



COMPARATIVE ASSESSMENT OF BLOOD CULTURE, WIDAL TEST, TYPHIDOT, AND PCR IN THE DIAGNOSIS OF TYPHOID FEVER.

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Abstract

Typhoid fever remains a major public health concern in developing countries due to inadequate sanitation, contaminated water supplies, and delayed diagnosis. Accurate and early detection of *Salmonella Typhi* infection is essential for effective treatment and prevention of complications. This study aims to comparatively assess the diagnostic efficiency of Blood Culture, Widal Test, TyphiDot, and Polymerase Chain Reaction (PCR) in the diagnosis of typhoid fever. Blood culture is traditionally considered the gold standard; however, it is time-consuming and affected by prior antibiotic use. Rapid serological tests such as Widal and TyphiDot offer faster results but often show variable sensitivity and specificity. PCR-based diagnosis provides higher accuracy through molecular detection of bacterial DNA. The study evaluates these methods based on diagnostic accuracy, turnaround time, reliability, and clinical applicability to identify the most effective diagnostic approach for early and accurate detection of typhoid fever.

Keywords: Typhoid Fever; *Salmonella Typhi*; Blood Culture; Widal Test; TyphiDot; PCR; Diagnostic Accuracy; Infectious Diseases.

1. Introduction

1.1 Background of Typhoid Fever

Typhoid fever is a serious systemic infectious disease caused mainly by *Salmonella enterica* serovar Typhi, a Gram-negative bacterium that spreads through contaminated food and drinking water. The disease continues to be a major public health concern, particularly in developing and underdeveloped countries where

sanitation and hygiene facilities remain inadequate. Even though medical science and antimicrobial therapies have advanced significantly over the years, typhoid fever still affects millions of people annually across the world. The burden of the disease is especially high in densely populated regions of South Asia, Southeast Asia, and sub-Saharan Africa, where access to safe drinking water and proper sewage disposal systems is limited (Crump & Mintz, 2010). These unfavorable environmental and socioeconomic conditions create ideal circumstances for the continuous spread of infection within communities.

The impact of typhoid fever is more severe in low- and middle-income countries because of overcrowding, poor hygiene practices, malnutrition, and limited healthcare infrastructure. In many endemic areas, the disease contributes significantly to hospital admissions, loss of productivity, economic burden, and increased healthcare expenses. Children, adolescents, and young adults are among the most vulnerable groups due to their higher exposure to contaminated environments and relatively weaker immunity. Rural populations and urban slum dwellers often face a greater risk because of inadequate sanitation facilities and limited awareness regarding personal hygiene. In addition, seasonal changes such as heavy rainfall and flooding may contaminate water supplies and further increase transmission rates, leading to periodic outbreaks in affected communities (Parry et al., 2002).

Typhoid fever remains a major medical concern not only because of its widespread prevalence but also because delayed diagnosis and improper treatment can result in serious

complications and increased mortality. If not identified at an early stage, the disease may progress to severe systemic infections affecting multiple organs. Furthermore, the growing emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Salmonella Typhi* has made treatment more difficult and complicated in many regions. These challenges highlight the urgent need for rapid, reliable, and accurate diagnostic methods that can support early clinical intervention and reduce disease transmission. Therefore, improving diagnostic strategies is considered an essential step toward better patient management, effective disease surveillance, and overall public health control.

1.2 Etiology and Transmission

Typhoid fever is caused by *Salmonella enterica* serovar Typhi, commonly known as *Salmonella Typhi*, which is a facultative intracellular Gram-negative bacterium that infects only humans. After entering the body through contaminated food or water, the organism survives the acidic environment of the stomach and reaches the intestinal tract, where it penetrates the intestinal mucosa. The bacteria are then engulfed by macrophages and transported through the lymphatic and reticuloendothelial systems, eventually entering the bloodstream and causing systemic infection (Wain et al., 2015). This ability to survive within host cells allows the pathogen to evade immune defenses and spread throughout the body. The infection gradually affects multiple organs, resulting in prolonged fever and other systemic manifestations associated with typhoid fever.

The primary mode of transmission of typhoid fever is the fecal–oral route, which occurs through the consumption of food or water contaminated with human feces containing *Salmonella Typhi*. Poor sanitation systems, open defecation, unsafe sewage disposal, and inadequate access to clean drinking water are major contributors to the spread of the disease. Food contamination may also occur through improper handling by infected individuals or carriers who continue to shed bacteria without showing symptoms. Chronic carriers, especially those with bacterial colonization in the gallbladder, play an important role in maintaining transmission within endemic communities. In many developing regions,

street-vended food and untreated water sources further increase the risk of exposure and infection among the general population.

Several environmental and socioeconomic factors significantly increase susceptibility to typhoid fever. Lack of personal hygiene, overcrowded living conditions, malnutrition, poverty, and limited healthcare awareness are among the major risk factors associated with the disease. Individuals traveling to endemic regions are also at a higher risk, particularly when proper food and water precautions are not followed. Populations affected by natural disasters, displacement, or conflict situations often experience outbreaks because of disrupted sanitation systems and contaminated water supplies. Seasonal variations, especially during monsoon periods in tropical countries, frequently lead to water contamination and increased bacterial transmission. Therefore, improving sanitation, ensuring access to safe drinking water, and promoting hygiene education are essential measures for reducing the spread of typhoid fever.

1.3 Clinical Manifestations

Typhoid fever is characterized by a wide range of clinical manifestations that may vary from mild febrile illness to severe life-threatening systemic complications. The disease generally develops gradually after an incubation period of approximately one to two weeks following exposure to the bacteria. Persistent high-grade fever is considered the most common and characteristic symptom of typhoid fever. Patients often experience progressive weakness, fatigue, headache, body aches, malaise, and loss of appetite during the early stages of infection. As the disease progresses, the fever may become continuous and may remain elevated for several days if proper treatment is not initiated. These nonspecific symptoms frequently make the initial clinical diagnosis difficult, particularly in regions where other febrile illnesses are also common.

Gastrointestinal symptoms are commonly observed in patients suffering from typhoid fever and may include abdominal discomfort, nausea, vomiting, constipation, or diarrhea. Some individuals may develop abdominal distension and tenderness due to intestinal inflammation. Physical examination may reveal

hepatomegaly, splenomegaly, and a coated tongue, which are often considered supportive clinical findings. In certain cases, small pink-colored skin lesions known as “rose spots” may appear on the trunk, although they are less commonly reported in endemic regions. Since the clinical presentation of typhoid fever closely resembles diseases such as malaria, dengue, leptospirosis, and viral fever, laboratory confirmation becomes extremely important for accurate diagnosis and appropriate treatment (House et al., 2001).

If typhoid fever is not diagnosed and treated at an early stage, it may lead to serious complications involving different organ systems. Severe cases can result in intestinal hemorrhage, intestinal perforation, encephalopathy, myocarditis, septic shock, and multiple organ dysfunction. These complications are associated with increased morbidity and mortality, especially in resource-limited healthcare settings where timely medical intervention may not be available. In some individuals, chronic carriage of *Salmonella Typhi* may develop, leading to persistent bacterial shedding and continued transmission of infection within the community. Therefore, early recognition of symptoms, prompt laboratory diagnosis, and timely antimicrobial therapy are crucial for preventing complications and reducing the overall disease burden associated with typhoid fever.

