Assessment of blood culture and tube agglutination serology test for the diagnosis of typhoid fever amongst malaria-negative patients: a one-year hospital-based study

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Abstract

Salmonella serotypes, including *Salmonella* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, and *S.* Paratyphi C, are responsible for the systemic, protracted febrile sickness known as typhoid fever. Various antibody-based tests are being used for diagnosing typhoid fever. This study was carried out to assess the performance of the widal test and blood culture for the diagnosis of typhoid fever among malaria-negative patients in a tertiary care hospital in east Delhi, India. The study was conducted from July 2021 to June 2022 in the Department of Microbiology of a tertiary care hospital in Delhi. Patients, including the adult and pediatric population, were evaluated for typhoid fever and participated in an observational, prospective study on febrile patients that was malaria-negative. Venous blood samples were obtained under strict aseptic conditions and further processed for widal serology and blood culture tests for typhoid fever. In our study, the prevalence of blood culture-positive *Salmonella* species was 0.3% (30/10,000 = 0.3%) Among antimicrobial susceptibility patterns, *S. Typhi* revealed the highest resistance rates for Ciprofloxacin (43.33%), Azithromycin (36.66%), and third-generation cephalosporins. Out of 30 blood culture-positive *Salmonella* Typhi of typhoid fever patients, 5 (17%) samples were negative for the Widal test. Among 30 samples, all were blood culture positive, but only 25 samples show Widal titer above the baseline *i.e.* >1:64. Although blood culture is the gold standard for the diagnosis of typhoid fever, the Widal test does play a role in the diagnosis and management of typhoid fever, especially in suspected cases when blood culture is negative, especially in government tertiary care hospitals.

Introduction

Salmonella serotypes, including *Salmonella Typhi, S. Paratyphi* A, *S. Paratyphi* B, *and S. Paratyphi* C, are responsible for the systemic, protracted febrile sickness known as typhoid fever. Typhoid fever only affects humans as reservoir hosts, and in endemic places, the disease is spread through contact with feces-contaminated water and food, especially by carriers who handle food. Typhoid fever is thought to cause over 21 million illnesses and more than 600,000 deaths annually, according to the World Health Organisation (WHO). In other words, in places with high population expansion, rising urbanization, and insufficient access to good water, infrastructure, and health systems, these instances are more likely to be seen in India, South and Central America, and Africa.[1,2]

Typhoid fever must be accurately diagnosed at an early stage to determine the etiological agent as well as to locate possible carriers who may be to blame for acute enteric fever epidemics.[3] Clinical signs and symptoms, serological markers, bacterial culture, antigen detection, and DNA amplification are all possible methods for diagnosing typhoid fever.[4,5] The most accurate diagnostic approaches involve the culture of blood, bone marrow, and stool.[6-8] Blood culture is regarded as the gold standard for diagnosis and has a diagnostic yield of 70-75% during the first week of illness and declining 20-30% later in the course of the disease.[9] The isolation from blood culture is more difficult due to the easy availability and widespread use of antibiotics in the community. Alternate methods such as bone marrow cultures may be required, which are invasive and difficult to carry out.[10] Thus one has to rely on serological diagnosis, which is the mainstay of diagnosis of typhoid fever in most laboratories.[11]

Various antibody-based tests are being used for diagnosing typhoid fever. The Widal test is the most frequently used test for diagnosing enteric fever since it is generally less expensive, simple to apply, and requires less training and equipment.[12,13] The efficacy of the widal test to diagnose enteric fever has been disputed for as long as it has been available, even though it has been in use for more than a century.[14] It traditionally relies on the proof of an increasing antibody titer in paired samples taken 10 to 14 days apart. This study was carried out to assess the performance of the Widal test and blood culture for the diagnosis of typhoid fever amongst malaria-negative patients in a tertiary care hospital in east Delhi, India.

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Ethical approval declaration: this study has been approved by the institutional ethical committee (IEC No. GTBHEC/APVL/2023/256-79).

Informed consent: we have not performed any extra tests apart from routine diagnostic methods (Widal test and blood culture) for the diagnosis of typhoid fever from the sample. We have processed those samples only which came into our microbiology laboratory for routine testing of typhoid fever. However, verbal consent from the respective patient was taken for this study.

Patient consent for publication: the manuscript does not contain any person's data in any form.

