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Clinico-immunological profile and antibiogram of Salmonella species causing enteric fever at a tertiary care hospital in Bihar: A hospital-based cross-sectional study.

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Abstract

Background:
Enteric fever continues to pose a significant public health challenge in Bihar, attributed to inadequate sanitation, restricted diagnostic capabilities, and the escalating risk of antibiotic resistance (AMR). Traditional diagnostic techniques, including blood culture and serological assays, have limitations in sensitivity and specificity, hence requiring enhanced diagnostic methodologies.

Objective:
To assess the clinico-immunological characteristics and antibiogram of Salmonella species responsible for enteric fever in a tertiary care facility in Bihar.

Methods:
This hospital-based cross-sectional observational study was performed at IGIMS, Patna, over a duration of 12 months. A total of 163 individuals, aged 12 years and older, with clinically suspected enteric fever were included. Blood specimens were obtained for culture and antibiotic susceptibility analysis. Immunological assays, specifically Widal and Typhidot, were conducted. Statistical analysis was conducted with Epi Info, with a p-value of less than 0.05 being significant.

Results:
Blood culture positivity was noted in 30.7% of instances. The Widal and Typhidot tests showed high positivity rates but low specificity. A notable correlation was identified between Widal test outcomes and blood culture results ($p < 0.05$). Multidrug-resistant (MDR) Salmonella strains were discovered, demonstrating a substantial association between culture-positive and resistance patterns ($p < 0.05$).

Conclusion:
Enteric fever is a major contributor to acute illness in Bihar. The restricted specificity of serological assays and the rise of multidrug-resistant bacteria underscore the necessity for shifting serology to automated identification and AST systems. Enhancing surveillance, advocating for judicious antibiotic utilisation, and using automated diagnostics are crucial for improving patient outcomes and managing antimicrobial resistance.

Recommendation:
Blood culture should be the principal diagnostic method, supplemented by serological testing. The implementation of rapid automated diagnostics and regular antibiogram testing is crucial. Prudent antibiotic utilisation, enhanced surveillance, and superior cleanliness are essential for managing enteric fever and addressing escalating antimicrobial resistance.

Keywords: *Blood culture, serological testing, enteric fever, antimicrobial resistance, antibiotic utilisation, Salmonella typhi*
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Introduction:

Enteric fever, predominantly induced by Salmonella typhi and Salmonella paratyphi, remains a considerable public health concern, particularly in areas characterised by inadequate sanitation, contaminated drinking water, and insufficient healthcare infrastructure, especially in rural and urban areas of India(1). The illness generally manifests with extended fever, abdominal pain, headache, fatigue, and gastrointestinal issues including diarrhoea as well as constipation. If not recognised and treated expeditiously, it may result in serious complications such as intestinal perforation and systemic infection (2).

The conventional gold standard for diagnosing enteric fever has been blood culture, facilitating the direct identification of the etiological agent. Blood culture possesses numerous limitations, such as diminished sensitivity resulting from prior antibiotic use, protracted result turnaround times, and the necessity for adequately equipped laboratory facilities (3). Alternative diagnostic instruments, like the Widal test, are commonly employed in resource-constrained environments but exhibit low specificity and cross-reactivity, resulting in false-positive outcomes. Inflammatory indicators such as C-reactive protein (CRP) may aid in diagnosis; however, they lack specificity and cannot definitively confirm infection (4).

The rising incidence of antimicrobial resistance (AMR) in Salmonella strains has exacerbated disease management challenges. Multidrug-resistant and severely drug-resistant strains restrict therapeutic alternatives and elevate the likelihood of treatment failure. In this setting, there is an increasing demand for rapid, dependable, and thorough diagnostic methodologies (5).

Automated diagnostic technologies have developed as effective alternatives, providing expedited pathogen detection and accurate antibiotic susceptibility testing. These solutions mitigate human error, expedite turnaround time, and augment clinical decision-making. Automated diagnostics facilitate prompt and precise treatment, significantly enhancing patient outcomes and addressing the worldwide challenge of antimicrobial resistance in enteric fever (6).

Materials and Methods

Study Design

This was a hospital-based cross-sectional observational study conducted to evaluate the clinico-immunological profile and antibiotic susceptibility pattern of *Salmonella* species among patients with clinically suspected enteric fever.