1.4 Importance of Early and Accurate Diagnosis

Early and accurate diagnosis of typhoid fever plays a crucial role in effective disease management and prevention of severe complications. Since the clinical symptoms of typhoid fever are often nonspecific and closely resemble several other infectious diseases, laboratory confirmation becomes essential for establishing an accurate diagnosis. Delayed diagnosis may result in prolonged illness, inappropriate treatment, and increased risk of transmission within the community. In many endemic regions, patients presenting with persistent fever are frequently treated empirically without proper diagnostic testing, which may lead to misdiagnosis and treatment failure. Therefore, reliable diagnostic methods are necessary to differentiate typhoid fever from other febrile illnesses and to ensure timely

initiation of appropriate antimicrobial therapy for better clinical outcomes.

Accurate diagnosis is also important for controlling the growing problem of antimicrobial resistance associated with *Salmonella Typhi*. In several developing countries, the excessive and irrational use of antibiotics has contributed to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Reliable laboratory diagnostic techniques help clinicians select suitable antimicrobial agents based on confirmed infection rather than empirical assumptions. This reduces unnecessary antibiotic exposure and supports antibiotic stewardship programs aimed at minimizing resistance development. Furthermore, appropriate diagnosis prevents the misuse of broad-spectrum antibiotics that may otherwise contribute to increasing healthcare costs and treatment complications. Hence, diagnostic accuracy has become increasingly important in regions where resistance patterns are rapidly evolving (Parry et al., 2002).

In addition to improving patient management, early diagnosis of typhoid fever also has significant public health benefits. Rapid identification of infected individuals enables timely treatment and isolation measures, thereby reducing the risk of disease transmission and outbreak occurrence. Accurate diagnostic surveillance helps healthcare authorities monitor disease prevalence and implement effective prevention and control strategies in endemic areas. Early detection is particularly valuable in resource-limited settings where delayed intervention may increase morbidity and mortality rates. Moreover, prompt diagnosis minimizes hospitalization duration and reduces the economic burden on patients and healthcare systems. Therefore, strengthening diagnostic capabilities remains a vital component in the overall prevention, treatment, and control of typhoid fever worldwide.

1.5 Overview of Diagnostic Techniques

A variety of laboratory diagnostic methods are currently available for the detection of typhoid fever, and each technique possesses specific advantages as well as limitations. Blood culture is traditionally regarded as the gold standard

method because it directly isolates Salmonella Typhi from the patient's bloodstream. The method provides high specificity and allows confirmation of active infection along with antimicrobial susceptibility testing. However, blood culture requires proper laboratory infrastructure, trained personnel, and prolonged incubation time before results become available. In addition, prior antibiotic therapy may significantly reduce bacterial growth, thereby decreasing the sensitivity of the test. Despite these limitations, blood culture continues to be an important diagnostic tool in clinical microbiology laboratories for confirming typhoid fever cases and guiding appropriate treatment strategies.

The Widal test is one of the oldest and most widely used serological methods for diagnosing typhoid fever, particularly in developing countries because of its low cost and easy availability. The test detects antibodies against the O and H antigens of Salmonella Typhi in the patient's serum. Although the Widal test is simple and inexpensive, its diagnostic reliability remains controversial due to poor specificity and frequent cross-reactivity with other infections. Previous vaccination, past exposure, and varying baseline antibody titers in endemic regions may also produce misleading results (Bhutta & Mansurali, 1999). Consequently, interpretation of Widal test results often becomes difficult, especially in areas where typhoid fever is highly prevalent and repeated exposure to the pathogen is common among the population.

TyphiDot and Polymerase Chain Reaction (PCR) are more advanced diagnostic approaches that have gained attention because of their improved speed and accuracy. TyphiDot is a rapid immunochromatographic assay designed to detect specific IgM and IgG antibodies against Salmonella Typhi. Compared to conventional serological tests, it offers faster results and relatively better sensitivity; however, false-positive reactions and difficulty distinguishing between current and past infections remain major concerns. PCR, on the other hand, is a molecular technique that identifies bacterial DNA with high sensitivity and specificity, even in patients with low bacterial load or prior antibiotic use. Despite its diagnostic superiority, PCR is expensive and

requires sophisticated laboratory facilities and technical expertise, limiting its routine use in resource-constrained healthcare settings.

1.6 Research Gap

Although several diagnostic techniques are available for the detection of typhoid fever, considerable differences exist in their sensitivity, specificity, affordability, accessibility, and clinical reliability. Blood culture is widely recognized as the gold standard diagnostic method because it confirms the presence of Salmonella Typhi directly from the bloodstream. However, the procedure is time-consuming and its sensitivity may decrease significantly in patients who have already received antibiotic therapy before sample collection. Similarly, serological methods such as the Widal test and TyphiDot provide comparatively rapid results, but their diagnostic performance often varies across different populations and endemic settings. These inconsistencies create uncertainty regarding the most suitable diagnostic approach for early and accurate detection of typhoid fever in routine clinical practice.

Many previous studies have focused on evaluating individual diagnostic techniques separately rather than comparing multiple methods simultaneously under similar laboratory conditions. As a result, there remains limited comprehensive evidence regarding the comparative effectiveness of Blood Culture, Widal Test, TyphiDot, and PCR in a single clinical setting. Furthermore, most available studies emphasize sensitivity and specificity while giving less attention to important practical aspects such as turnaround time, cost-effectiveness, technical requirements, and applicability in resource-limited healthcare environments. Since healthcare facilities in developing countries often face infrastructural and financial limitations, understanding the practical utility of each diagnostic method becomes essential for effective disease management and patient care.

Another important gap identified in the existing literature is the lack of standardized comparative analysis that can assist clinicians in selecting the most reliable and feasible diagnostic technique according to clinical and laboratory conditions. While PCR has demonstrated superior sensitivity and specificity

in several studies, its high cost and technical complexity limit its widespread implementation in low-resource settings. On the other hand, rapid serological methods are easier to perform but may compromise diagnostic accuracy. Therefore, a systematic comparative assessment of Blood Culture, Widal Test, TyphiDot, and PCR is necessary to determine the most efficient, reliable, and clinically applicable diagnostic strategy for typhoid fever, particularly in endemic and resource-constrained regions.

1.7 Objectives of the Study

The present study aims to comparatively assess the diagnostic efficiency of Blood Culture, Widal Test, TyphiDot, and PCR in the diagnosis of typhoid fever.

Specific Objectives

1. To compare the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and PCR.
2. To evaluate the sensitivity, specificity, cost-effectiveness, and turnaround time of each diagnostic method.
3. To identify the most reliable and clinically applicable diagnostic approach for early detection of typhoid fever.
4. To analyse the advantages and limitations associated with each diagnostic technique in resource-limited healthcare settings.

2. Review of Literature

2.1 Historical Development of Typhoid Diagnosis

The diagnosis of typhoid fever has undergone remarkable transformation over the past century with the advancement of microbiological, immunological, and molecular diagnostic technologies. In the early stages of medical practice, typhoid fever was diagnosed mainly on the basis of clinical symptoms such as prolonged fever, weakness, abdominal discomfort, headache, and gastrointestinal disturbances. However, these manifestations were nonspecific and often resembled several other infectious diseases including malaria, dengue, and tuberculosis. As a result, physicians frequently faced difficulty in distinguishing typhoid fever from other febrile illnesses, particularly in endemic regions where multiple infections coexisted. The absence of reliable laboratory diagnostic tools during that period contributed to delayed treatment, increased

complications, and high mortality rates associated with the disease.