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Materials and Methods

The study was conducted from July 2021 to June 2022 in the Department of Microbiology under the Bacteriology and Serology laboratory of a tertiary care Hospital, in Delhi. Patients including the adult and pediatric population were evaluated for typhoid fever and participated in an observational, prospective study on febrile patients which was malaria negative. The clinical symptom of typhoid fever, which is a fever that occurred two or more days before admission and was also accompanied by other clinical symptoms of typhoid fever, was checked on patients through a physician. The study comprised febrile patients whose presumptive clinical diagnosis was enteric fever and not started on antibiotics enrolled and their blood culture and serology sample was sent for a Widal test in the laboratory.

Blood sample collection and inoculation

Venous blood samples of 8-10 mL from adults and 1-3 mL from children were obtained under strict aseptic conditions. Each 8 to 10 mL blood sample was placed into a blood culture bottle with 50 mL of brain heart infusion broth for adults, and 30 mL of blood culture broth for pediatric patients. The blood culture sample will be delivered right away to the bacteriology laboratory, where it will spend the night being incubated at 37°C in an ambient atmosphere. Following manual subculture onto 5% sheep blood agar and Mac-Conkey agar incubation for 24 hours, 48 hours, and a seventh day, the samples were processed. Following the established process in our laboratory, the obtained growth was recognized using colony morphology, gram stain of the isolated colonies, common microbiological tests, and biochemical assays.[15] All isolated blood culture *Salmonella* species isolates from suspected cases of enteric fever were confirmed from *Salmonella* antisera (*Salmonella* Sero-Quick, SSI Diagnostic) for grouping of serotype.

Widal test

Widal test also known as the tube agglutination serology test, was done by tube agglutination method using colored TYPHOCHECK reagent (Tulip Diagnostic pvt. Ltd.). Anti-salmonella antibodies in the patient's blood react with the colored, smooth, TYPHOCHECK antigen solutions to produce agglutination when they are combined with the patient's serum. The maximum dilution of serum that causes visible agglutination is determined by the antibody titer of the patient's serum using TYPHOCHECK antigen suspensions.

Antimicrobial susceptibility testing of Salmonella species isolates

By using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates, the antibiotic susceptibility pattern of the salmonella species *Salmonella* Typhi isolates, isolated from blood culture was assessed. The antibiotics disc including Ampicillin (10 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg), Chloramphenicol (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg) and Trimethoprim-sulfamethoxazole (1.25/23.75 µg) was placed. The results were recorded according to the latest recommended Clinical Laboratory Standard Institute (CLSI) guidelines.[21]

Results

In our study, the prevalence of *Salmonella* species was 0.3% (30/10,000=0.3%) in clinically suspected cases of typhoid fever. Among 30 blood culture-positive febrile patients, 21 (70%) and 9 (30%) were females and males respectively. The female: male ratio was (2:1). The age range of patients was 2–28 years with a mean of 9.8 and a median of 7 years of age. Typhoid fever was more prevalent in the age group of 1-10 years (63.33%) and females (70%). Moreover, in the present study, typhoid fever was more prevalent in pediatric Intensive care unit (PICU)patients (33.33%) than in other departments (Table 1). Amongst antimicrobial susceptibility patterns, *S.* Typhi revealed the highest resistance rate for Ciprofloxacin 13(43.33%) and Azithromycin 11(36.66%) followed by Tetracycline 4(13.33%), Ceftriaxone 2(6.66%), Cefotaxime 2 (6.66%), Cotrimoxazole 2 (6.66%), and Chloramphenicol 1 (3%). On the other hand, all *S.* Typhi isolates were susceptible to Meropenem and Piperacillin-Tazobactam. (Figure 1) Furthermore, 6 (20%) isolates of *S.* Typhi were resistant to more than two different groups of class

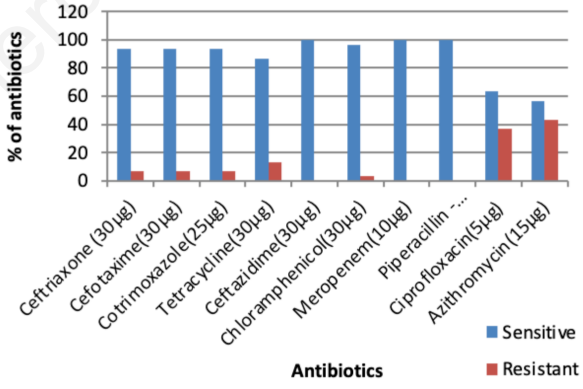

Figure 1. Antibiotic profile of *S.* Typhi blood culture isolates among clinically suspected cases of typhoid fever in the study group (n=30).

