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Original Article

Predictive Value of Clinical and Laboratory Findings in the Diagnosis of Enteric Fever

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ABSTRACT

Background: Enteric fever remains endemic in Pakistan, where limited access to blood culture and increasing antimicrobial resistance complicate early diagnosis and treatment. Clinical signs and routine laboratory parameters are commonly used in resource-limited settings, but their predictive value against confirmed non-enteric febrile illnesses remains uncertain. Objective: To identify independent clinical and laboratory predictors of culture-confirmed enteric fever and evaluate the diagnostic performance of individual and combined findings. Methods: This retrospective diagnostic case–control study was conducted at Pak-Emirates Military Hospital, Rawalpindi, from January 2021 to December 2022. Sixty patients with culture-confirmed Salmonella Typhi or Salmonella Paratyphi infection were compared with 58 patients with microbiologically, serologically, antigenically, or molecularly confirmed non-enteric febrile illnesses. Clinical findings, hematological parameters, liver enzymes, and Widal titres were analyzed using group comparisons and multivariable logistic regression. Diagnostic performance was assessed using sensitivity, specificity, positive predictive value, and negative predictive value. Results: Salmonella Typhi accounted for 42/60 cases, with multidrug resistance in 28/42 and extensively drug-resistant phenotype in 8/42 isolates. Splenomegaly, relative bradycardia, rose spots, thrombocytopenia, elevated aspartate aminotransferase, and Widal O titre ≥1:160 were independent predictors of enteric fever. The model showed good discrimination, with an area under the receiver operating characteristic curve of 0.84. Combined findings showed high specificity but low sensitivity. Conclusion: Clinical and routine laboratory findings may support rule-in diagnosis of enteric fever when present but cannot replace microbiological confirmation or reliably exclude disease when absent. Prospective validation is required before these predictors can be used as a formal diagnostic algorithm. Keywords: Enteric fever; Salmonella Typhi; diagnostic prediction; Widal test; thrombocytopenia; relative bradycardia; Pakistan

INTRODUCTION

Enteric fever, caused by Salmonella enterica serovar Typhi and serovars Paratyphi A, B, and C, remains a major public health challenge in low- and middle-income countries where inadequate water, sanitation, and hygiene infrastructure sustain fecal–oral transmission. The disease continues to impose a substantial global burden, with South Asia contributing disproportionately to incident cases, complications, and mortality (1). Pakistan is among the most affected countries, where enteric fever remains an important cause of bacteremia, particularly in urban and peri-urban populations exposed to overcrowding, contaminated water supplies, and limited sanitation. The emergence and dissemination of multidrug-resistant and extensively drug-resistant Salmonella Typhi strains have further complicated management

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by narrowing effective treatment options and increasing the consequences of delayed or inappropriate empirical therapy (2).

Although blood culture remains the reference standard for confirming enteric fever, its availability, sensitivity, and timeliness are limited in many endemic healthcare settings. Culture yield is affected by prior antibiotic exposure, timing of sample collection, blood volume, and laboratory capacity, while advanced molecular or next-generation rapid diagnostic platforms are not routinely accessible in peripheral facilities. Consequently, clinicians frequently rely on a combination of clinical features and routine laboratory findings when evaluating patients with prolonged fever. However, the clinical syndrome of enteric fever overlaps substantially with other febrile illnesses prevalent in Pakistan, including brucellosis, dengue fever, scrub typhus, urinary tract infection, pneumonia, and tuberculosis. This diagnostic overlap can lead to both under-recognition of enteric fever and unnecessary broad-spectrum antibiotic use in non-enteric febrile illnesses, thereby worsening antimicrobial selection pressure.

Classic clinical signs such as splenomegaly, hepatomegaly, rose spots, and relative bradycardia, along with laboratory abnormalities including leukopenia, thrombocytopenia, eosinopenia, elevated transaminases, and Widal agglutinin titres, are commonly associated with enteric fever. However, their individual and combined predictive value remains uncertain in routine clinical practice, especially when assessed against microbiologically confirmed alternative diagnoses rather than healthy controls. This distinction is important because diagnostic predictors that appear useful in uncomplicated comparisons may perform poorly in real febrile populations where inflammatory, hematological, and hepatic abnormalities are shared across multiple infections. In endemic settings, the Widal test is particularly difficult to interpret because background exposure, prior infection, vaccination, and serological cross-reactivity can produce false-positive results, reducing its value as a standalone diagnostic test.

