

# Diagnosis of Enteric Fever

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## ABSTRACT

Enteric fever is a community-acquired generalized bacteremic infection of the tropical countries where health infrastructure and hygienic conditions are compromised to a great extent due to poor public health facilities and low socioeconomic status. It is associated with the involvement of the reticuloendothelial system, intestinal lymphoid tissue, and even the gallbladder. Ever-increasing antimicrobial resistance, increasing number of cases in younger children, and increasing paratyphoid cases are the current challenges. Though the management of typhoid fever is straight forward, it poses a number of diagnostic challenges and dilemma due to many reasons which include lack of sensitivity and specificity of clinical features and CBC parameters, overreliance on serology despite its limitations, non-feasibility of culture methods, underutilization of blood culture despite its availability, and poor yield due to prior antibiotic therapy. Classic presentation may not be always there. CBC has more of a negative predictive value. Blood culture is the gold standard but may not always be feasible and results are available by day 4. A rapid, point-of-care test with very good sensitivity and specificity is needed to detect acute cases as well as carriers.

**Keywords:** Blood culture, CBC, Serological tests, Typhoid.

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## INTRODUCTION

Enteric fever is a community-acquired generalized bacteremic infection of the tropical countries where health infrastructure and hygienic conditions are compromised to a great extent due to poor public health facilities and low socioeconomic status.

It is caused by bacteria named *Salmonella Typhi*, *S. Paratyphi A*, *S. Paratyphi B*, and *S. Paratyphi C*. India including South-Central Asia has an incidence setting of roughly 622 cases/100,000/per year suggesting to be in a high incidence setting group. India carries high disease burden with 30–40% of global burden having 7–9 million cases per year with 500–700 cases per 1 lakh population/year.<sup>1</sup> Ever-increasing antimicrobial resistance, increasing number of cases in younger children, and increasing paratyphoid cases are the current challenges.<sup>2,3</sup> Current epidemiologic profile suggests that children between 5 years and 15 years are the most vulnerable group and increasing incidence of typhoid fever is seen even in younger age groups which are more prone to severe disease, complications, and hospitalization. Children below the age of 4 years contribute nearly more than a quarter number of cases.<sup>4</sup>

Though the management of typhoid fever is straight forward it poses several diagnostic challenges and dilemmas due to many reasons which include lack of sensitivity and specificity of clinical features and CBC parameters, overreliance on serology despite its limitations, non-feasibility of culture methods, underutilization of blood culture despite its availability, and poor yield due to prior antibiotic therapy.

## DIAGNOSIS

It mainly depends upon history, clinical findings, and laboratory investigations.

## CLINICAL FEATURES

Typhoid should be suspected in any child living in a typhoid endemic area presenting with fever without focus, with or without toxemia, and/or other findings. The typical clinical

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features are malaise, fever, with or without chills with weakness poor oral intake and even abdominal pain. Fever is the most consistent presentation of typhoid fever. The onset of fever is gradual, initially, the fever is low grade, then rises progressively in a stepwise manner and by the second week it is often high and sustained at 39–40°C (102–104°F). During convalescence fever diminishes also in stepwise fashion over several days. In most cases, there is no definite pattern of fever, more so in pediatric patients. Large number of cases will have abdominal symptoms with fever such as abdominal pain, anorexia, vomiting, and sometimes diarrhea. Though diarrhea is common in typhoid fever it usually does not cause dehydration. During the second week of illness, high fever is sustained, and prostrations, fatigue, anorexia, cough and abdominal symptoms like distension, tenderness and gurgling (typhlitis) increase in severity. Abdominal symptoms are more common in children. Constipation is more likely in older children and in later course of disease. Hepatosplenomegaly and coated tongue usually are late features and associated with systemic toxemia. In the second week, the symptoms become more severe with toxicity, lethargy, and at times delirium or coma may supervene. A coated tongue, tender abdomen, anemia, and hepatosplenomegaly are common. Relative bradycardia is manifested in older children and adults but is not a consistent finding. Rose spots, which are blanching, erythematous papules may appear on 7–10 days in 10–20% of individuals. If the course remains uncomplicated, symptoms start abating by 2–4 weeks,