Table 1. Socio-demographic characteristics and distribution of blood culture isolates of *S.* Typhi in febrile patients.

Variables	Category	Number of positive blood culture isolates for *S.* Typhi. n=30 (%)
Sex	Male	9 (30)
	Female	21 (70)
Age (in years)	1-10	19 (63.33)
	>10	11 (36.66)
Departments	PICU	10 (33.33)
	Medicine	9 (30)
	Pediatrics	4 (13.33)
	Surgery	3 (10)
	MCH	2 (6.66)
	MICU	2 (6.66)

PICU, pediatrics intensive care unit; MICU, multidisciplinary adult intensive care unit; MCH, maternity and child health.

Table 2. Multidrug resistance patterns among *S.* Typhi isolated from clinically suspected cases of typhoid fever.

Antibiotics resistant	Resistance isolate of *Salmonella Typhi* N=6 (%)
CTX+CTR+CIP	2
COT+TET+CIP	2
TET+CIP+AZT	2

CTX, cefotaxime, CTR, ceftriaxone, CIP, ciprofloxacin, COT, cotrimoxazole, TET, tetracycline, AZT, azithromycin.

Table 3. Correlation between Widal test and blood culture in typhoid fever patients (n=30).

Tests	Widal test (+)	Widal test (-)	
Blood culture (+)	25	5	p<0.098
Blood culture (-)	0	30	Chi-square test not significant

p<0.05 is statistically significant.

drugs or Multidrug Resistance (MDR). (Table 2)

In the present study, tube agglutination serology test /widal test was carried out for all the clinically proven typhoid cases. The cut-off value of the Widal test was considered as 1:64 for both TO and TH. Out of 30 blood culture-positive cases of typhoid fever, 33.33% cases have shown an antibody titer of ≥ 128, 23.33% cases have shown an antibody titer of ≥ 64, 13.33% cases have showed an antibody titer of ≥ 256, 13.33% cases have shown an antibody titer of ≥ 512. Out of 30 positive samples for blood culture; only 5 (17%) samples were negative for the Widal test. Amongst 5 negative Widal tests the blood culture was positive for *Salmonella* Typhi. This was not statistically significant. (p<0.098, chi-square test)

Discussion

In the present study, the prevalence of *S.* Typhi among febrile illness patients at tertiary care government hospitals was 0.3%. This finding was much lower than the study conducted in Shashemene Ethiopia 5%,[15] Central Ethiopia (4.1%),[16] in Indonesia (15.5%)[17] and Lalitpur 4.1%.[18] Similar findings were also reported in India 2.5%[19] and Nepal 1.2%.[20] This difference might be due to the geographic setting of the study district, the disparity in the study population, time of the studies. Moreover, the mode of the laboratory investigation technique disparity also affects the result. The finding of this study shows most of the isolates of *S.* Typhi were sensitive to ceftriaxone. A similar finding was reported in a study done in Bangladesh and Lalitpur, Nepal which shows 100% sensitivity to ceftriaxone.[21,22] In this study *S.* Typhi susceptible to chloramphenicol was observed in 29 (96.66%) cases. This finding was similar to a study done in India which shows 87.4% of *S.* Typhi was sensitive to chloramphenicol.[23]

The Widal test is still the widely used serological test for typhoid fever. Here the antibody against antigens O and H are detected. In this study, a Widal test was carried out for all the clinically proven typhoid cases. The cut-off value of the Widal test was considered as 1:64 for both TO and TH antigens. Present study about 33.33% of cases with a fever of more than a week showed an antibody titer of ≥128. A study done by Shukla *et al.*,[24] also found that 44.2% had TO titter of ≥160 in a single sample collected from patients suspected to have typhoid in an endemic area of South India. Second specimens are often not sent to the laboratory to verify the rising titter. It is possible that the Widal test would have performed better if paired sera were tested to demonstrate the rising titers. Patients rarely return for follow-up once treated so obtaining paired sera in a routine clinical setting is unlikely. Clinicians cannot wait for results from two samples and hence widely rely on "positive" Widal tests done on a single serum sample.

Typhoid fever diagnostic evaluations conducted on hospitalized patients provide little insight into the application of diagnostic tests in the community health care setting. However, it is the primary health care level where sensitive, specific, rapid, cheap, and user-friendly typhoid diagnostic kits are most required. It is in this context that high-grade fever is important: in areas of malaria and typhoid endemicity where malaria rapid diagnostic test (RDT) yields a negative result, there may be clinical signs and symptoms such as the severity of fever that can help determine the value of conducting a typhoid diagnostic test without negatively impacting patient outcome.