The introduction of typhoid conjugate vaccine into Pakistan's routine immunization program has strengthened prevention against Salmonella Typhi, but it does not address Salmonella Paratyphi infections and does not eliminate the immediate need for practical diagnostic strategies in symptomatic patients (3). At the same time, ongoing reports of antimicrobial resistance in Salmonella Typhi and changing serovar patterns in Pakistan highlight the need for locally relevant diagnostic evidence that can support rational empirical treatment while microbiological confirmation is pending (4). In this context, a parsimonious set of clinical and laboratory predictors may help clinicians identify patients with a high probability of enteric fever, particularly as a rule-in approach in settings where culture is delayed or unavailable.

This study aimed to identify independent clinical and routine laboratory predictors of culture-confirmed enteric fever among patients presenting with prolonged fever and to evaluate the diagnostic performance of individual and combined findings against microbiologically confirmed non-enteric febrile illnesses. The primary research question was whether selected bedside clinical signs and routinely available laboratory parameters can provide clinically useful predictive value for culture-confirmed enteric fever in a Pakistani tertiary-care setting.

MATERIAL AND METHODS

This retrospective diagnostic case–control study was conducted at Pak-Emirates Military Hospital, Rawalpindi, Pakistan, using medical records of patients admitted to the emergency and inpatient medicine units between January 2021 and December 2022. The study compared patients with culture-confirmed enteric fever against patients with microbiologically, serologically, or molecularly confirmed non-enteric febrile illnesses in order to evaluate the predictive value of selected clinical and routine laboratory findings for the diagnosis of enteric fever. The study design was selected because the objective was to assess diagnostic predictors among patients who presented with a clinically overlapping syndrome of prolonged fever rather than to estimate population incidence or prevalence.

Patients aged 12 years or older were screened for eligibility if they presented with fever of at least 38.0°C persisting for more than four days and had at least one associated clinical feature suggestive of systemic febrile illness, including nausea, vomiting, abdominal pain, headache, anorexia, cough, rose spots, or documented relative bradycardia. The enteric fever group included patients in whom Salmonella Typhi or Salmonella Paratyphi was isolated from blood culture or another sterile-site specimen. The non-enteric fever control group included patients in whom Salmonella Typhi and Salmonella Paratyphi were not isolated and an alternative infectious diagnosis was established through microbiological, serological, antigen-based, or molecular testing. Patients were excluded if they had received systemic antibiotics for more than 48 hours before blood culture collection, had incomplete clinical or laboratory records, or had no microbiological, serological, or molecular confirmation of the final diagnosis. Patients with alternative infections were eligible for the control group only when those diagnoses were confirmed and accounted for the febrile illness; therefore, these conditions were not treated as exclusions when they constituted the confirmed non-enteric comparator diagnosis.

The final study sample comprised 118 patients, including 60 patients with culture-confirmed enteric fever and 58 patients with confirmed non-enteric febrile illnesses. The enteric fever group included 32 males and 28 females with a mean age of 28.8 ± 10.5 years. The non-enteric fever control group included 30 males and 28 females with a mean age of 30.6 ± 12.2 years. Cases and controls were identified from hospital records according to the predefined diagnostic criteria, and data were extracted from electronic and paper-based medical records using the same clinical and laboratory variable definitions for both groups.

The clinical predictors extracted from records were hepatomegaly, splenomegaly, rose spots, and relative bradycardia. Relative bradycardia was operationally defined for this study as a documented pulse rate below 100 beats per minute in the presence of fever of at least 38.5°C at the same clinical assessment. Although this definition provides a reproducible bedside threshold for retrospective record extraction, it was interpreted as an operational study definition rather than a complete physiological assessment of pulse–temperature dissociation. Laboratory variables included total leukocyte count, platelet count, eosinophil count, hemoglobin concentration, alanine aminotransferase, aspartate aminotransferase, Widal O agglutinin titre, and Widal H agglutinin titre. Leukopenia was defined as total leukocyte count below 4,300/mm³, leukocytosis as total leukocyte count above 10,800/mm³, thrombocytopenia as platelet count below 150,000/mm³, eosinopenia as eosinophil count below 40/mm³, anemia as hemoglobin below 11 g/dL, and elevated transaminases as alanine aminotransferase or aspartate aminotransferase above 40 U/L. Widal O and H titres of at least 1:160 were treated as suggestive serological thresholds for analysis, while higher titre combinations were interpreted cautiously because single Widal titres may be affected by background exposure and cross-reactivity in endemic settings.

