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Blood culture versus molecular and serological methods in the diagnosis of enteric fever: A hospital-based prospective follow-up comparative study.

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Abstract

Background:

Typhoid fever is a life-threatening systemic infection that is usually presented with non- specific symptoms and signs**.** Typhoid and paratyphoid fever are both caused by S. *Typhi* and *S. Paratyphi*, which are gram-negative, rod-shaped, flagellated, motile, and non-spore-forming bacteria in the family *Enterobacteriaceae.* Its diagnosis is usually confirmed by blood culture and the Widal test. The delayed results of microbiologic examination and the unreliable results of the Widal test suggest a rapid and reliable method for the diagnosis of typhoid fever. The objective of the study was to compare different diagnostic modalities used in enteric fever.

Material and Methods:

This prospective follow-up study was conducted in the Departments of Microbiology, LHMC, and Associated Hospitals, and the Department of Medicine in LHMC from April 2024 to August 2025**.** A total of 132 clinically suspected typhoid fever cases of adult age groups from both sexes attending were studied. The blood collected aseptically was used for blood culture, and a serum sample was used for the Widal test, and plasma for the PCR test.

Results:

Out of 132 blood samples studied, 5 cases were positive by blood culture. Widal test showed sensitivity and specificity of 20% and 82.7%, and the PCR test showed sensitivity and specificity of 80% and 90%, respectively, on blood culture-proven cases. Maximum positive cases were seen in July and November.

Conclusion:

Using blood culture as the gold standard, PCR showed a higher sensitivity compared to the Widal test. The Widal test is easy to get and cheap, but because it's not very accurate in places where the disease is endemic, integrating molecular diagnostics with the usual culture-based methods could help in early detection of enteric fever.

Recommendation:

PCR-based molecular diagnostics should be incorporated as an adjunct to conventional methods for the early and accurate diagnosis of Enteric fever.

Keywords: *Enteric fever, typhoid, Polymerase chain reaction, Widal test, Blood culture, Diagnosis*
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Introduction

Typhoid and paratyphoid fever are both widespread, systemic infections by S. *Typhi* and *S. Paratyphi A1*. *S. Typhi* and *S. Paratyphi* are gram-negative, rod-shaped, flagellated, motile, and non-spore-forming bacteria in the family *Enterobacteriaceae*. *S.typhi* and *S.paratyphi* are transmitted through ingestion of fecally contaminated food or water. Variation in factors that influence these modes of transmission, such as host behavior or environmental factors, can result in fluctuating or complex seasonal dynamics 1, 2. It is basically a life-threatening systemic infection more frequently seen in developing countries due

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to overcrowding and poor sanitation, causing a major public health problem 3. In individuals with typhoid fever, the microorganisms initially travel through the digestive system and, in the long run, enter the circulatory system. The subsequent sickness is often non-specific and clinically unclear from other febrile diseases.3

Considering globally, an estimated 11-20 million individuals get typhoid and 5 million cases of paratyphoid fever happen every year, and approximately 1, 28,000 to 1, 61,000 individuals die from it Signs and symptoms of typhoid fever develop gradually, with the incubation period generally being 7 to 14 days.4The sickness starts gradually, with a gradual increase in fatigue and fever that rises daily from low grade to as high as 38°C to 40°C through the third to fourth day after the disease. Fever is generally lowest, it's most reduced in the first part of the day and at its most elevated in the early evening or at night. In severe cases, it can lead to serious complications and death.

Lab diagnosis of typhoid fever requires isolation and identification of S. Typhi. The finding of typhoid fever, as of now, depends upon the isolation of the microorganism by blood culture. Typhoid fever is generally diagnosed by using blood, bone marrow, stool, and urine according to the illness. Blood cultures are considered the gold standard for diagnosis of typhoid fever and are best done within seven days of the beginning of symptoms (5, 4. The Widal test has been traditionally employed as a rapid serologic screening test, but it remains a serological test with a moderate sensitivity and specificity.

Specific IgM antibodies serve as a marker for recent contamination and can be identified 2 to 3 days after the beginning of symptoms5. The Typhidot test is a dot ELISA kit for the detection of IgM and IgG antibodies against the outer membrane protein (OMP) of Salmonella *typhi*. PCR-based amplification of *S. typhi* genomic targets from typhoid fever patients is additionally done.

The present study was undertaken to compare the sensitivity and specificity of the Widal test, PCR, and the blood culture in the diagnosis of typhoid fever.