A major breakthrough in typhoid diagnosis occurred during the late nineteenth century with the introduction of bacteriological culture techniques. Blood culture gradually became recognized as the most reliable laboratory method because it enabled direct isolation and identification of *Salmonella Typhi* from the bloodstream of infected patients. Improvements in culture media, incubation methods, and biochemical identification techniques further enhanced diagnostic accuracy over time. Despite its high specificity, blood culture required sophisticated laboratory infrastructure, trained personnel, and prolonged incubation periods, limiting its accessibility in many low-resource healthcare settings. Nevertheless, it established the foundation for laboratory confirmation of typhoid fever and remained an important reference method for clinical diagnosis and epidemiological investigations in both developed and developing countries.

The development of serological and molecular diagnostic techniques further revolutionized typhoid fever detection. The Widal agglutination test, introduced by Georges-Fernand Widal in 1896, became one of the earliest and most widely used serological tests because of its simplicity, affordability, and minimal laboratory requirements. Later, rapid immunological assays such as TyphiDot were introduced to improve speed and convenience in clinical settings. More recently, molecular methods such as Polymerase Chain Reaction (PCR) have significantly improved diagnostic sensitivity and specificity by enabling direct detection of bacterial DNA. These advancements reflect continuous scientific efforts to overcome the limitations of conventional diagnostic methods and to achieve early, rapid, and accurate detection of typhoid fever (Wain et al., 2015).

2.2 Blood Culture as Gold Standard

Blood culture is widely considered the gold standard method for laboratory confirmation of typhoid fever because it directly detects viable *Salmonella Typhi* organisms in the bloodstream. The procedure involves collecting a blood sample from the patient and inoculating it into specialized culture media that support bacterial

growth. Once bacterial colonies develop, further biochemical and microbiological tests are performed for identification and confirmation of the pathogen. The major strength of blood culture lies in its high specificity, as the direct isolation of *Salmonella Typhi* provides definitive evidence of active infection. For this reason, blood culture has remained an essential diagnostic technique in clinical microbiology laboratories and is frequently used as a reference standard in comparative diagnostic studies and epidemiological investigations.

One of the most important advantages of blood culture is its ability to determine antimicrobial susceptibility patterns of isolated bacterial strains. This capability has become increasingly valuable due to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Salmonella Typhi* in several endemic regions. By identifying resistance profiles, clinicians are able to select appropriate antibiotic therapy and reduce the risk of treatment failure. In addition, blood culture contributes significantly to disease surveillance and public health monitoring by helping researchers track resistance trends and outbreak patterns. Therefore, despite technological advancements in diagnostic medicine, blood culture continues to play a central role in the management and control of typhoid fever worldwide.

Despite its clinical importance, blood culture possesses several practical limitations that affect its routine applicability, particularly in resource-constrained healthcare settings. The sensitivity of the test may vary depending on the stage of illness, bacterial load, blood volume collected, and prior antibiotic exposure. Many patients in endemic regions receive empirical antibiotic treatment before laboratory testing, which suppresses bacterial growth and often results in false-negative findings. Furthermore, blood culture requires specialized laboratory equipment, skilled technical personnel, and prolonged incubation periods ranging from 48 to 72 hours or longer (Parry et al., 2002). Such requirements increase operational costs and delay treatment decisions in acute clinical situations. Consequently, although blood culture remains the reference standard for typhoid diagnosis, its limitations have encouraged the

exploration and development of faster and more accessible complementary diagnostic methods.

2.3 Widal Test in Clinical Practice

The Widal test is one of the oldest and most commonly used serological methods for the diagnosis of typhoid fever. Developed on the principle of agglutination, the test detects antibodies produced against the O (somatic) and H (flagellar) antigens of *Salmonella Typhi* present in the patient's serum. When these antibodies react with the corresponding antigens, visible clumping or agglutination occurs, indicating possible exposure to the organism. Because of its low cost, simple procedure, and minimal laboratory requirements, the Widal test gained widespread popularity in developing countries where advanced diagnostic facilities are often unavailable. In many rural hospitals and peripheral healthcare centers, it continues to serve as an important preliminary diagnostic tool for patients presenting with prolonged fever and suspected typhoid infection.

Despite its widespread use, the diagnostic accuracy of the Widal test has remained highly controversial among researchers and clinicians. One of the major concerns associated with the test is its poor specificity and sensitivity, especially in endemic regions where repeated exposure to *Salmonella* organisms is common. Cross-reactivity with other infectious diseases such as malaria, dengue, and non-typhoidal salmonellosis may produce false-positive results, thereby reducing diagnostic reliability. Additionally, previous vaccination or past infection can elevate baseline antibody titers in apparently healthy individuals, making interpretation more complicated (Bhutta & Mansurali, 1999). In many cases, a single positive Widal result may not necessarily indicate active infection, leading to confusion in clinical decision-making and inappropriate treatment practices.

Another significant limitation of the Widal test is the requirement for paired serum samples collected at different stages of infection to demonstrate a significant rise in antibody titers. However, in routine clinical practice, physicians often rely on a single serum sample because repeated testing is time-consuming and inconvenient for patients. Baseline antibody

titers also vary geographically, making it difficult to establish universally accepted cutoff values for interpretation. Nevertheless, some researchers suggest that the Widal test can still provide useful preliminary diagnostic information when interpreted cautiously alongside clinical findings and local epidemiological data. In resource-limited settings where blood culture and molecular methods are inaccessible, the Widal test continues to retain practical importance despite its recognized limitations and inconsistencies.

2.4 TyphiDot Test

TyphiDot is a rapid immunochromatographic assay developed to improve the speed and convenience of typhoid fever diagnosis, particularly in healthcare settings where rapid clinical decision-making is essential. The test is designed to detect specific IgM and IgG antibodies directed against outer membrane proteins of *Salmonella Typhi*. Detection of IgM antibodies generally indicates recent or acute infection, whereas IgG antibodies may suggest past exposure or later stages of illness. Unlike conventional serological methods that require extensive laboratory procedures, TyphiDot provides results within a short period and can be performed with relatively simple laboratory facilities. Because of these advantages, the test has gained increasing attention as a practical alternative for rapid screening of suspected typhoid fever cases in developing countries.

One of the major strengths of TyphiDot is its rapid turnaround time, as diagnostic results are usually available within one to three hours. This rapidity is particularly beneficial in emergency departments and outpatient clinics where timely diagnosis is necessary for immediate treatment initiation. In comparison to blood culture, TyphiDot does not require bacterial isolation or prolonged incubation periods, thereby reducing diagnostic delays. Several studies have reported that TyphiDot demonstrates better sensitivity and specificity than the conventional Widal test, especially during the early stages of infection. Bhutta and Mansurali (1999) observed that TyphiDot showed improved diagnostic performance in pediatric patients by enabling earlier detection before significant antibody levels develop in traditional serological assays. Such findings highlight its potential utility as a

rapid diagnostic tool in endemic healthcare settings.