Blood cultures were obtained during the febrile phase, preferably before antibiotic administration, using standard aseptic technique. For adult patients, a minimum blood volume of 10 mL was inoculated into each culture bottle, with proportionally reduced volumes used for adolescent patients according to institutional protocol. Blood culture processing was performed using automated blood culture systems, including BACTEC FX, BACTEC 9000 series, or BacT/ALERT 3D platforms. Culture bottles flagged as positive underwent Gram staining and subculture on blood agar and eosin methylene blue agar. Isolates were identified using the VITEK 2 Compact automated system, with biochemical confirmation where required using standard reactions such as triple sugar iron, urease, citrate, and lysine decarboxylase testing. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute guidance applicable during 2021–2022, with assessment of resistance to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, third-generation cephalosporins, and azithromycin. Multidrug resistance was defined as resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, while extensively drug-resistant phenotype was defined as multidrug resistance with additional resistance to fluoroquinolones and third-generation cephalosporins.

Widal tube agglutination testing was performed according to institutional laboratory procedures. Anti-O and anti-H agglutinin titres were recorded when available and interpreted in relation to the clinical and microbiological diagnosis rather than as standalone evidence of enteric fever. Because single Widal titres may yield false-positive results in endemic populations and in patients with other Gram-negative or intracellular infections, Widal results were analyzed as candidate predictors and in selected combinations with objective clinical and laboratory findings rather than as an independent reference standard.

Data extraction was performed using predefined variables to reduce information bias and ensure uniform ascertainment across cases and controls. The reference diagnosis for enteric fever was isolation of Salmonella Typhi or Salmonella Paratyphi from blood culture or another sterile-site specimen. The reference diagnosis for controls required confirmation of a non-enteric infectious etiology by microbiological, antigen-based, serological, or molecular methods. Restricting controls to confirmed alternative diagnoses was intended to reduce misclassification bias; however, this approach may introduce spectrum bias because the control group represents hospitalized febrile illnesses rather than the full range of community febrile presentations. Potential confounding was addressed analytically by comparing candidate predictors between groups and entering statistically significant univariate predictors into a multivariable logistic regression model to identify variables independently associated with culture-confirmed enteric fever.

Continuous variables were summarized as mean ± standard deviation when approximately normally distributed and compared using the independent samples t-test; non-normally distributed variables were compared using the Mann–Whitney U test. Categorical variables were summarized as frequencies and percentages and compared using the chi-square test or Fisher's exact test, as appropriate. Candidate clinical and laboratory predictors with statistically significant univariate associations were entered into multivariable logistic regression to estimate adjusted odds ratios with 95% confidence intervals. Diagnostic performance was assessed by calculating sensitivity, specificity, positive predictive value, and negative predictive value for individual findings and selected clinically plausible combinations of findings. Because the study used a case–control structure with a fixed proportion of enteric fever cases and non-enteric controls, positive and negative predictive values were interpreted as sample-specific estimates requiring prospective validation in populations with real-world disease prevalence. Model discrimination was assessed using the area under the receiver operating characteristic curve. All analyses were performed using SPSS version 26.0 or R version 4.2.0, with a two-tailed significance threshold of $p < 0.05$.

The study used retrospective hospital record data and laboratory reports. Patient confidentiality was maintained during data extraction and analysis by using de-identified study variables. Data were checked for completeness, internal consistency, and agreement between clinical records and laboratory reports before analysis. Culture-confirmed case definitions, confirmed comparator diagnoses, standardized laboratory thresholds, and uniform extraction of predictor variables were used to support reproducibility and minimize differential classification between groups.

RESULTS

A total of 118 patients were included, comprising 60 patients with culture-confirmed enteric fever and 58 patients with confirmed non-enteric febrile illnesses. The enteric fever group included 32 males and 28 females, with a mean age of 28.8 ± 10.5 years. The non-enteric fever control group included 30 males and 28 females, with a mean age of 30.6 ± 12.2 years. There was no statistically significant difference between groups for age or sex distribution.

Table 1. Baseline Characteristics of the Study Groups

Variable	Enteric Fever (n = 60)	Non-Enteric Fever (n = 58)	p-value
Age, years, Mean ± SD	28.8 ± 10.5	30.6 ± 12.2	0.386

Variable	Enteric Fever (n = 60)	Non-Enteric Fever (n = 58)	p-value
Male, n (%)	32 (53.3)	30 (51.7)	0.824
Female, n (%)	28 (46.7)	28 (48.3)	0.824

SD: standard deviation.