Study Setting

The study was conducted at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, a tertiary-care hospital. Patients attending the outpatient and inpatient departments of various specialties with febrile illness or clinical features suggestive of enteric fever were assessed for eligibility and enrolled in the study. The study was conducted over a period of **12 months**.

Study Population

The study population comprised patients attending the outpatient and inpatient departments of various specialties at IGIMS, Patna, who presented with fever or symptoms suggestive of enteric fever. A total of 163 clinically suspected enteric fever patients aged 12 years and above were enrolled during the study period.

Sample Size Determination

A total of **163 participants** were included in the study. The sample size was determined based on the number of eligible patients available during the study period.

Sampling Procedure

Patients who fulfilled the eligibility criteria and presented to the hospital during the study period were considered for inclusion. A total of 163 eligible patients were enrolled.

Inclusion criteria

- Patients aged **12 years and above**.
- Patients presenting with fever and other clinical features suggestive of enteric fever.
- Patients who provided consent to participate.
- Patients who underwent routine immunological testing as part of standard clinical care.

Exclusion criteria

- Patients with immunocompromised conditions.
- Patients receiving immunosuppressive therapy.
- Individuals who did not provide consent.

Variables

The variables assessed included demographic characteristics such as age and sex, diagnostic findings including blood culture, Widal test, and Typhidot results, and microbiological findings including antibiotic susceptibility and multidrug-resistant (MDR) status.

Data Collection Tools and Methods

Blood samples were collected from patients with clinically suspected enteric fever for blood culture and antibiotic susceptibility testing. Additional immunological investigations, including the Widal test and Typhidot test, were performed as part of routine diagnostic procedures. Demographic and relevant clinical information was recorded for each participant.

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Blood culture results were categorized as positive or negative. For culture-positive samples, antibiotic susceptibility testing was performed, and isolates were categorized according to their susceptibility patterns. The study also assessed the relationship between blood culture findings, Widal test results, and antibiotic susceptibility patterns.

Validity and Reliability

Standard laboratory procedures were followed for blood culture, serological testing, and antibiotic susceptibility testing. Data were recorded systematically using predefined study variables to maintain consistency and minimize errors during data collection and analysis.

Study Procedure

Patients presenting with fever or other clinical features suggestive of enteric fever were assessed according to the predefined eligibility criteria. After enrollment and consent, blood samples were collected for culture and antibiotic susceptibility testing. Widal and Typhidot tests were also performed as part of the diagnostic evaluation. The demographic, clinical, immunological, culture, and antibiotic susceptibility findings were recorded for each participant. The collected data were subsequently analyzed statistically.

Bias

Potential selection and information biases were considered during the study. Predefined inclusion and exclusion criteria were applied consistently to identify eligible participants. Standardized procedures were used for the collection and processing of blood samples and for the performance of diagnostic investigations. Data were recorded systematically to minimize errors in data collection and classification.

Statistical Analysis

Statistical analysis was performed using Epi Info software. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using means and standard deviations (SD). The **Chi-square test** was used to assess associations between categorical variables. A *p*-value of **<0.05** was considered statistically significant.

Ethical Consideration and Informed Consent

The study was conducted in accordance with applicable ethical principles. Written informed consent was obtained from the participants before enrollment.

Results

A total of 163 patients with clinically suspected enteric fever, aged 12 years and above, were assessed and enrolled during the 12-month study period. All enrolled participants met the predefined inclusion criteria and underwent the required diagnostic investigations, including blood culture, Widal testing, and Typhidot testing. No participants were excluded after enrollment, and all 163 participants were included in the final analysis. Among the 163 clinically suspected enteric fever patients, blood culture was negative in 113 (69.3%) cases and positive in 50 (30.7%) cases. Of the 113 blood culture-negative patients, 54 (47.8%) were Widal negative and 59 (52.2%) were Widal positive. Among the 50 blood culture-positive patients, 34 (68.0%) showed Widal positivity, while 16 (32.0%) were Widal negative. Overall, Widal positivity was observed in 93 (57.1%) patients, whereas 70 (42.9%) patients were Widal negative.

A statistically significant association was observed between blood culture findings and Widal test results (χ^2 test, $p = 0.03$). Patients with positive blood cultures were significantly more likely to demonstrate Widal positivity compared to blood culture-negative patients. However, a substantial proportion of blood culture-negative cases also showed positive Widal results, suggesting limited specificity of the Widal test when used as a standalone diagnostic tool.