Materials and methods

Study Design

Prospective follow-up study

Study Period

April 2024- August 2025

Study Area

Departments of Microbiology and Department of Medicine in Lady Hardinge Medical College (LHMC) and Smt. Sucheta Kriplani Hospital.

Study Population

Patients admitted with acute febrile illness in the ward of the medicine department and the emergency department in Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital.

Inclusion Criteria

- Adult patients of more than 18 years of age

Patients with a fever of 2 weeks or shorter in duration with a body temperature of more than 38 °C who are admitted to the hospital.

- The cause of illness is undiagnosed after medical history and physical examination.

Exclusion Criteria

1. Fever due to any other causes, like connective tissue disease, malignancy, or an immunocompromised condition.

Sample Size:

Considering the prevalences 60% of enteric fever, confidence interval of 95%, and precision of 9%, the sample size came out to be 113, using the formula:

$n = Z^2P(1-P)/d^2$,

Where n is the sample size, Z is the statistic corresponding to the level of confidence expected prevalence. Thus, the proposed sample size for the study is 113 as per the calculation.

For the present study, we will take a convenience sample of **132** for the Enteric fever patients.

Blood culture bottles were incubated in the BACTEC automated blood culture system at 37°C and monitored for up to 5 days. Bottles flagged positive were subjected to Gram staining and subculture. Subcultures were made on MacConkey's agar and blood agar. The growth of *S. typhi* was identified by the standard biochemical tests, and it was confirmed by MALDI TOF. The Widal test was done by using the standard procedure, which was confirmed by the tube agglutination method, and it was considered positive when a titer of equal to or more than 1:160 was observed. DNA was extracted from plasma using the commercial DNA extraction kit according to the manufacturer's

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instructions. The presence of *Salmonella* spp. was confirmed by conventional PCR targeting the tetrathionate reductase (*ttr*) gene, a conserved gene located within the Salmonella pathogenicity island 2 (SPI-2). The data was tabulated in Microsoft Excel. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Bias

Efforts were made to minimize potential sources of bias throughout the study. Selection bias was reduced by including consecutive adult patients presenting with clinically suspected enteric fever who fulfilled the predefined inclusion criteria during the study period. Information bias was minimized by using standardized laboratory procedures for all diagnostic tests, including blood culture, Widal test, and PCR. Blood culture was considered the reference standard for comparison of diagnostic modalities. To reduce observer bias, the interpretation of laboratory results was carried out independently according to predefined criteria.

Data Collection

Demographic, clinical, and laboratory data were collected using a structured case record form. Variables of interest included age, sex, duration of fever, clinical symptoms, blood culture results, Widal test results, and PCR findings.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using appropriate statistical methods. Categorical variables were expressed as frequencies and percentages. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios, and diagnostic accuracy were calculated for the Widal test and PCR using blood culture as the reference standard.

Ethical Consideration

The study was approved by the Institutional Ethics Committee of Lady Hardinge Medical College and Hospitals, New Delhi, India. Ethical approval was obtained before commencement of the study.

- **Approval date:** [22/04/2024]
- **Ethical clearance number:** [LHMC/IEC/PG Thesis /64]

Written informed consent was obtained from all participants before enrolment in the study. Participants were informed regarding the purpose of the study, procedures involved, confidentiality of data, and their right to withdraw from the study at any stage without affecting their treatment. Confidentiality and anonymity of patient information were strictly maintained throughout the study.