Although blood culture is the gold standard for diagnosis of typhoid fever, Widal tests do play a role in the diagnosis and management of typhoid fever, especially in suspected cases when blood culture is negative. It is likely that the Widal test will remain in use in tertiary government set-ups and many other low-income settings for the foreseeable future, despite its known limitations in such settings.

References

1. Willke A, Ergonul O, Bayar B: Widal test in diagnosis of typhoid fever in Turkey. Clin Diagn Lab Immunol 2002;9:938-41.
2. Crump JA, Luby SP, Mintz ED: The global burden of typhoid fever. Bull World Health Organ 2004;82:346-53.
3. Gopalakrishnan V, Sekhar WY, Soo EH, et al. Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to salmonella typhi. Singapore Med J 2002;43:354-8.
4. Nsutebu EF, Martins P, Adiogo D. Prevalence of typhoid fever in febrile patients with symptoms clinically compatible with typhoid fever in Cameroon. Trop Med Int Health 2003;8:575-8.
5. Onyekewere CA. Typhoid fever: misdiagnosis or over diagnosis. Niger Med Pract 2007;51:76-9.
6. Wain J, Hosoglu S. The laboratory diagnosis of enteric fever. J Infect Dev Ctries 2008;2:421-5.
7. Parry CM, Tuyet HNT, Diep TS, et al. Value of a single-tube Widal test in diagnosis of typhoid fever in Vietnam. J Clin Microbiol 1999;37:2882-6.
8. Wain J, Diep TS, Be Bay PV, et al. Specimens and culture media for the laboratory diagnosis of typhoid fever. J Infect Dev Ctries 2008;2:469-74.

9. Krishna S, Desai S, Anjana VK, Paranthaaman RG. Typhidot (IgM) as a reliable and rapid diagnostic test for typhoid fever. Ann Tropical Med Public Health 2011;4:42.
10. Olsen SJ, Pruckler J, Bibb W, et al. Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol 2004;42: 1885-9.
11. Begum Z, Hossain MA, Shamsuzzaman AK, et al. Evaluation of Typhidot (IgM) for early diagnosis of typhoid fever. Bangladesh J Med Microbiol 2009;3: 10-3.
12. Ley B, Mtove G, Thriemer K, et al. Evaluation of the Widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hospital in Tanzania and a comparison with previous studies. BMC Infect Dis 2010;10:180.
13. Beyene G, Asrat D, Mengistu Y, et al. Typhoid fever in Ethiopia. J Infect Dev Ctries 2008;2:448-53.
14. Olopoenia LA, King AL. Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med J 2000;76:80-4.
15. Laxminarayan R, Matsoso P, Pant S, et al. Access to effective antimicrobials: a worldwide challenge. Lancet 2016;387:168-75.
16. Murray CK, Gasser RA, Jr., Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clinical Microbiol Rev 2008;21:97-110.
17. Wongsrichanalai C, Barcus MJ, Muth S, et al. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am Soc Trop Med Hygiene 2007;77:119-27.
18. Bell D, Peeling RW. Evaluation of rapid diagnostic tests: malaria. Nat Rev Microbiol 2006;4:S34-8.
19. WHO. Report of a Joint WHO/USAID Informal Consultation. New perspectives: malaria diagnosis. Geneva, Switzerland: World Health Organization; 1999.
20. Banoo S, Bell D, Bossuyt P, et al. Evaluation of diagnostic tests for infectious diseases: general principles. Nat Rev Microbiol 2006;4:S20-2.
21. Kalpana P, Sharma VK, Maharjan R. Prevalence and antibiotic sensitivity test of salmonella serovars from enteric fever suspected patients visiting Alka Hospital, Lalitpur. Am J Microbiol 2015;6:40-43.
22. Globally TD-RI. Final report and recommendations. Review on Antimicrobial Resistance Report. 2016.
23. Tewari R, Jamal S, Dudeja M. Antimicrobial resistance pattern of Salmonella enterica servars in southern Delhi. Int J Community Med Public Health 2015;2:254-8.
24. Shukla S, Patel B, Chitnis DS. 100 years of Widal test & its reappraisal in an endemic area. Indian J Med Res 1997;105:53-7.