Table 1: Demographic Characteristics

Gender	Number of Patients	Mean Age (years)
Female	88	38.09
Male	75	36.23

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Figure 1: Distribution of male and female patients in the study

Table 2: Blood Culture vs Widal Test

Blood Culture	Widal Negative	Widal Positive
Negative	54	59
Positive	16	34

p-value = 0.03 (Significant)

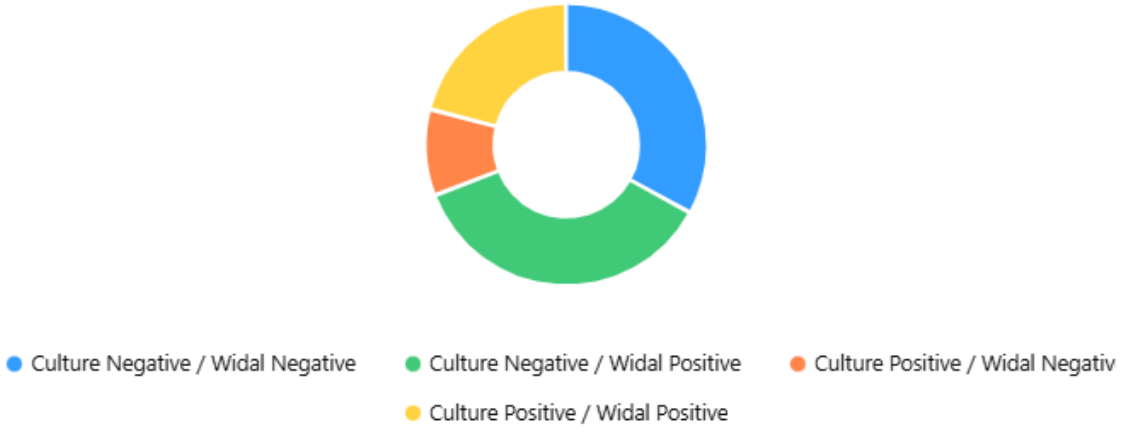

Figure 2: Distribution of blood culture and Widal test results

Table 3 presents the relationship between blood culture status and antibiotic susceptibility pattern among the study participants. Of the 163 patients evaluated, 46 (28.2%) isolates were identified as multidrug-resistant (MDR), whereas 117 (71.8%) were antibiotic-sensitive. Among the 113 blood culture-negative cases, 36 (31.9%) were associated with MDR strains and 77 (68.1%) were antibiotic-sensitive. In contrast, among the 50 blood culture-positive cases, 10 (20.0%) demonstrated MDR patterns while 40 (80.0%) remained sensitive to commonly used antibiotics. Statistical analysis revealed a significant association between blood culture status and antibiotic susceptibility pattern (Chi-square test, $p = 0.02$), indicating that resistance patterns differed significantly between the two groups.

Figure 3 compares the positivity rates of the diagnostic tests employed in the study. Typhidot demonstrated the highest positivity rate, followed by the Widal test, whereas blood culture showed the lowest positivity. These findings suggest that serological tests are more sensitive

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screening tools; however, blood culture remains essential for definitive diagnosis and antimicrobial susceptibility testing.

Table 3: Comparison of Blood Culture Positivity with Antibiotic Susceptibility Pattern (AST)

Blood Culture Status	MDR Resistant	Antibiotic Sensitive	Total
Negative	36	77	113
Positive	10	40	50
Total	46	117	163

Chi-square test p-value = 0.02 (Statistically Significant)