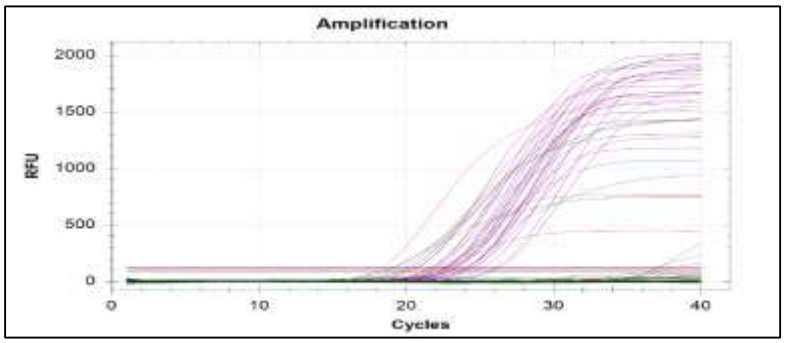

Figure 1: PCR complete run

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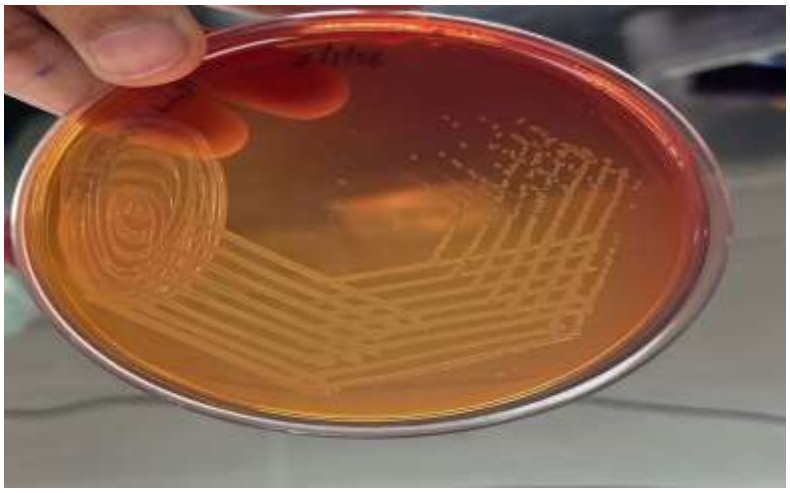

Figure 2 Salmonella positive subculture on MacConkey agar.

Results

This study was conducted in the Department of Microbiology, Lady Hardinge Medical College, and Smt. Sucheta Kriplani Hospital, New Delhi, between April 2024 and August 2025. A total of 148 patients with acute febrile illness were initially screened for eligibility during the study period. Among them, 132 patients fulfilling the inclusion criteria and providing informed consent were enrolled in the study. Sixteen patients were excluded due to confirmed alternative diagnoses, incomplete clinical details, or inadequate sample collection. All enrolled participants underwent blood culture, Widal test, and PCR analysis, and complete laboratory data were available for all included cases. Therefore, all 132 participants were included in the final analysis.

Patients screened (n=148)
↓
Excluded (n=16)
- Alternative diagnosis (n=9)
- Incomplete data (n=5)
- Inadequate sample (n=2)
↓
Included in study (n=132)
↓
Blood culture performed (n=132)
Widal test performed (n=132)
PCR performed (n=132)
↓
Final analysis (n=132)

Sociodemographic profile of study subjects

Table 1: Age and sex distribution of study subjects (n=132)

Age Group (in years)	Sex distribution		Total
	Male	Female	
18-25	16(28.1%)	41(71.9%)	57(43%)
26-35	11(24.4%)	34(75.6%)	45(34.1%)
36-45	8(57.1%)	6(42.9%)	14(10.6%)
46-55	7(87.5%)	1(12.5%)	8(6.1%)
56 and above	2(25%)	6(75%)	8(6.1%)

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Total	44(33.3%)	88(66.7%)	132

The mean age of enrolled patients was 31.7 (95% CI: 29.47-33.84) years, the median age was 26 years, while the mode was 24 years. The age range of admitted patients was 18- 83 years with a standard deviation of 12.8 years. The maximum number of patients was in the age group 18-25 years (57; 43.1%) with a male to female ratio of 1:2.2.

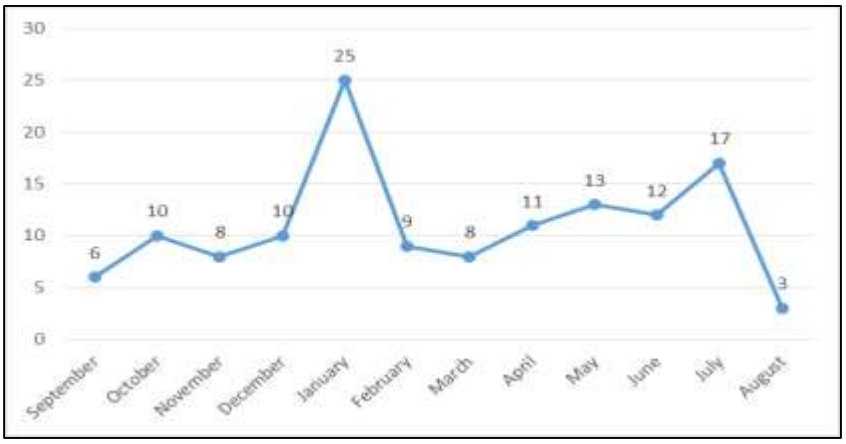

Figure 3. Monthwise distribution of enrolled patients.