The two groups were demographically comparable. The mean age differed by 1.8 years, and the male-to-female distribution was nearly identical between groups, supporting subsequent comparison of clinical and laboratory predictors without major baseline imbalance.

Among the 60 culture-confirmed enteric fever cases, Salmonella Typhi was isolated in 42 patients, Salmonella Paratyphi A in 14 patients, and Salmonella Paratyphi B in 4 patients. Among the 58 non-enteric febrile controls, the most frequent alternative diagnoses were brucellosis, dengue fever, scrub typhus, urinary tract infection, pneumococcal pneumonia, and tuberculous meningitis.

Table 2. Microbiological Profile of Enteric Fever Cases and Non-Enteric Febrile Controls

Diagnosis or Isolate	n (%)
Enteric fever isolates (n = 60)	
Salmonella Typhi	42 (70.0)
Salmonella Paratyphi A	14 (23.3)
Salmonella Paratyphi B	4 (6.7)
Non-enteric febrile diagnoses (n = 58)	
Brucellosis	18 (31.0)
Dengue fever	14 (24.1)
Scrub typhus	10 (17.2)
Urinary tract infection due to Escherichia coli	8 (13.8)
Pneumococcal pneumonia	5 (8.6)
Tuberculous meningitis	3 (5.2)

Salmonella Typhi accounted for 70.0% of enteric fever isolates, while Salmonella Paratyphi A represented nearly one-quarter of cases. The control group was composed of clinically relevant febrile comparators, with brucellosis, dengue fever, and scrub typhus together accounting for 72.3% of non-enteric febrile diagnoses.

Antimicrobial susceptibility testing showed a high burden of resistance among Salmonella Typhi isolates. Of the 42 Salmonella Typhi isolates, 28 were multidrug-resistant and 8 were extensively drug-resistant. Among the 18 Salmonella Paratyphi isolates, all were susceptible to third-generation cephalosporins, while reduced fluoroquinolone susceptibility was observed in 10 isolates.

Table 3. Antimicrobial Resistance Profile of Salmonella Isolates

Resistance Profile	Denominator	n (%)
MDR Salmonella Typhi	42	28 (66.7)
XDR Salmonella Typhi	42	8 (19.0)
Third-generation cephalosporin-susceptible Salmonella Paratyphi	18	18 (100.0)
Reduced fluoroquinolone susceptibility in Salmonella Paratyphi	18	10 (55.6)

MDR: multidrug-resistant; XDR: extensively drug-resistant.

After denominator correction, multidrug resistance was present in two-thirds of Salmonella Typhi isolates, while approximately one-fifth were extensively drug-resistant. This resistance pattern strengthens the clinical importance of early diagnostic stratification and careful empirical antibiotic selection.

Clinical examination findings showed clear differences between enteric fever cases and non-enteric febrile controls. Hepatomegaly, splenomegaly, rose spots, and relative bradycardia were significantly more common in enteric fever, whereas non-specific symptoms such as nausea or vomiting, abdominal pain, headache, anorexia, and cough did not differ significantly between groups.

Table 4. Clinical Findings in Enteric Fever Cases and Non-Enteric Febrile Controls

Clinical Finding	Enteric Fever (n = 60), n (%)	Non-Enteric Fever (n = 58), n (%)	p-value
Hepatomegaly	34 (56.7)	12 (20.7)	<0.001
Splenomegaly	38 (63.3)	10 (17.2)	<0.001
Rose spots	18 (30.0)	3 (5.2)	<0.001
Relative bradycardia	22 (36.7)	6 (10.3)	0.001
Nausea/vomiting	42 (70.0)	36 (62.1)	0.347
Abdominal pain	40 (66.7)	30 (51.7)	0.089
Headache	44 (73.3)	38 (65.5)	0.334
Anorexia	46 (76.7)	40 (69.0)	0.321
Cough	16 (26.7)	14 (24.1)	0.743

Splenomegaly was present in 63.3% of enteric fever cases compared with 17.2% of controls, while hepatomegaly was present in 56.7% and 20.7%, respectively. Rose spots were less frequent but highly discriminatory, occurring in 30.0% of enteric fever cases and only 5.2% of controls. Relative bradycardia was also significantly more common in enteric fever, although it was present in only 36.7% of cases. Common systemic symptoms had limited discriminatory value because they were frequent in both groups.