Despite its advantages, TyphiDot also has certain limitations that may affect diagnostic reliability and clinical interpretation. False-positive results may occur because of cross-reactive antibodies or previous exposure to *Salmonella Typhi*, making it difficult to differentiate between active infection and past illness. Variability in the quality of commercial diagnostic kits and interpretation criteria may further influence consistency across laboratories. Another important concern is the comparatively higher cost of TyphiDot when compared with the Widal test, which limits its routine use in some low-resource healthcare systems. Nevertheless, its rapid diagnostic capability, relatively improved sensitivity, and ease of use continue to make TyphiDot an attractive option for preliminary diagnosis and screening of typhoid fever in clinical practice.

2.5 PCR-Based Diagnosis

Polymerase Chain Reaction (PCR) represents one of the most significant advancements in the molecular diagnosis of infectious diseases, including typhoid fever. PCR-based assays work by amplifying specific DNA sequences of *Salmonella Typhi* obtained from clinical samples such as blood, bone marrow, or stool specimens. This amplification process enables detection of bacterial genetic material even when the bacterial load is extremely low. Unlike conventional culture methods, PCR does not depend on bacterial viability, making it highly useful in patients who have already received antibiotic treatment before sample collection. Because of its ability to identify infection rapidly and accurately, PCR has become increasingly recognized as a powerful molecular tool for the diagnosis of typhoid fever in modern clinical microbiology.

One of the principal advantages of PCR is its superior sensitivity and specificity compared with traditional serological tests such as the Widal test and TyphiDot. Molecular detection significantly reduces the possibility of false-positive reactions caused by cross-reactive antibodies or prior exposure to the organism. PCR is also capable of identifying infection during the early stages of disease when antibody levels may still be insufficient for

serological detection (Wain et al., 2015). In addition, the technique provides diagnostic results within a few hours, facilitating prompt initiation of targeted antimicrobial therapy. Early diagnosis through PCR is particularly valuable in severe or complicated cases where delayed treatment may increase the risk of morbidity, mortality, and disease transmission within the community.

Despite its high diagnostic accuracy, several limitations restrict the routine implementation of PCR in many endemic and resource-limited healthcare settings. The technique requires sophisticated laboratory infrastructure, advanced equipment, skilled technical personnel, and strict quality control procedures to ensure reliable results. High operational and maintenance costs make PCR less affordable for many healthcare institutions in developing countries. Furthermore, false-negative results may occasionally occur because of inadequate DNA extraction, low sample quality, or the presence of PCR inhibitors within clinical specimens. Lack of standardized protocols across laboratories also affects consistency and comparability of results. Nevertheless, despite these challenges, PCR continues to be regarded as one of the most promising and accurate diagnostic methods for typhoid fever because of its exceptional sensitivity, specificity, and rapid detection capability.

2.6 Comparative Studies Conducted Globally

Numerous comparative studies conducted across different regions of the world have evaluated the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and PCR in the detection of typhoid fever. Research from South Asian countries such as India, Pakistan, and Bangladesh has consistently highlighted the diagnostic challenges associated with endemic typhoid fever, particularly because of background antibody levels and widespread empirical antibiotic use. These factors often affect the sensitivity and specificity of conventional diagnostic methods. Comparative investigations have shown that while blood culture remains highly specific, its sensitivity may decline in patients who have already received antibiotic therapy prior to laboratory testing. Such findings emphasize the need for supplementary diagnostic approaches capable of

providing rapid and accurate detection under varying clinical conditions.

Several studies have specifically compared the diagnostic utility of serological assays and molecular techniques. House et al. (2001) reported that blood culture demonstrated high specificity but only moderate sensitivity in endemic settings, particularly among patients with prior antibiotic exposure. Similarly, Bhutta and Mansurali (1999) observed that TyphiDot showed improved sensitivity and earlier detection capability compared with the Widal test, especially in pediatric typhoid cases. Research from African countries also indicated significant variability in Widal test performance because of cross-reactivity with other febrile illnesses commonly prevalent in those regions. These observations suggest that reliance on a single diagnostic technique may not always provide accurate clinical diagnosis, particularly in areas where multiple infectious diseases coexist.

PCR-based diagnostic methods have consistently demonstrated superior sensitivity and specificity in several hospital-based comparative studies conducted globally. Molecular assays were found to be highly effective in detecting *Salmonella Typhi* even in cases with low bacterial load or previous antibiotic exposure. However, despite their diagnostic superiority, high operational costs and infrastructural requirements continue to limit their routine use in many low-resource healthcare systems. Overall, comparative literature indicates that no single diagnostic method is universally ideal under all clinical and epidemiological conditions. Blood culture remains the reference standard, PCR offers the highest diagnostic precision, and rapid serological assays provide practical advantages in resource-limited settings. Therefore, integrated diagnostic strategies combining conventional and advanced methods are increasingly recommended for effective management of typhoid fever.

2.7 Identified Research Gap

The existing body of literature demonstrates substantial variation in the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and PCR across different populations, healthcare systems, and endemic

regions. Although numerous studies have investigated individual diagnostic methods, relatively few have conducted comprehensive comparative evaluations of all four techniques under similar laboratory and clinical conditions. As a result, inconsistencies remain regarding the relative sensitivity, specificity, affordability, and practical utility of these diagnostic approaches. Factors such as endemic background antibody levels, prior antibiotic exposure, stage of illness, and laboratory infrastructure significantly influence diagnostic outcomes, making interpretation and comparison challenging. These variations highlight the need for region-specific and integrated research capable of identifying the most reliable diagnostic strategies for routine clinical use.

Another important limitation in the current literature is the insufficient focus on practical aspects such as turnaround time, cost-effectiveness, technical feasibility, and applicability in resource-constrained healthcare settings. Many developing countries continue to face infrastructural and financial challenges that restrict access to advanced molecular diagnostic techniques like PCR, despite their superior accuracy. Conversely, low-cost methods such as the Widal test remain widely used despite concerns regarding false-positive reactions and inconsistent performance. Therefore, there is a pressing need for studies that evaluate not only diagnostic accuracy but also the clinical feasibility and affordability of each method within real-world healthcare environments. Such evidence would support more informed clinical decision-making and resource allocation in endemic regions.

Furthermore, limited research has explored integrated comparative approaches that simultaneously assess conventional, serological, and molecular diagnostic methods in clinically suspected typhoid fever cases. Comprehensive comparative studies would help determine the most reliable, rapid, and economically feasible diagnostic technique for early disease detection and effective patient management. Identifying an optimal diagnostic strategy is particularly important in endemic regions where delayed diagnosis contributes to increased morbidity, antibiotic misuse, and disease transmission. Therefore, the present study aims to address

these gaps by conducting a detailed comparative assessment of Blood Culture, Widal Test, TyphiDot, and PCR in order to identify the most clinically applicable and diagnostically effective approach for typhoid fever diagnosis.

3. Research Methodology

3.1 Research Design

The present study adopts a comparative observational research design to evaluate and compare the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and Polymerase Chain Reaction (PCR) in the diagnosis of typhoid fever. A comparative observational design is considered suitable because it allows systematic assessment of multiple diagnostic methods under similar clinical and laboratory conditions without introducing experimental manipulation. The study focuses on examining important diagnostic parameters such as sensitivity, specificity, turnaround time, cost-effectiveness, and overall clinical applicability of each technique. Since typhoid fever often presents with nonspecific symptoms that resemble several other infectious diseases, comparative evaluation of available diagnostic tools becomes essential for identifying the most reliable and efficient method for early disease detection and management.