although lethargy and malaise may persist for 1–2 months. Erosion of blood vessels may cause intestinal hemorrhage and extension of necrosis through the bowel wall may result in perforation. Complications occur in 10–15% of patients and usually seen in second and third week of the infection. Relapse rates are 5–20% and occur 2–3 weeks after the fever subsides or stopping antibiotics. It mimics the original illness except that it is milder and has a shorter duration. The morbidity and toxemia associated with MDR strain is higher in children due to greater virulence of the organisms.

In neonates, the clinical manifestations begin within 72 hours presenting either with hypothermia or with hyperthermia, vomiting, diarrhea, abdominal distention, seizures, jaundice, hepatomegaly, and failure to thrive. Infection is also transmitted vertically.

## LABORATORY DIAGNOSIS

### Hematological

Complete blood counts are non-specific, often useful to make alternative diagnosis and is more of a negative predictive value. Usually Hb is unaffected. However, a drop in HB indicates prolonged toxemia, poor oral intake, and some degree of bone marrow suppression. Acute and significant fall in Hb may be due to complications like GI hemorrhage, hemolysis, or even hemophagocytosis.

Anemia because of the disease per se is rare but may be present some time as a late feature. If there is significant anemia, search for alternative diagnoses like malaria.

Leukopenia is seen in only 20–25% of cases and hence absence of leukopenia does not rule out typhoid fever. There is relative lymphopenia and absence of eosinophils is quite characteristic. Though eosinopenia is described as an independent predictor of enteric fever for a long, it is found only in 30–50% of cases as per recent studies.<sup>5,6</sup> Absence of eosinopenia does not rule out typhoid fever but is a useful marker to differentiate viral and bacterial fevers.

The presence of normal WBC count or leukocytosis with or without eosinopenia does not rule out typhoid fever.

Thrombocytopenia usually occurs by the end of the first week of illness. Association of fever with thrombocytopenia in typhoid fever is important in our country where various viral infections like dengue and scrub typhus are common and present with overlapping complaints. Transient thrombocytopenia may be seen in 10–15% cases after 1 week.

### Biochemical

There is a mild elevation of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) but seldom exceeds 2–3 times the normal levels. Serum bilirubin may be mildly elevated especially in infants and younger children. Prothrombin time and activated partial thromboplastin time (APTT) are also mildly prolonged. Normal liver enzymes levels are seen in many cases and do not rule out typhoid fever. Typhoid hepatitis is not uncommon and closely mimics an anicteric form of viral hepatitis. Serum ALT:LDH ratio is <9 in typhoid hepatitis and >9 in acute viral hepatitis in children.<sup>7</sup>

### C-reactive Protein

It is advised as a part of workup in all cases of fever without focus, fever with toxemia, and prolonged pyrexia. It provides a useful

marker for bacterial infection and is not specific for any infection including typhoid fever.

## CULTURES

### Blood Culture

Patients with typhoid or paratyphoid fever have bacteremia. Hence, blood culture is the gold standard for the diagnosis of typhoid and paratyphoid fever. *Salmonella* are the easiest to grow. Blood culture diagnostic sensitivity is characterized by heterogeneity, ranging between 40% and 87%<sup>8</sup> with an average sensitivity of about 50% and drops considerably with prior antibiotic treatment and duration of illness. The highest yield is in the first week of illness (90%). Thereafter, it is 75% in second week, 60% in third week, and 25% in fourth week.<sup>9</sup> The sample should be inoculated immediately at the time of withdrawing the blood. In case the sample has to be transported, it should be done at the room temperature. Since blood contains about 0.3 cfu/mL of bacteria, the optimum volume of blood for culture is 10 mL in adults and 5 mL in children to maintain a blood broth ratio of 1:5 to 1:10.<sup>9</sup> It is estimated that sensitivity of blood culture as a typhoid diagnostic method increases from 51% for 2 mL of blood to 65% for 10 mL, or by 3% for each additional milliliter.<sup>10</sup> Table 1 represents guide to volumes of blood to be collected for blood culture according to age of the patient.