Routine laboratory findings also differed between groups. Leukopenia, thrombocytopenia, eosinopenia, elevated AST, elevated ALT, Widal O titre ≥1:160, and Widal H titre ≥1:160 were significantly associated with enteric fever. Leukocytosis was significantly more common among non-enteric febrile controls.

Table 5. Laboratory Findings in Enteric Fever Cases and Non-Enteric Febrile Controls

Laboratory Finding	Enteric Fever (n = 60), n (%)	Non-Enteric Fever (n = 58), n (%)	p-value
Leukopenia, <4,300/mm³	24 (40.0)	8 (13.8)	0.002
Normal WBC, 4,300–10,800/mm³	28 (46.7)	32 (55.2)	0.341
Leukocytosis, >10,800/mm³	8 (13.3)	18 (31.0)	0.016
Thrombocytopenia, <150,000/mm³	32 (53.3)	10 (17.2)	<0.001
Eosinopenia, <40/mm³	44 (73.3)	26 (44.8)	0.002
Anemia, Hb <11 g/dL	28 (46.7)	22 (37.9)	0.312
Elevated AST, >40 U/L	36 (60.0)	14 (24.1)	<0.001
Elevated ALT, >40 U/L	30 (50.0)	18 (31.0)	0.032
Widal O titre ≥1:160	46 (76.7)	22 (37.9)	<0.001
Widal H titre ≥1:160	38 (63.3)	18 (31.0)	0.001

WBC: white blood cell count; Hb: hemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Thrombocytopenia was observed in 53.3% of enteric fever cases compared with 17.2% of controls, while elevated AST was observed in 60.0% and 24.1%, respectively. Eosinopenia was frequent among enteric fever cases but also occurred in nearly half of controls, reducing its specificity. Elevated ALT was significantly more common in enteric fever, correcting the earlier inconsistency in which ALT was described as non-significant despite a reported p-value of 0.032. Widal O and H titres were also significantly more frequent in enteric fever but remained positive in a substantial proportion of controls, supporting cautious interpretation in endemic settings.

The diagnostic performance of individual findings is presented in Table 6. Sensitivity was highest for Widal O titre ≥1:160 and eosinopenia, while specificity was highest for rose spots, relative bradycardia, leukopenia, splenomegaly, and thrombocytopenia. Positive and negative predictive values are sample-specific because the case–control design fixed the proportion of enteric fever cases and controls.

Table 6. Diagnostic Performance of Individual Clinical and Laboratory Findings for Enteric Fever

Finding	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Hepatomegaly	56.7 (44.1–68.4)	79.3 (67.2–87.7)	73.9 (59.7–84.4)	63.9 (52.4–74.0)
Splenomegaly	63.3 (50.7–74.4)	82.8 (71.1–90.4)	79.2 (65.7–88.3)	68.6 (57.0–78.2)
Rose spots	30.0 (19.9–42.5)	94.8 (85.9–98.2)	85.7 (65.4–95.0)	56.7 (46.8–66.1)
Relative bradycardia	36.7 (25.6–49.3)	89.7 (79.2–95.2)	78.6 (60.5–89.8)	57.8 (47.5–67.5)
Leukopenia	40.0 (28.6–52.6)	86.2 (75.1–92.8)	75.0 (57.9–86.7)	58.1 (47.6–68.0)
Thrombocytopenia	53.3 (40.9–65.4)	82.8 (71.1–90.4)	76.2 (61.5–86.5)	63.2 (51.9–73.1)

Finding	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Eosinopenia	73.3 (61.0–82.9)	55.2 (42.5–67.3)	62.9 (51.1–73.2)	66.7 (52.5–78.3)
Elevated AST	60.0 (47.4–71.4)	75.9 (63.5–85.0)	72.0 (58.3–82.5)	64.7 (52.8–75.0)
Widal O titre ≥1:160	76.7 (64.6–85.6)	62.1 (49.2–73.4)	67.6 (55.8–77.6)	72.0 (58.3–82.5)
Widal H titre ≥1:160	63.3 (50.7–74.4)	69.0 (56.2–79.4)	67.9 (54.8–78.6)	64.5 (52.1–75.3)

CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; AST: aspartate aminotransferase. Confidence intervals were derived from the reported aggregate counts. PPV and NPV are sample-specific estimates and require validation in cohorts with real-world disease prevalence.

