84.1%) resistant to chloramphenicol, 32 isolates (50.8%) resistant to azithromycin, 29 isolates (46.0%) resistant to ceftriaxone, and 18 isolates (28.6%) resistant to cefotaxime (Table 5). These resistance rates are similar to those previously reported in Yemen for Gram-negative enteric bacteria, including *Salmonella* species. The mean resistance rate for a wide range of tested antibiotics ranged from 22.5% to 98.1%, with cefazolin exhibiting the highest resistance rate (98.1%), followed by amoxicillin (87.2%) and then cefixime (83%)[19-21]. As for Gram-negative bacteria, resistance rates to narrow-spectrum antibiotics ranged from 2.3% for colistin sulphate to 84.8% for aztreonam[22-25]. Other bacteria exhibit very high multidrug resistance in aerobic colonisation of the oral cavity[26], in methicillin-resistant *S. aureus* (MRSA) with methicillin-sensitive *S. aureus* (MRSA) among *S. aureus*[27], in clinical samples from military patients at Medical Complex 48 in Sana'a[28], in pathogenic bacteria in wounds of patients in Sana'a hospitals[29], in *Vibrio cholerae*[30], and in bacteria in facial and oral abscesses of dental origin[31].

Limitations of the study

The limitations of determining the prevalence of *S. typhi* and *S. paratyphi* in stool samples from patients with Widal test positive often involve issues with diagnostic accuracy, cross-reactivity, and study design, according to the context of epidemiological studies carried out in Dhamar Governorate and comparable studies in Yemen. Stool culture is helpful, although it is less sensitive than bone marrow or blood culture, particularly in patients who have already begun antibiotic treatment or are in the early stages of fever. Additionally, a lot of Yemeni patients use antibiotics or self-medicate before giving faeces samples, which seriously impedes bacterial culture.

CONCLUSIONS

The Widal test is a primary screening tool that is sensitive but not reliable for diagnosing typhoid fever due to common false positives. In contrast, stool culture is a specialised confirmatory tool but less sensitive. The study highlighted a higher prevalence of *S. paratyphi* compared to *S. typhi*, raising concerns about the role of chicken products. Isolation of

Salmonella was more frequent in females. All age groups are susceptible to *Salmonella* infections, particularly those aged 21-30. Ciprofloxacin and Ofloxacin showed greater sensitivity against *Salmonella* isolates, while Cefotaxime had a high resistance rate. Antimicrobial therapy should follow sensitivity testing.

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AUTHOR'S CONTRIBUTION

Al-Arashi IA: writing original draft, methodology, investigation. **Khalil EHA:** conceptualization, data organization. **Al-Shamahy HA:** formal analysis, data curation, conceptualisation. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The empirical data used to support the study's conclusions are available upon request from the corresponding author.

CONFLICT OF INTEREST

Regarding this project, there are no conflicts of interest.

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