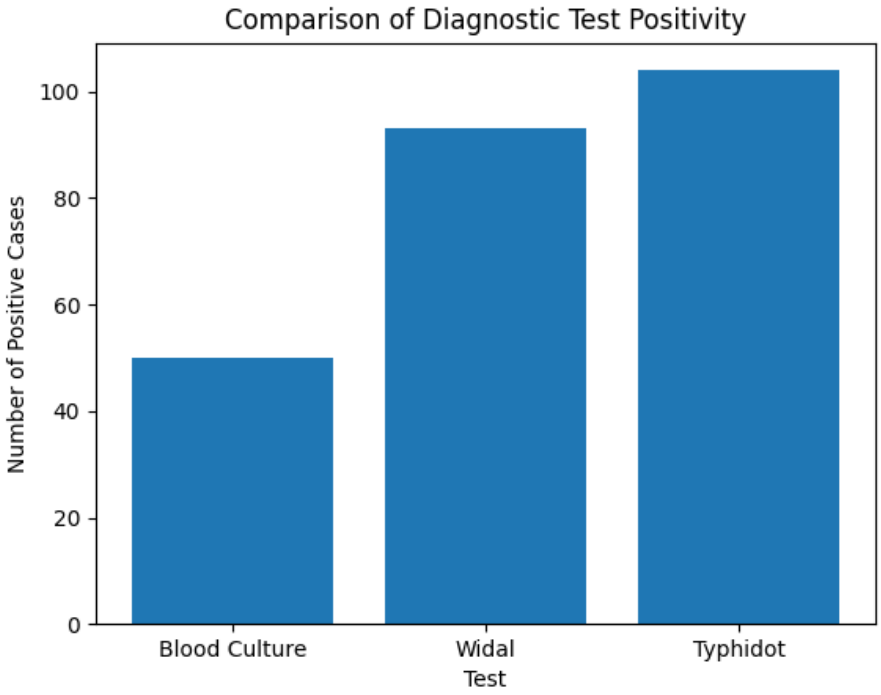

Figure 3: Comparison of diagnostic test positivity

Discussion

This study emphasises the persistent incidence of enteric fever in Bihar, highlighting the broader challenges faced in a number of resource-constrained settings where poor sanitation, contaminated drinking water, and limited access to treatment promote the spread of illness. The frequency of cases among young and middle-aged adults is consistent with their increased exposure risk due to social and professional mobility(7). Rather than an actual epidemiological trend, the somewhat higher proportion of female patients seen in this study could be attributed to differences in healthcare-seeking behaviour, cultural effects, or simple sample variability (8). Although the blood culture positivity rate of 30.7% is consistent with findings from similar studies, its sensitivity is still insufficient. The main cause of this restriction is prior empirical antibiotic use before hospital admission, which reduces bacterial burden and affects culture production. Although blood culture is the gold standard for diagnosis, its efficacy in urgent clinical decision-making is limited by its lengthy procedure and infrastructure requirements (9). On the other hand, serological tests such as Typhidot

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and Widal have shown high positive rates, making them useful as rapid screening tools. However, false-positive results are caused by their reduced specificity, cross-reactivity with other diseases, and inability to distinguish between past and current infections (10).

The present study revealed an alarming rise in multidrug-resistant (MDR) *Salmonella* isolates. Antibiotic susceptibility testing showed that a considerable proportion of culture-positive isolates exhibited resistance to commonly prescribed antibiotics. This trend may be attributed to irrational antibiotic usage, incomplete treatment courses, and over-the-counter availability of antimicrobials in resource-limited settings (11). The statistically significant association between blood culture positivity and MDR pattern suggests that resistant strains are increasingly responsible for clinically significant enteric fever infections. Similar findings have been reported in recent Indian studies, where resistance to first-line drugs such as ampicillin, chloramphenicol, and cotrimoxazole has been rising steadily. Routine AST plays an essential role in guiding targeted antimicrobial therapy and preventing unnecessary antibiotic exposure. Early detection of resistant strains can reduce treatment failure, shorten hospital stay, and limit the spread of resistant organisms (12).

Therefore, periodic regional antibiogram surveillance should be integrated into hospital infection control policies. Although it shouldn't be used in isolation, the statistically significant link between Widal test results and blood culture positivity indicates that it is still a useful additional diagnostic tool. Typhidot requires further thorough validation to confirm its reliability across diverse populations, even if it has shown enhanced sensitivity (13). One particularly concerning finding is the emergence of multidrug-resistant (MDR) Salmonella bacteria(14). The significant correlation between resistance patterns and culture-positive cases highlights the growing threat of antibiotic resistance, which complicates treatment procedures and increases morbidity. This emphasises how crucial it is to conduct routine antibiotic susceptibility testing in order to guide focused treatment (15).

Automated diagnostic tools are a major advancement in this regard. By providing quick, accurate pathogen identification and reliable antibiogram data, these devices can significantly reduce diagnostic delays and support evidence-based treatment decisions. Incorporating them into routine clinical practice has the potential to improve patient outcomes, decrease inappropriate antibiotic use, and eventually slow the spread of antimicrobial resistance (16).