Maximum enrolment of patients was in January [25(18.9%)], followed by July [17(12.8%)], with a mean of 11.0 (95% CI 7.81-14.19) and minimum in September [6(4%)] and August [3(2%)]. The range for seasonal variation is 3 to 25, with a standard deviation of 5.64 (p value for equal distribution across months <0.05 (0.00082) shows significant difference).

Out of 132 suspected cases, 5(3.7%) were positive by blood culture, 23 (17.4%) by Widal, and 16 (12.1%) by PCR (Table-2). Out of 5 blood culture positive cases, which have been taken as a gold standard, were compared with the Widal test, where only 1 showed positive results, and 4 were negative (Table-3). The remaining 22(of 23 cases), which were blood culture negative, showed positive results with the Widal test. Widal test has a sensitivity of 20%, specificity of 82.7%, and positive predictive value of 4.3% and negative predictive value of 96.3% in comparison with blood culture results (Table-5). Out of 5 blood culture positive cases, which have been taken as a gold standard, were compared with the PCR, where 4 showed positive results, and only one case was negative by PCR (Table-3). The remaining 12 (of 16), which were blood culture negative, showed positive results with PCR. PCR has a sensitivity of 80 %, specificity of 90.6%, and positive predictive value of 25% and negative predictive value of 99.1% in comparison with blood culture results (Table-5).

Table 2: Results of Blood culture, Widal, and PCR

Results	**Blood culture**		**Widal**		**PCR**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Positive	5	3.7	23	17.4	16	12.1
Negative	127	96.3	109	82.6	116	87.9
Total	132	100	132	100	132	100

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Table 3: Comparison of the Widal test with the blood culture

		Blood culture		Total
		Positive	Negative	
Widal	Positive	1	22	23
	Negative	4	105	109
Total		5	127	132

Table 4: Comparison of PCR with Blood culture

		Blood culture		Total
		Positive	Negative	
PCR	Positive	4	12	16
	Negative	1	115	116
Total		5	127	132

Table 5: Validity of Widal and PCR as a diagnostic tool in comparison with blood culture

Parameter	Widal	PCR
Sensitivity	20%	80%
Specificity	82.7%	90.6%
Predictive value of a positive test	4.35%	25%
Predictive value of a negative test	96.3%	99.1%
Likelihood ratio for positive test	1.15	8.5
Likelihood ratio for a negative test	0.97	0.22
Accuracy	80.3%	90.1%

Discussion

Enteric fever caused by *Salmonella enterica serovar Typhi* is still a major public health problem in countries where it is endemic, like India. The overlapping clinical manifestation with other causes of acute febrile illness can delay getting a clear diagnosis. Emergence of multidrug-resistant strains of *Salmonella enterica serovar typhi* has only added to the burden of the disease. Any delay in diagnosis and inappropriate therapy increases the risk of outcome 6. In this study, the blood culture positivity rate among patients who were clinically suspected was 3.7%. Culture positivity in other studies has quoted sensitivity ranging from 8.9% - 43%7,8,9. The Widal and PCR tests showed a positivity rate of 17.4% and 12.1 %, respectively. This discrepancy shows the diagnostic gap between what doctors suspect and what microbiological tests confirm in areas where the disease is endemic (10).

The relatively low culture positivity in the study group might be because some patients had taken antibiotics earlier, came in late, or had low levels of bacterial load in their blood. These factors have been commonly mentioned in areas where the disease is widespread11. Although Blood culture is considered the reference standard for diagnosis, under routine field conditions, its sensitivity usually declines substantially compared with lab-controlled settings (10). Recent data from the Indian Council of Medical Research antimicrobial resistance network shows a decline in isolation rates, probably because many people take antibiotics before going to the hospital (12).

The Widal test in this study had a low sensitivity of just 20% and a very low positive predictive value of 4.35%, while it showed a moderate specificity of 82.7%. The low sensitivity shows that serology missed a lot of cases confirmed by culture, and the low PPV means there were many false positives. These results show the concerns regarding background antibody titer, cross-reactivity, and inability to differentiate between a current infection and past infection13. A study done by Maha et al. found that the sensitivity and specificity of the Widal test were 81% and 71%, respectively, with 34% PPV and 96% NPV14. A study

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done by Rahman et al. also reported the sensitivity and specificity of the Widal test as 81% and 71% respectively15. Recent studies in India show that Widal test results are still quite unreliable, especially in areas where typhoid is common. Sensitivities in these populations range from 25% to 50%, according to Kumar et al16. This variability makes it less reliable when used as a diagnostic modality. The interpretation of the Widal test remains problematic to this day, with different studies reporting different cut-offs, and for this reason, the test has lost some popularity in recent years as technical skills are required for its performance, and interpretation, different sensitivity and specificity rates are obtained even in the same region17.