Widal O titre ≥1:160 showed the highest sensitivity at 76.7%, but specificity was limited at 62.1%. Eosinopenia also showed relatively high sensitivity but poor specificity. Rose spots had the highest specificity at 94.8% and the highest positive predictive value among individual findings, but sensitivity was only 30.0%. These findings indicate that rose spots, relative bradycardia, splenomegaly, leukopenia, and thrombocytopenia are more useful for ruling in enteric fever when present than for excluding the disease when absent.

Multivariable logistic regression identified splenomegaly, relative bradycardia, rose spots, thrombocytopenia, elevated AST, and Widal O titre ≥1:160 as independent predictors of culture-confirmed enteric fever. The model demonstrated good discrimination, with an area under the receiver operating characteristic curve of 0.84.

Table 7. Independent Predictors of Culture-Confirmed Enteric Fever

Predictor	Adjusted OR	95% CI	p-value
Splenomegaly	7.42	3.12–17.64	<0.001
Relative bradycardia	14.28	4.86–41.98	<0.001
Rose spots	8.56	2.28–32.14	0.002
Thrombocytopenia	6.84	2.94–15.92	<0.001
Elevated AST, >40 U/L	4.18	1.86–9.38	0.001
Widal O titre ≥1:160	3.62	1.58–8.30	0.002

OR: odds ratio; CI: confidence interval; AST: aspartate aminotransferase. The dependent variable was culture-confirmed enteric fever. Reference categories were absence of the listed predictor.

Relative bradycardia showed the strongest independent association with enteric fever, with adjusted odds more than 14 times higher among affected patients. Rose spots, splenomegaly, and thrombocytopenia also showed strong independent associations. Elevated AST and Widal O titre ≥1:160 remained statistically significant predictors, although Widal O had limited standalone specificity and should therefore be interpreted as an adjunctive marker rather than a confirmatory test.

Combined clinical and laboratory findings were evaluated as high-specificity rule-in patterns. The reported combinations showed high specificity but low sensitivity. However, the originally reported positive and negative predictive values for some combined findings were not fully concordant with the stated sensitivity, specificity, and group denominators. Therefore, the reported PPV and NPV values should be verified against the original 2 × 2 data before final submission.

Table 8. Rule-In Diagnostic Performance of Combined Clinical and Laboratory Findings

Combined Finding	Sensitivity (%)	Specificity (%)	Reported PPV (%)	Reported NPV (%)	Verification Status
Splenomegaly + rose spots	23.3	98.3	87.5	55.1	Requires dataset verification
Splenomegaly + rose spots + relative bradycardia	18.3	98.3	84.6	53.2	Requires dataset verification
Splenomegaly + rose spots + relative bradycardia + Widal O titre ≥1:200	21.7	96.6	81.3	54.2	Requires dataset verification
Splenomegaly + rose spots + relative bradycardia + Widal O titre ≥1:200 + normal WBC	16.7	98.3	90.9	53.1	Requires dataset verification

Combined Finding	Sensitivity (%)	Specificity (%)	Reported PPV (%)	Reported NPV (%)	Verification Status
Splenomegaly + rose spots + relative bradycardia + Widal O titre ≥1:200 + leukopenia	15.0	98.3	90.0	52.8	Requires dataset verification

PPV: positive predictive value; NPV: negative predictive value; WBC: white blood cell count.

The combined findings demonstrated very high specificity, ranging from 96.6% to 98.3%, but low sensitivity, ranging from 15.0% to 23.3%. These combinations may be clinically useful as rule-in indicators when present, but they are unsuitable as screening tools because most culture-confirmed enteric fever cases would be missed. Increasing the number of required findings improved diagnostic certainty in a small subset of patients but reduced overall case detection.