An automated blood culture system (Bactec) enhances the recovery rate of bacteria. Poor yield in blood culture may be due to various factors like inadequate laboratory media, the inadequate volume of blood taken for culture, prior use of antibiotics, and the time of blood collection. Earlier, it was thought that clot cultures may be better as they obviate the inhibitory effect of serum, but it has now been found that there is no distinct advantage.

Bone marrow aspiration culture: Since *Salmonella* is predominantly an intracellular organism, bone marrow cultures have a very high yield with sensitivity between 80% and 95%. Furthermore, it has the advantage that it also provides definitive diagnosis for patients who have already received antibiotics, have a long history of illness, and negative blood culture with the recommended volume of blood. The concentration of bacteria in bone marrow is 5 times (9 cfu/mL) than that of peripheral blood in the first week and rising to 150 times in the third week. While bone marrow culture has been shown to be approximately 50% more sensitive than blood culture, it is an invasive procedure that is impractical in most of the clinical settings.<sup>11</sup> Hence, this modality makes it more suitable for investigating pyrexia unknown origin (PUO) cases, where typhoid is a frequent diagnosis. Whenever

**Table 1:** Age of the patient and volume of blood recommended for blood culture

Patient age	Blood volume for culture bottles containing 40 mL of broth (pediatric)	Blood volume for culture bottles containing 80 mL of broth (adult)
3 months <2 years	1–2 mL	–
2 years – <5 years	2–3 mL	
5 < 15 years	5–10 mL	
Adult (> 15 years)	–	8–10 mL

Adapted from WHO vaccine-preventable diseases surveillance standards



bone marrow aspiration is performed due to any reason, please remember to send it for culture as well.

### Stool Culture

Stool culture is not recommended for the diagnosis of acute enteric fever. Stool culture is positive in 30% of cases and that too only during the second and third week. A brief period of asymptomatic fecal shedding occurs following *Salmonella* infections; and some of these patients will progress to long-term, asymptomatic carriers. A stool culture may thus be used for the detection of chronic carriers and to monitor fecal shedding in patients following acute typhoid fever. Because of irregular shedding, several stool specimens are required. It needs to be processed within 2 hours after collection, or can be stored at 4°C (in case of delay) in refrigerator. Rectal swab culture has still poorer sensitivity.

### Urine Culture

It is also not recommended because of poor sensitivity.

Skin snip cultures from rose spots: This also has a high yield and has shown positivity in 63% of cases. However, it is not very much in practice.

## ANTIMICROBIAL SENSITIVITY TESTING

Antimicrobial sensitivity testing is one of the most important advantages for sending blood culture. It will help reduce empiric antibiotic therapy. Second, in resource-restricted areas where culture facilities are not available, such type of antibiogram will be used to guide antibiotic therapy in suspected and clinically diagnosed cases. *Salmonella* Typhi and *S. Paratyphi* A should be tested for their susceptibility to ampicillin, chloramphenicol, co-trimoxazole, nalidixic acid, ciprofloxacin or pefloxacin, ceftriaxone, and azithromycin. This panel may be expanded based on local resistance patterns or prescribing practices. There is an increase in MIC of quinolones and are associated with a high incidence of clinical failure. The current MIC for quinolones are still below the National Committee for Clinical Laboratory Standards (NCCLS) susceptibility breakpoint, and accordingly laboratory report will report as *Salmonella* to be quinolone sensitive. So, nalidixic acid resistance is used as a surrogate marker for the high MIC of quinolones. Recently, increase in MICs to ceftriaxone in India has been reported since recent past but still, as of now, ceftriaxone and azithromycin have regained their sensitivity against *Salmonella* serovars. It is important to note the issues of *in vivo* and *in vitro* mismatch. Typhoid bacilli are intracellular organisms and hence aminoglycosides are not going to work despite their apparent *in vitro* susceptibility.