Generalizability

The findings of this study may apply to tertiary care hospital settings with patient populations and diagnostic practices similar to those of the study site. The observed patterns of blood culture positivity, serological test results, and antimicrobial susceptibility may provide useful information for clinicians and microbiology laboratories managing suspected enteric fever in comparable settings. However, differences in patient characteristics, local epidemiology, antimicrobial prescribing practices, laboratory facilities, and patterns of antimicrobial resistance may influence the applicability of these findings to other hospitals and geographic regions. Therefore, the findings should be interpreted primarily within the context of tertiary care settings in Bihar and similar healthcare environments.

Conclusion

Enteric fever continues to pose a considerable public health concern in Bihar, mostly attributable to insufficient sanitation, protracted diagnosis, and the rising incidence of antibiotic resistance. Although blood culture remains the gold standard for diagnosis, its limitations—such as low sensitivity and delayed results—require the utilisation of additional immunological tests like Widal and Typhidot. Nonetheless, these tests have poor specificity and may yield false-positive results, hence diminishing their trustworthiness when utilised in isolation.

The study demonstrates a significant association between blood culture positivity and antimicrobial resistance patterns among *Salmonella* isolates. The emergence of multidrug-resistant strains emphasises the urgent need for routine antibiotic susceptibility testing, antimicrobial stewardship, and region-specific antibiogram surveillance to improve treatment outcomes and limit the spread of resistant enteric fever pathogens. Enhancing regular surveillance systems, advocating for judicious antibiotic utilisation, and incorporating automated diagnostic technology into clinical practice are imperative measures. These measures will facilitate prompt diagnosis, direct appropriate treatment, and ultimately mitigate the impact of antibiotic resistance while enhancing patient outcomes.

Limitations

This study has certain limitations. First, it was conducted at a single tertiary care hospital in Bihar, which may limit the generalizability of the findings to other geographic regions and healthcare settings. Second, the study included patients with clinically suspected enteric fever, and the diagnostic performance of serological tests may have been influenced by factors such as previous exposure, cross-reactivity, or prior antibiotic use. Third, the sensitivity of blood culture may have been affected by

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prior empirical antibiotic therapy before hospital presentation. Finally, the study was conducted over a limited study period and therefore may not fully represent longer-term variations in Salmonella epidemiology and antimicrobial resistance patterns. Further multicentric studies involving larger populations and longer surveillance periods are recommended to validate these findings.

Recommendation:

This study recommends the continued use of blood culture as the primary diagnostic approach for enteric fever, with serological tests such as Widal and Typhidot serving exclusively as supplementary tools rather than conclusive assays. A pressing necessity exists to enhance laboratory infrastructure to facilitate prompt and precise diagnoses, encompassing the use of automated diagnostic technologies for swift pathogen identification and antibiotic susceptibility assessment.

Routine antimicrobial susceptibility testing ought to be instituted to inform targeted therapy and reduce antibiotic overuse. Clinicians must follow judicious antibiotic prescribing procedures to mitigate the increasing prevalence of multidrug-resistant Salmonella strains.

Moreover, public health initiatives including the enhancement of sanitation, provision of safe drinking water, and improvement of disease surveillance systems are crucial. Continuous surveillance and region-specific antimicrobial strategies must be established to efficiently manage and control enteric fever.

List of abbreviations:

MDR	Multidrug-resistant
AMR	Antibiotic resistance
SD	Standard deviation

Conflict of interest

Author declares no conflict of interest

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

R K Priyanka: Conceptualization, methodology, data collection, laboratory investigations, data analysis, and manuscript preparation.

Randhir Kumar: Study supervision, methodology, interpretation of results, and critical review of the manuscript.

Archana: Laboratory investigations, data collection, and critical review of the manuscript.

Amit Mishra: Clinical supervision, patient recruitment, interpretation of clinical findings, and critical review of the manuscript.

Rakesh Kumar: Laboratory supervision, interpretation of microbiological findings, and critical review of the manuscript.

Namrata Kumari: Study supervision, interpretation of results, and critical review and final approval of the manuscript.

All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Data Availability

The data generated and/or analyzed during the present study are not publicly available but may be made available from the corresponding author on reasonable request, subject to appropriate institutional and ethical considerations.

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