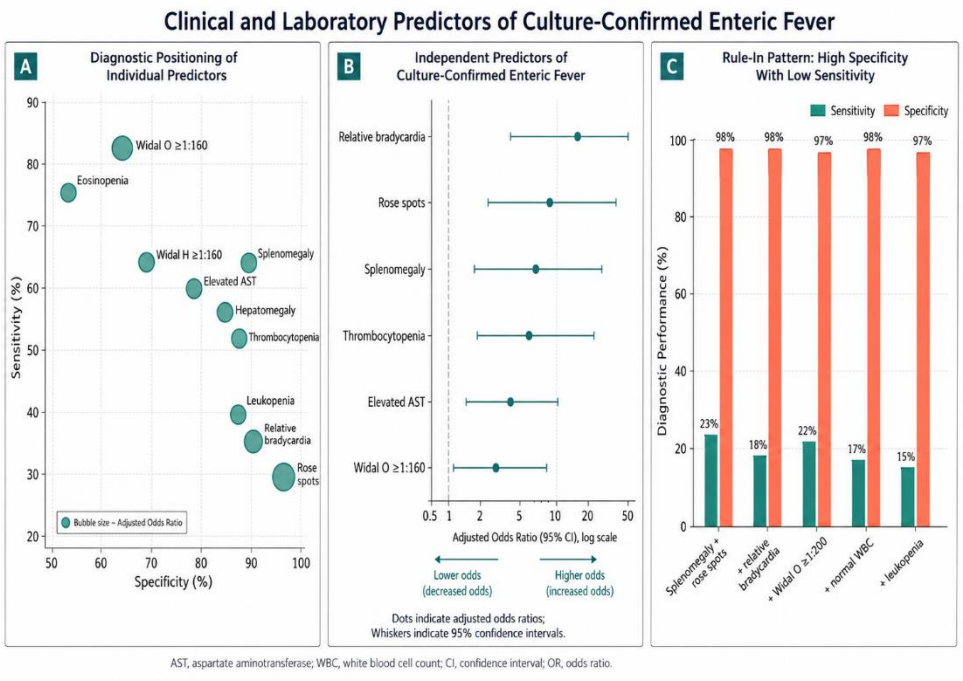

Figure 1: Panel A maps the sensitivity and specificity of individual clinical and laboratory predictors, with bubble size reflecting positive predictive value. Panel B presents adjusted odds ratios with 95% confidence intervals for independent predictors of culture-confirmed enteric fever. Panel C shows that combined clinical-laboratory rule-in patterns maintain very high specificity but markedly reduce sensitivity, indicating usefulness for confirming suspicion rather than screening or exclusion.

DISCUSSION

This retrospective diagnostic case–control study evaluated the predictive value of selected clinical and routine laboratory findings for culture-confirmed enteric fever among patients presenting with prolonged febrile illness. The main finding was that splenomegaly, relative bradycardia, rose spots, thrombocytopenia, elevated aspartate aminotransferase, and Widal O titre ≥1:160 were independently associated with enteric fever. Among these predictors, relative bradycardia showed the strongest independent association, followed by rose spots, splenomegaly, and thrombocytopenia. However, the diagnostic performance analysis demonstrated an important clinical distinction: individual and combined findings generally provided high specificity but insufficient sensitivity, indicating that these parameters may support rule-in decision-making when present but cannot reliably exclude enteric fever when absent.

The predominance of Salmonella Typhi among culture-confirmed cases is consistent with the continuing epidemiological burden of typhoidal salmonellosis in Pakistan and South Asia. Salmonella Paratyphi A also represented a substantial proportion of cases, which is clinically relevant because typhoid conjugate vaccines target Salmonella Typhi but do not provide direct protection against paratyphoid fever. The antimicrobial resistance profile further emphasizes the clinical importance of

accurate early diagnostic stratification. After correction of the denominator, multidrug resistance was present in 66.7% of Salmonella Typhi isolates and extensively drug-resistant phenotype in 19.0%, underscoring the need to reduce unnecessary empirical antibiotic exposure while ensuring that true enteric fever cases receive appropriate therapy. This finding aligns with regional concerns regarding resistant Salmonella Typhi strains and the ongoing challenge of antimicrobial stewardship in endemic settings (5,6).

The clinical findings most strongly associated with enteric fever were biologically plausible and consistent with the established systemic pathophysiology of typhoidal infection. Splenomegaly and hepatomegaly may reflect reticuloendothelial involvement, while rose spots and relative bradycardia are classically described features of enteric fever. In this study, rose spots had the highest specificity among individual predictors, but their low sensitivity limits their practical value as a screening sign. Similarly, relative bradycardia showed a strong adjusted association with enteric fever, but it was present in only a minority of cases. Therefore, these clinical signs should be interpreted as high-value supportive findings when present, rather than expected or required features of enteric fever.

Routine laboratory findings added useful diagnostic information. Thrombocytopenia and elevated aspartate aminotransferase were independently associated with culture-confirmed enteric fever, while leukopenia and eosinopenia were significantly more common in cases on univariate analysis. These findings are clinically relevant because complete blood count and liver enzyme testing are widely available even where blood culture access is limited. Nevertheless, the diagnostic performance of these markers remained imperfect. Eosinopenia and Widal O titre ≥1:160 showed higher sensitivity than several clinical signs, but both had limited specificity. Conversely, leukopenia, thrombocytopenia, and elevated aspartate aminotransferase were more specific but did not achieve sufficient sensitivity to function as standalone diagnostic tools. This pattern supports their use as components of a structured clinical assessment rather than as independent confirmatory or exclusionary tests.