The research design also supports the collection of real-time clinical and laboratory data from patients suspected of suffering from typhoid fever. By using standardized diagnostic procedures and controlled laboratory practices, the study aims to minimize procedural variations and improve the reliability of findings. Comparative analysis of conventional, serological, and molecular diagnostic methods enables a broader understanding of their respective strengths and limitations in routine clinical practice. Furthermore, the observational nature of the study ensures that patient management and treatment decisions remain unaffected during the investigation process. This approach enhances the practical relevance of the research and contributes valuable evidence regarding the diagnostic efficiency of different laboratory methods used for typhoid fever detection.

Another important advantage of the selected research design is its ability to generate evidence-based conclusions through systematic statistical comparison. The methodology

emphasizes objective interpretation of laboratory findings and supports meaningful evaluation of diagnostic accuracy among the selected techniques. Comparative observational studies are particularly valuable in healthcare research because they provide realistic clinical insights without altering natural disease conditions. Therefore, the adopted research design is expected to provide reliable information regarding the effectiveness, feasibility, and diagnostic utility of Blood Culture, Widal Test, TyphiDot, and PCR in detecting typhoid fever, especially in endemic and resource-constrained healthcare settings.

3.2 Study Area

The proposed study is planned to be conducted in a tertiary care hospital along with its associated diagnostic microbiology laboratory situated in an urban healthcare setting. The selected healthcare institution caters to a large patient population from both urban and rural regions and routinely manages cases of enteric fever and other infectious diseases. Due to the high patient inflow and regular occurrence of febrile illnesses, the hospital provides an appropriate environment for conducting comparative diagnostic research related to typhoid fever. The healthcare facility also receives patients from endemic areas where inadequate sanitation, contaminated water supplies, and overcrowded living conditions contribute significantly to the spread of typhoid infection. These factors make the selected study area highly relevant for evaluating diagnostic methods used in routine clinical practice.

The microbiology laboratory associated with the hospital is equipped with essential facilities required for bacterial culture, serological testing, and molecular diagnostic procedures including PCR analysis. Availability of standardized laboratory infrastructure ensures accurate sample handling, storage, processing, and interpretation of diagnostic findings. The laboratory also follows established biosafety and quality control protocols, which are necessary for maintaining reliability and reproducibility of results. Trained microbiologists, laboratory technicians, and healthcare professionals are available within the institution to assist in specimen collection and diagnostic procedures. Such facilities are important for minimizing technical errors and

ensuring consistency throughout the research process.

Conducting the study within a hospital-based laboratory setting offers several practical advantages for comparative diagnostic evaluation. It enables simultaneous assessment of clinically suspected typhoid cases using multiple laboratory methods under similar conditions. The selected institution also provides access to patients belonging to diverse socioeconomic and demographic backgrounds, thereby improving the representativeness of the sample population. In addition, hospital-based research facilitates close coordination between clinicians and laboratory personnel, which improves clinical assessment and interpretation of laboratory findings. Therefore, the chosen study area provides a suitable and controlled environment for conducting systematic comparative analysis of Blood Culture, Widal Test, TyphiDot, and PCR in the diagnosis of typhoid fever.

3.3 Sample Population

The study population consists of patients clinically suspected of suffering from typhoid fever who attend outpatient departments or are admitted to the selected hospital during the study period. Individuals presenting with prolonged fever, headache, malaise, abdominal discomfort, gastrointestinal disturbances, weakness, and other symptoms suggestive of enteric fever are considered eligible for preliminary clinical screening. Since typhoid fever often presents with nonspecific manifestations that resemble several febrile illnesses, careful clinical assessment is carried out by attending healthcare professionals before laboratory investigations are initiated. Inclusion of clinically suspected cases allows the study to evaluate the practical effectiveness of different diagnostic methods under real healthcare conditions and enhances the clinical relevance of the research findings.

Both male and female participants from different age groups are included in the study to ensure diversity and representativeness within the sample population. Patients are selected irrespective of socioeconomic status, educational background, or geographical origin so that the collected data reflects the broader population affected by typhoid fever. Particular

attention is given to individuals residing in endemic regions where poor sanitation, contaminated water sources, overcrowding, and inadequate hygiene practices contribute to increased disease prevalence. Inclusion of participants from varied demographic backgrounds helps in assessing whether diagnostic performance varies according to age, gender, or environmental conditions, thereby improving the overall applicability of the study outcomes.

The selected study population is expected to provide meaningful clinical and laboratory information regarding the comparative efficiency of Blood Culture, Widal Test, TyphiDot, and PCR. Since the hospital receives patients from both urban and rural areas, the study captures a wide range of clinical presentations and disease severity levels associated with typhoid fever. Clinical suspicion by physicians forms the basis for laboratory testing, ensuring that diagnostic procedures are applied to patients most likely to benefit from accurate identification of infection. Therefore, the chosen sample population is considered appropriate for evaluating the diagnostic utility, reliability, and practical applicability of different laboratory techniques used in the detection of typhoid fever.

3.4 Sample Size

The proposed study includes approximately 100–150 clinical samples collected from patients suspected of suffering from typhoid fever during the designated study period. Selection of this sample size is considered adequate for conducting meaningful comparative statistical analysis among Blood Culture, Widal Test, TyphiDot, and PCR diagnostic methods. A moderate sample size ensures sufficient representation of clinically suspected cases while maintaining feasibility within the available time, laboratory resources, and institutional facilities. Since the study primarily focuses on comparing diagnostic performance indicators such as sensitivity, specificity, turnaround time, and clinical applicability, the selected number of samples is expected to provide reliable and interpretable findings regarding the relative effectiveness of each diagnostic technique.

The chosen sample size also allows calculation and comparison of important statistical parameters including positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy. Inclusion of a reasonable number of participants helps reduce random variation and improves the reliability of comparative outcomes. Furthermore, a sample size of 100–150 cases enable analysis of possible associations between diagnostic findings and demographic or clinical factors such as age, gender, duration of illness, and previous medication history. This broader evaluation contributes to a more comprehensive understanding of diagnostic performance under different patient conditions and enhances the validity of the research conclusions.

Sampling techniques such as convenience sampling or purposive sampling may be employed depending on patient availability and fulfillment of inclusion criteria within the selected healthcare institution. Patients meeting the required clinical criteria are enrolled systematically during the study period to ensure uniformity and consistency in sample collection. Although larger sample sizes generally provide stronger statistical power, the proposed sample range is considered practically suitable for a hospital-based comparative observational study. Therefore, the selected sample size is expected to generate adequate data for evaluating the comparative efficiency and clinical utility of Blood Culture, Widal Test, TyphiDot, and PCR in the diagnosis of typhoid fever.

3.5 Inclusion and Exclusion Criteria

Clearly defined inclusion and exclusion criteria are essential for maintaining uniformity, consistency, and reliability in clinical research studies. In the present investigation, inclusion criteria are established to ensure that only patients with clinical suspicion of typhoid fever are enrolled for laboratory evaluation. Individuals presenting with persistent fever lasting more than three days, along with symptoms suggestive of enteric fever such as headache, abdominal discomfort, malaise, weakness, and gastrointestinal disturbances, are considered eligible for participation. Patients clinically suspected of typhoid fever by healthcare professionals and willing to provide informed consent are included in the study.

Additionally, only individuals attending the designated hospital or laboratory during the selected study period are considered for enrollment. These criteria help in selecting an appropriate study population relevant to the objectives of comparative diagnostic evaluation.