The Widal test has been used for over a century in developing countries, but its diagnostic utility has some limitations due to low sensitivity, specificity, and positive predictive value18. Decreased sensitivity is due to the long latent period after which the test may become positive. The reason for decreased specificity is due to prior infection, vaccination with the TAB vaccine, or cross-reaction with other gram-negative infections.

On the other hand, PCR did a lot better in this study, with a sensitivity at 80% and a specificity at 90.6%, and it has a really good negative predictive value of 99.1%. The likelihood ratio for a positive test (8.5) strengthens its diagnostic accuracy. Molecular tests that focus on genes like ttr or fliC help detect infections better, even when someone has had some treatment already. These results suggest that PCR, as a nucleic acid-based method, can reliably detect low levels of bacterial DNA even in patients who have received antibiotics before sample collection. This helps overcome one of the principal limitations of blood culture methods19. Recent data at Indian tertiary care centres showed similar findings, with PCR doing better than Widal, especially in patients who had already taken antibiotics, according to Sharma et al20, 21.

In South India, Reddy et al. evaluated a real-time PCR protocol targeting the *fliC* gene and reported a sensitivity of 79.5% and specificity of 89.7% versus blood culture, showing the trend seen in urban Northern Indian cohorts22. That study further emphasized that PCR remained useful even in low-bacteraemia samples, supporting its role as a supplementary diagnostic tool in peripheral laboratory settings where culture facilities are limited or want an early test report.

However, despite the demonstrated advantages, PCR implementation still faces some barriers. High costs, requirements for specialized equipment and trained personnel, and concerns about standardization across laboratories have limited widespread adoption. The reproducible high accuracy reported across multiple Indian studies highlights the value of integrating PCR into routine diagnostic pathways, especially in hospitals where enteric fever burden is high.

This study found that the most affected group was among young adults, which lines up with what we see in urban Indian areas, where enteric fever mostly affects people in the working-age group. The spikes seen in January and July might be linked to changes in the environment and sanitation that affect how the disease spreads. The National Centre for Disease Control has also kept track and noticed seasonal increases during the monsoon and early winter months (23). The overall diagnostic accuracy was highest for PCR, coming in at 90.1% compared to Widal's 80.3%, reaffirming that molecular methods are a reliable diagnostic method.

Generalizability

The findings of this study may be generalized to tertiary care hospitals and other healthcare settings in enteric fever endemic regions with similar demographic and epidemiological characteristics. The study highlights the limitations of conventional serological methods and supports the utility of molecular diagnostics for early diagnosis in resource-limited settings. However, generalizability may be limited in non-endemic regions and primary healthcare settings where disease prevalence and laboratory infrastructure differ.

Conclusion

This study shows that PCR has much better sensitivity and specificity than the Widal test when compared to blood culture. The Widal test is easy to get and cheap, but because it's not very accurate in places where the disease is endemic, doctors can't rely on it for diagnosis. Integrating molecular diagnostics with the usual culture-based methods could help in early detection of enteric fever, suggest better use of antibiotics, and strengthen the management strategies in India.

Limitations

This study had certain limitations. First, the number of blood culture-positive cases was relatively low, which may have affected the precision of diagnostic accuracy estimates. Second, the study was conducted at a single tertiary care center, which may limit external validity.

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Recommendation

PCR-based molecular diagnostic methods should be integrated with conventional blood culture techniques for improved early diagnosis of enteric fever in endemic settings. Routine reliance on the Widal test alone should be discouraged because of its poor diagnostic performance. Larger multicentric studies are recommended to further evaluate cost-effective molecular diagnostic methods suitable for resource-limited settings.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

- Concept and study design: All authors
- Data collection: Dr.Vikash Yadav
- Laboratory investigations: Dr. Vikash Yadav
- Data analysis and interpretation: Dr. Vikash Yadav, Dr. Preeti Thakur
- Manuscript drafting: All Authors
- Manuscript review and final approval: All authors

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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