### Serological Tests

Although serological tests are commonly used in many settings, current evidence suggests that these tests are limited by their poor sensitivity and specificity, and so are inappropriate.<sup>12</sup>

#### Widal Test

This has been a widely used and abused test. It has many limitations. It is a serological test that measures agglutinating antibody levels against "O" and "H" antigens. O antibodies which are predominantly immunoglobulin M (IgM) appear usually on the sixth to eighth day and "H" antibodies which are IgM and immunoglobulin G (IgG) appear on the tenth to twelfth day after the onset of disease. A high titer in the first sample collected at the end of the first week

is highly suggested. A rising titer in a paired sample taken 2 weeks apart is also diagnostic but by that time the clinical tests become rather irrelevant to a clinician. Currently, available typhoid vaccines do not affect the Widal test. The antibody titer of both (and not either) "H" and "O" antibodies in the range of 1 in 160 dilutions are taken as significant cut-offs.

### LIMITATIONS

- In endemic countries like India, there exists a baseline antibody level in the population following repeated exposures to *Salmonella* infection; hence, interpretation of Widal titers becomes difficult unless these baseline values are known.
- Due to the previous antimicrobial treatment Widal be falsely negative.
- *S. Typhi* shares "O" and "H" antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriaceae, so a false-positive test may occur.
- Widal test may also be false-positive in malaria, typhus, sepsis with other organisms, cirrhosis, etc.
- Widal test is moderately sensitive and specific. Even in blood culture-proven cases, it may be negative in nearly 30% of cases.

There are two methods of doing the Widal test: (1) the tube test and (2) the slide test. The tube test takes 6 hours while the slide test takes 2–5 minutes. However, the tube test is far better than the slide test and should be preferred over the slide test. The tube test can detect the antibody in the concentration of 1:1,280 while the slide test can detect it only up to 1:320 concentration.

As a general protocol, the blood culture is the modality of choice and serological tests are to be avoided as far as possible.

### Typhi Dot/Enzyme Immunoassay Test (EIA)

It is based on the detection of both IgM and IgG against a 50 kDa outer membrane protein antigen of *S. Typhi*. It is a simple, rapid, economic test with high specificity, sensitivity. However, in endemic areas because of a persistent high IgG level following typhoid infection, this test cannot differentiate between acute and convalescent cases. It may also give false-positive results because of previous infection with *S. Typhi* or current reinfection in which secondary immune response significantly boosts IgG production and IgM becomes undetectable. To increase the diagnostic efficacy, modified typhidot has been developed, called "Typhidot M" in which IgG is totally inactivated in the serum sample and only IgM is detected. However, this test is not available in India.

### IgM Strip/Dipstick Test

This test is the most commonly used currently and has replaced the Widal and Typhidot tests. It is based on the binding of *S. Typhi* specific IgM antibodies to *S. Typhi* lipopolysaccharide antigen (LPS). It can be done on serum or whole blood and requires incubation for 3 hours at room temperature. Evaluation studies suggest that this method is 65–77% sensitive and 95–100% specific. It is a rapid and simple alternative for the diagnosis of typhoid fever where culture facilities are not available. Moreover, this test does not require trained persons or equipment.

### IDL TUBEX Test

It is a rapid and simple test that can detect IgM antibodies against O9 antigen specifically found in group D *Salmonella* within 2 minutes. It detects IgM and not IgG and is thus suggestive of recent infection.

It is not positive with other serotypes including *S. Paratyphi* and thus has good specificity. A study showed that in a single blood sample collected on admission to hospital sensitivity of TUBEX test was 69.8% as compared with bone marrow culture and 86.5% as compared with blood culture.

Table 2 describes the limitations of serological tests and its implications in clinical practice.