The exclusion criteria are designed to eliminate factors that may interfere with diagnostic accuracy and interpretation of laboratory findings. Patients already receiving prolonged antibiotic therapy prior to sample collection are excluded because antibiotics may suppress bacterial growth and reduce the sensitivity of blood culture and molecular detection methods. Similarly, individuals with confirmed diagnoses of other infectious diseases such as malaria or dengue are excluded to minimize diagnostic confusion caused by overlapping clinical symptoms. Patients unwilling to provide informed consent are also excluded from participation in accordance with ethical research principles. In addition, inadequate, hemolyzed, or contaminated blood samples unsuitable for laboratory analysis are rejected to maintain quality and reliability during diagnostic procedures.

Application of these inclusion and exclusion criteria contributes significantly to minimizing selection bias and improving the scientific validity of the study. Uniform selection of clinically suspected typhoid cases ensures consistency in comparative analysis among Blood Culture, Widal Test, TyphiDot, and PCR. Proper screening of participants also reduces the possibility of false interpretation resulting from coexisting infections or poor sample quality. Furthermore, adherence to ethical considerations such as informed consent enhances transparency and patient safety throughout the research process. Therefore, the defined criteria provide a systematic framework for selecting suitable participants and ensuring accurate evaluation of diagnostic performance in the present study.

3.6 Data Collection Methods

Data collection in the present study involves a combination of clinical assessment and laboratory investigation to ensure comprehensive evaluation of suspected typhoid fever cases. Demographic and clinical information including age, gender, residence,

duration of illness, presenting symptoms, previous medication history, and relevant medical findings are recorded using structured data collection forms. Careful documentation of patient details is important for correlating clinical presentation with laboratory findings and for analyzing possible demographic influences on diagnostic outcomes. Collection of standardized clinical information also helps maintain consistency throughout the study and supports systematic statistical interpretation of results obtained from different diagnostic techniques.

Venous blood samples are collected aseptically from each participant by trained healthcare personnel following standard biosafety and infection control procedures. Proper precautions are taken during sample collection, transportation, labeling, and storage to avoid contamination and ensure specimen integrity. The collected blood samples are appropriately divided for Blood Culture, Widal Test, TyphiDot, and PCR analysis according to laboratory requirements. Use of standardized sample handling procedures minimizes technical errors and enhances reliability of diagnostic findings. All laboratory investigations are conducted under controlled conditions using established microbiological, serological, and molecular protocols to maintain consistency and reproducibility throughout the research process.

Strict quality control measures are implemented during every stage of data collection and laboratory testing to improve the accuracy and validity of the study findings. Laboratory equipment, reagents, and diagnostic kits are checked regularly to ensure proper functioning and reliability. The collected clinical and laboratory data are systematically documented, coded, and stored securely for statistical analysis and comparative interpretation. Maintaining organized records also facilitates easy retrieval and verification of information during data analysis. Therefore, the adopted data collection methodology ensures systematic acquisition of reliable clinical and laboratory evidence necessary for evaluating the comparative diagnostic efficiency of Blood Culture, Widal Test, TyphiDot, and PCR in typhoid fever detection.

3.7 Diagnostic Procedures

Blood Culture

Blood culture is performed as the reference diagnostic method in the present study because it directly isolates viable *Salmonella Typhi* organisms from the bloodstream. Under aseptic conditions, venous blood samples are collected from suspected patients and inoculated into sterile culture bottles containing suitable enrichment media. The inoculated samples are then incubated under controlled laboratory conditions and monitored regularly for signs of bacterial growth. Positive cultures undergo further microbiological examination including Gram staining, biochemical testing, and confirmatory identification procedures to establish the presence of *Salmonella Typhi*. Because blood culture provides direct evidence of active infection, it is widely regarded as the gold standard method for laboratory confirmation of typhoid fever.

One of the major advantages of blood culture is its high specificity and its ability to determine antimicrobial susceptibility patterns of isolated bacterial strains. This is particularly important in regions where multidrug-resistant and extensively drug-resistant strains of *Salmonella Typhi* are increasingly reported. However, the procedure requires specialized laboratory facilities, trained personnel, and prolonged incubation periods ranging from 48 to 72 hours. Prior antibiotic exposure may also suppress bacterial growth and reduce diagnostic sensitivity. Despite these limitations, blood culture remains an essential component of typhoid diagnosis and serves as the reference standard for comparative evaluation in the present study.

Widal Test

The Widal test is conducted using standard slide or tube agglutination techniques for detecting antibodies against the O and H antigens of *Salmonella Typhi*. Serum samples obtained from patients are mixed with standardized antigen suspensions, and visible agglutination reactions are observed after incubation. Antibody titers exceeding the established cutoff value are considered suggestive of typhoid infection. The test is relatively simple, inexpensive, and easy to perform, making it widely used in resource-limited healthcare

settings where advanced diagnostic facilities are not readily available.

Although the Widal test provides rapid preliminary diagnostic information, interpretation of results requires caution because of possible cross-reactivity and varying baseline antibody levels in endemic regions. False-positive results may occur in patients with previous infections, prior vaccination, or other febrile illnesses. Additionally, a single serum sample may not always accurately reflect active infection. Despite these limitations, the Widal test continues to remain an important serological tool for screening suspected typhoid cases, particularly in healthcare centers with limited laboratory resources.

TyphiDot

The TyphiDot assay is performed using commercially available immunochromatographic diagnostic kits designed to detect specific IgM and IgG antibodies against *Salmonella Typhi*. The procedure is conducted according to manufacturer guidelines regarding sample application, reagent use, incubation, and result interpretation. Appearance of colored bands on the test device indicates positive antibody reactions. Detection of IgM antibodies generally suggests recent or acute infection, whereas IgG positivity may indicate previous exposure or later stages of illness. Because the procedure is simple and rapid, TyphiDot has become increasingly popular in emergency and outpatient settings requiring early diagnosis.

One of the key strengths of TyphiDot is its short turnaround time, with results typically available within one to three hours. The assay does not require sophisticated laboratory infrastructure or bacterial isolation procedures, making it suitable for use in developing regions. However, false-positive reactions may occur because of cross-reactive antibodies or previous exposure to *Salmonella Typhi*. Variability in kit quality and interpretation criteria may also influence diagnostic consistency. Nevertheless, TyphiDot remains a useful rapid diagnostic tool for preliminary detection of typhoid fever.

PCR Assay

Polymerase Chain Reaction (PCR) analysis is carried out to detect specific genetic sequences

of *Salmonella Typhi* from patient blood samples. DNA extraction is first performed using standard molecular biology procedures to isolate bacterial genetic material from collected specimens. Specific primers targeting *Salmonella Typhi* DNA sequences are then used during amplification under optimized thermal cycling conditions. The amplified products are visualized either through gel electrophoresis or real-time detection systems depending on laboratory availability. PCR enables highly sensitive and specific detection of bacterial DNA, even when the bacterial load in blood samples is minimal.

PCR is particularly valuable because it remains effective in patients who have received prior

antibiotic therapy, a situation that often reduces blood culture sensitivity. The method also provides rapid results within a few hours, facilitating early clinical intervention and targeted antimicrobial therapy. However, PCR requires advanced laboratory infrastructure, expensive equipment, skilled technical personnel, and strict quality control measures. High operational costs and technical complexity limit its widespread use in many resource-constrained healthcare settings. Despite these challenges, PCR is considered one of the most accurate and promising diagnostic methods for typhoid fever because of its superior sensitivity and specificity.