The Widal test remained significantly associated with enteric fever in the multivariable model, but its interpretation requires caution. Although Widal O titre ≥1:160 had the highest individual sensitivity in this study, specificity was modest, and positive titres were also present in a considerable proportion of non-enteric febrile controls. This finding is expected in endemic settings, where prior exposure, background antibody levels, vaccination, and serological cross-reactivity may reduce diagnostic specificity. The inclusion of brucellosis and scrub typhus among controls strengthens the real-world relevance of this observation because these conditions may present with overlapping clinical and laboratory features and can complicate interpretation of serological tests. Therefore, Widal testing should not be used as a standalone diagnostic basis for enteric fever but may provide incremental value when interpreted alongside objective clinical findings and routine laboratory abnormalities (7,8).

The combined diagnostic patterns demonstrated very high specificity but low sensitivity. This means that combinations such as splenomegaly with rose spots, with or without relative bradycardia and supportive laboratory findings, may be useful for increasing confidence in enteric fever when present. However, their low sensitivity means that most culture-confirmed cases would not be captured by these restrictive combinations. Accordingly, the combined findings should be described as rule-in patterns rather than diagnostic algorithms. The present data do not support their use as screening tools, exclusion criteria, or definitive clinical decision rules. Future studies should prospectively evaluate whether these predictors can be incorporated into a validated scoring system or triage algorithm that balances diagnostic accuracy with antimicrobial stewardship.

The model's area under the receiver operating characteristic curve of 0.84 suggests good discrimination between culture-confirmed enteric fever and confirmed non-enteric febrile illnesses in this sample. However, the model should be interpreted cautiously because it was derived from a relatively small, single-center retrospective dataset. The number of predictors in relation to the number of cases may increase the risk of model overfitting, and no external validation cohort was available. In addition,

positive and negative predictive values are sample-specific because the case–control design fixed the proportion of enteric fever cases and non-enteric controls. These values may differ substantially in primary care, emergency department, pediatric, or community settings where disease prevalence and differential diagnoses vary.

This study has several limitations. First, the retrospective design limited control over data completeness, clinical documentation, timing of investigations, and uniform assessment of physical findings such as relative bradycardia and rose spots. Second, the study was conducted at a single tertiary-care hospital, which may limit generalizability to peripheral healthcare settings and community-level febrile illness. Third, although blood culture was used as the reference standard, its sensitivity is affected by blood volume, timing of collection, prior antibiotic exposure, and laboratory processing. Fourth, the control group consisted of microbiologically or serologically confirmed alternative febrile illnesses, which improves diagnostic specificity but may introduce spectrum bias because it does not represent all patients presenting with undifferentiated fever. Fifth, confidence intervals and validation procedures are needed for all diagnostic combinations before they can be recommended for clinical use. Finally, the reported positive and negative predictive values for combined findings require confirmation from original 2 × 2 cross-tabulations before final publication.

Despite these limitations, the study provides clinically useful evidence from a Pakistani setting where enteric fever remains endemic and antimicrobial resistance is a major therapeutic concern. The findings support a pragmatic diagnostic interpretation: classic clinical signs and routine laboratory abnormalities can increase suspicion of enteric fever, especially when high-specificity findings coexist, but they cannot replace microbiological confirmation. Prospective multicenter validation is needed to determine whether these predictors can be converted into a reliable diagnostic score or rule-in tool for resource-limited settings.

CONCLUSION

In this retrospective diagnostic case–control study, splenomegaly, relative bradycardia, rose spots, thrombocytopenia, elevated aspartate aminotransferase, and Widal O titre ≥1:160 were independently associated with culture-confirmed enteric fever. Individual and combined clinical-laboratory findings showed useful rule-in value because of their high specificity, but their low sensitivity limits their ability to exclude enteric fever when absent. Widal testing contributed adjunctive diagnostic information but remained insufficient as a standalone test in an endemic setting with microbiologically confirmed alternative febrile illnesses. These findings support the use of structured clinical and laboratory risk stratification while awaiting culture confirmation, but prospective multicenter validation is required before these predictors can be adopted as a formal diagnostic algorithm.

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