Rapid serological tests correlate poorly with blood culture results. They are far inferior to blood culture. However, such tests may be useful for rapidly diagnosing typhoid fever in emergencies—e.g., during outbreaks, when pretest probability would be high. The Widal test has several limitations and even if it is requested for in the second week of the illness, it loses its clinical relevance.

**Antigen Detection Tests**

Using counter immune electrophoresis, agglutination and immunoassay techniques to detect *Salmonella* somatic/flagellin/Vi antigen have been developed. They are unable to detect *Salmonella* Paratyphi infection and Vi antigen negative strains of *S. Typhi* and have variable sensitivity and specificity.

**Polymerase Chain Reaction (PCR)<sup>13</sup>**

This is a modern modality and may not be feasible for routine use. It is highly sensitive and specific for the early and reliable diagnosis. Nested PCR (N-PCR) and Q-PCR provide better yield. Nested PCR is more sensitive and can detect even 3–5 bacilli [flagellin (fliC) gene]. The sensitivity was found to be 100% whereas the specificity was 76.9% (76% in the first week of illness and 54% in the second week).<sup>14</sup>

Table 3 describes a summary of laboratory tests and important remarks for the commonly performed tests in the diagnosis of typhoid fever.

**SUMMARY**

Enteric fever continues to be a major health problem in our settings due to the lack of safe drinking water and poor sanitation facilities. It is one disease where treatment is straightforward but has a great diagnostic challenge. The classic presentation may not be always there. CBC has more of a negative predictive value. Blood culture is the gold standard but may not always be feasible and

results are available by day 4. Serological tests and rapid tests are widely ordered despite their manifold limitations. An ideal test should be rapid, specific as well as sensitive for detection of true infection and carrier stage. Coagglutination tests have also been used for the detection of antigens in urine and serum. Recently, DNA probes for the detection of *S. Typhi* in blood are also been an area of research. However, all these newer modalities have not obtained significant acceptance. As we move ahead for a possible typhoid elimination with the use of improved infrastructure and typhoid conjugate vaccine, we need to reconsider the role of newer typhoid diagnostics.

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**Table 3:** Summary of laboratory tests and remarks

	Test		Remarks
CBC	Normal or low leukocyte count with eosinopenia Thrombocytopenia in some cases at the end of the first week of illness	Highly non-specific Leukocytosis and eosinophilia do not rule out typhoid	Helps in the evaluation of alternative diagnoses such as malaria, dengue, and other cases of bacteremia
Blood culture	Gold standard	Highly specific Gives antibiotic susceptibility too	Must be sent always before antibiotic Cost-effective in the long run Longer turnaround time
Bone marrow culture	Highly sensitive Sensitivity more than blood culture	Useful diagnostic tests even in late stages of the illness even with prior antibiotic therapy	Invasive and impractical to offer. Should be performed in all patients with prolonged pyrexia if routine investigations have failed to establish a diagnosis
Widal test	Wide range of limitations	False-positive and false-negatives	Not to be recommended Far inferior to blood culture
Rapid test	Not standardized yet	False-positive in endemic areas. Does not differentiate past infection, carrier, recent infection	Point-of-care test during outbreaks only Positive results to be confirmed by blood culture
CRP	As a workup for FWS/FUO		

**Table 2:** Limitations of serological tests and its implications in clinical practice

- Serological tests for typhoid fevers are reported to be non-specific and confusing.
- They are poorly standardized.
- They have more negative predictive value than positive predictive value.
- Positive tests in endemic areas do not confirm the diagnosis.
- Positive tests to be correlated clinically and confirmed by culture.
- If a clinical scenario is suggestive of typhoid fever:
  - Start antibiotic after sending blood C/S.
  - Stop if an alternative diagnosis available.
  - If culture is negative, alternative diagnosis is not available and the child is febrile and toxic, continue to treat as a clinically suspected enteric fever and continue your attempts for diagnosis.



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