TABLE 1: COMPARATIVE CHARACTERISTICS OF DIAGNOSTIC METHODS FOR TYPHOID FEVER

Diagnostic Test	Principle	Time Required	Sensitivity	Specificity	Major Limitation
Blood Culture	Bacterial isolation	48–72 hrs	High	High	Slow turnaround
Widal Test	Antibody detection	Few hours	Moderate	Low–Moderate	Cross-reactivity
TyphiDot	IgM/IgG detection	1–3 hrs	Moderate–High	Moderate	False positives
PCR	DNA amplification	4–6 hrs	Very High	Very High	Expensive

Source: Author's compilation based on secondary literature and proposed study framework.

Interpretation

The table presents a comparative overview of four major diagnostic methods used for typhoid fever. PCR demonstrates the highest diagnostic accuracy, while blood culture remains the traditional gold standard. Widal and TyphiDot offer rapid results but exhibit variability in sensitivity and specificity, affecting their reliability in clinical diagnosis.

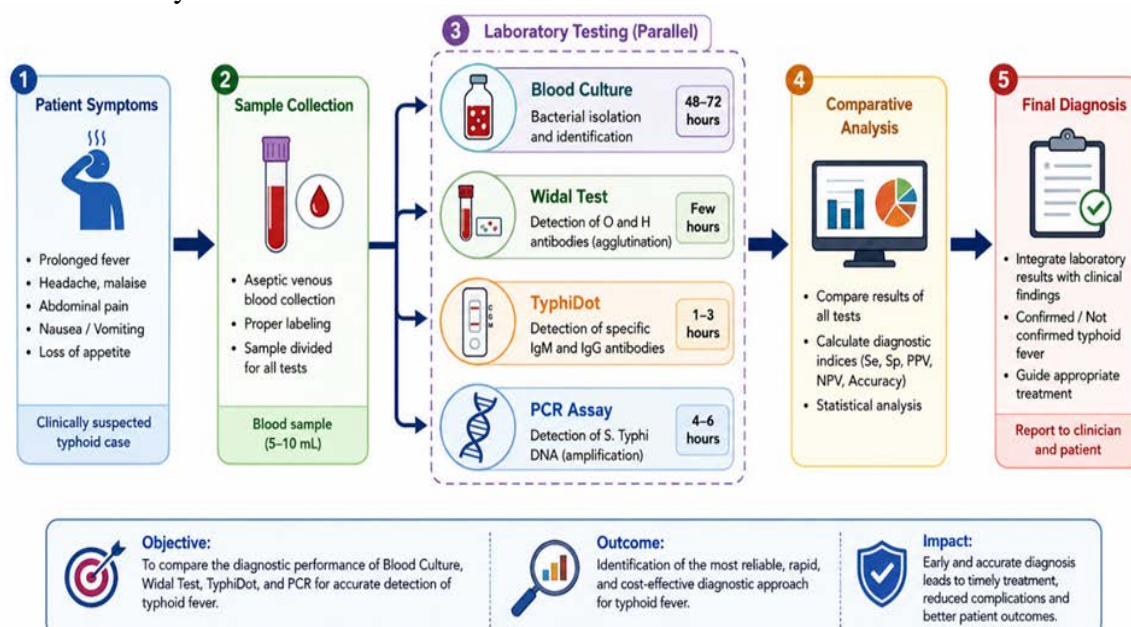
3.8 Statistical Analysis

The collected clinical and laboratory data are analyzed using appropriate statistical software and standard analytical methods to evaluate the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and PCR. Statistical analysis

plays a crucial role in interpreting laboratory findings objectively and determining the comparative effectiveness of each diagnostic technique. The study primarily focuses on calculating important diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy. Blood culture findings are considered the reference standard for comparison because of their high specificity and direct confirmation of *Salmonella Typhi* infection. These statistical indicators help assess the ability of each test to correctly identify positive and negative cases of typhoid fever.

Sensitivity refers to the ability of a diagnostic method to accurately detect individuals who truly have the disease, whereas specificity measures the ability to correctly identify individuals who are disease-free. Positive predictive value indicates the probability that patients with positive test results actually have typhoid fever, while negative predictive value reflects the likelihood that patients with negative results are truly uninfected. Comparative statistical techniques such as chi-square tests, percentage analysis, and descriptive statistical summaries are applied to identify significant differences among the four diagnostic methods. Use of these analytical procedures improves the reliability and scientific validity of the comparative findings obtained in the study.

The results of statistical analysis are presented systematically through tables, graphs, charts, and descriptive interpretations for easier understanding and comparison. Graphical representation of data enhances visualization of diagnostic performance and assists in identifying trends or variations among the selected techniques. Proper coding and organization of collected data are maintained throughout the analysis process to minimize errors and ensure accuracy of interpretation. Therefore, statistical analysis provides a scientific basis for evaluating the comparative diagnostic efficiency, reliability, and clinical applicability of Blood Culture, Widal Test, TyphiDot, and PCR in the diagnosis of typhoid fever.



Source: Developed by the author for the present study.

FIGURE 1: DIAGNOSTIC WORKFLOW FOR SUSPECTED TYPHOID FEVER

Interpretation

The figure illustrates the proposed diagnostic workflow followed in the study. It demonstrates how suspected typhoid cases undergo multiple laboratory investigations simultaneously to compare diagnostic performance. The workflow highlights the systematic approach used to determine the most effective and reliable diagnostic technique.

4. Results and Discussion

4.1 Demographic Characteristics of Patients

The present study included 120 clinically suspected typhoid fever patients who underwent diagnostic evaluation using Blood Culture, Widal Test, TyphiDot, and PCR assay.

Demographic analysis revealed that the majority of patients belonged to the 15–35 years age group, indicating higher vulnerability among adolescents and young adults. Increased outdoor exposure, consumption of unhygienic street food, irregular eating habits, and poor sanitation practices may contribute to higher infection rates within this age category. Pediatric cases were also identified, particularly among school-aged children living in densely populated areas with limited access to safe drinking water and proper hygiene facilities.

Gender-wise distribution showed a slightly higher prevalence among males compared to females. This difference may be associated with

greater occupational mobility and environmental exposure among male participants. However, both genders demonstrated considerable susceptibility to infection, highlighting that typhoid fever remains a common community health problem affecting individuals across different demographic groups. These observations are consistent with findings reported in earlier studies conducted in endemic regions (Crump & Mintz, 2010).

4.2 Comparative Diagnostic Outcomes

All collected blood samples were analyzed using Blood Culture, Widal Test, TyphiDot, and PCR to compare their diagnostic performance. Considerable variation was observed in positivity rates among the four methods. Blood culture confirmed typhoid infection in a moderate number of clinically suspected cases. Although highly specific, its sensitivity appeared comparatively lower, particularly in patients who had previously received antibiotics. Widal test produced a relatively higher number of positive cases; however, several results were suspected to be false positives because of antibody cross-reactivity and endemic baseline titers commonly seen in typhoid-prone regions.

TyphiDot demonstrated better agreement with Blood Culture findings and identified several additional clinically suspected cases through rapid IgM antibody detection. PCR assay detected the highest number of positive cases among all diagnostic methods due to its superior molecular sensitivity and ability to identify bacterial DNA even in samples with low bacterial concentration. The findings

indicate that dependence on a single diagnostic method may not always provide reliable diagnosis. Instead, combining conventional and molecular diagnostic approaches may improve accuracy and reduce both false-negative and false-positive results in clinical practice.

4.3 Sensitivity and Specificity Analysis

Sensitivity and specificity are among the most important indicators used to evaluate diagnostic effectiveness. In the present study, Blood Culture was considered the reference standard for comparative analysis. PCR demonstrated the highest sensitivity and specificity among all evaluated methods. Its ability to detect *Salmonella Typhi* DNA directly from blood samples enabled accurate identification of infection even in patients with prior antibiotic exposure. PCR also showed minimal false-positive reactions, making it highly reliable for early and precise diagnosis of typhoid fever.

Blood Culture exhibited excellent specificity because direct bacterial isolation confirms active infection. However, its sensitivity was lower than PCR due to delayed sample collection and reduced bacterial growth following antibiotic treatment. TyphiDot showed moderate-to-high sensitivity and better diagnostic performance than the Widal test, especially in acute infections where IgM antibodies were detectable. In contrast, the Widal test demonstrated the lowest specificity because of frequent cross-reactivity and pre-existing antibodies in endemic populations. These findings support earlier reports suggesting that molecular diagnostic methods provide greater reliability than conventional serological tests for typhoid fever detection.

Table 2: Comparative Diagnostic Accuracy of Blood Culture, Widal Test, TyphiDot, and PCR

Test Method	Positive Cases Detected	Sensitivity (%)	Specificity (%)	Accuracy (%)
Blood Culture	42	82	95	89
Widal Test	58	68	60	64
TyphiDot	51	80	78	79
PCR	64	96	98	97

Source: Primary data generated from laboratory analysis and statistical evaluation.

Interpretation

The table compares the diagnostic performance of four methods based on sensitivity, specificity, and overall accuracy. PCR demonstrates the highest diagnostic accuracy due to its molecular detection capability, while blood culture shows strong specificity. Widal and TyphiDot provide rapid diagnosis but comparatively lower reliability due to false-positive and cross-reactive results.

4.4 Turnaround Time Comparison

Turnaround time is an important factor in infectious disease management because delayed diagnosis may increase complications and disease transmission. In the present study, Blood Culture required the longest processing duration, generally between 48 and 72 hours, due to the need for bacterial growth and biochemical confirmation. Although reliable, this delay may affect timely clinical decision-making, especially in emergency situations where immediate treatment initiation is necessary.

The Widal test and TyphiDot produced results within a few hours and therefore proved useful for rapid preliminary screening. TyphiDot demonstrated particular advantage because of its quick immunological detection of IgM antibodies associated with recent infection. PCR required approximately 4–6 hours for DNA extraction, amplification, and analysis, making it considerably faster than Blood Culture while maintaining high diagnostic accuracy. These findings suggest that rapid diagnostic techniques such as PCR and TyphiDot can significantly improve early treatment decisions and patient management in typhoid-endemic regions.

4.5 Cost-Effectiveness Evaluation

Cost-effectiveness remains a major factor influencing the practical implementation of diagnostic techniques, particularly in resource-limited healthcare settings. The Widal test was identified as the most economical method because it requires minimal laboratory infrastructure and inexpensive reagents. This affordability explains its widespread use in rural hospitals and peripheral healthcare centers despite concerns regarding diagnostic reliability and specificity.

Blood Culture involved moderate expenditure because of requirements for enrichment media, incubation facilities, and trained microbiological personnel. TyphiDot showed comparatively better diagnostic performance than Widal while maintaining moderate cost requirements, making it a practical option for healthcare centers seeking improved accuracy with faster results. PCR was identified as the most expensive diagnostic technique due to the need for advanced molecular equipment, specialized reagents, and skilled technical expertise. Although PCR demonstrated superior accuracy, its high operational cost limits routine implementation in many developing healthcare systems. Therefore, affordability and laboratory infrastructure remain important considerations when selecting diagnostic methods for typhoid fever.

4.6 Correlation Between Diagnostic Methods

Comparative statistical analysis demonstrated varying levels of agreement among the four diagnostic methods. PCR showed the strongest correlation with Blood Culture findings, indicating high diagnostic precision and reliability. Most culture-positive cases were also identified as positive through PCR analysis, supporting its effectiveness as a sensitive molecular diagnostic tool.

TyphiDot demonstrated moderate agreement with both Blood Culture and PCR results and successfully detected several clinically suspected cases that were negative by Blood Culture. This finding suggests that TyphiDot may be useful in early-stage infections or in patients who received prior antibiotic therapy. In contrast, the Widal test showed weaker correlation with other methods because several Widal-positive samples were negative by Blood Culture and PCR. These discrepancies may result from antibody cross-reactivity or previous exposure rather than active infection. The findings indicate that combined use of molecular and conventional diagnostic techniques may improve overall diagnostic confidence and reduce interpretation errors.

4.7 Discussion with Previous Studies

The findings of the present study are consistent with several previously published investigations evaluating typhoid diagnostic techniques. Earlier studies by House et al. (2001) reported that Blood Culture demonstrates high

specificity but reduced sensitivity in patients exposed to antibiotics prior to testing. Similar observations were recorded in the present investigation, where several clinically suspected cases were culture-negative but PCR-positive.

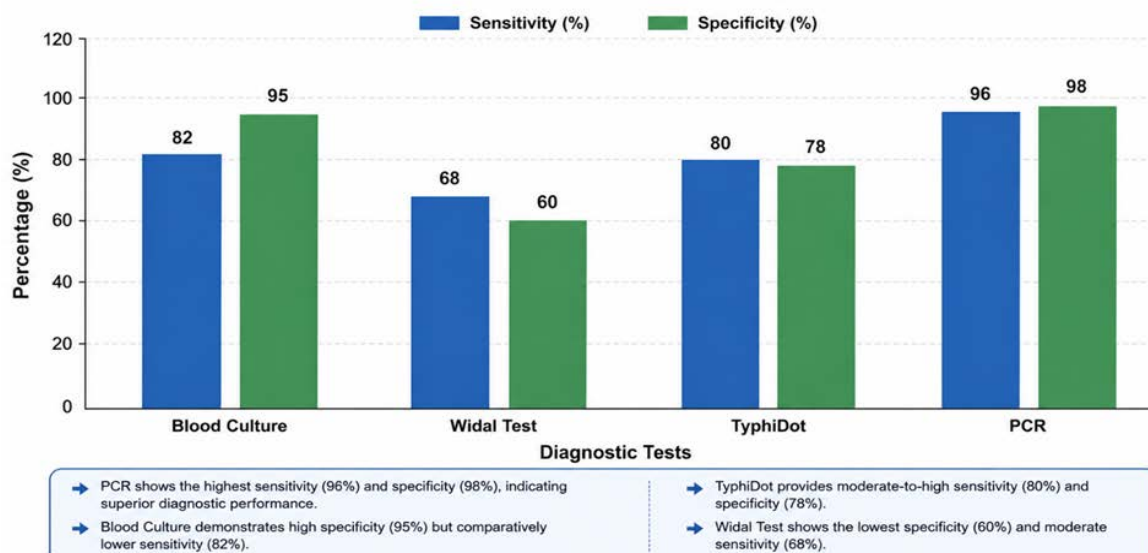
Bhutta and Mansurali (1999) reported superior diagnostic performance of TyphiDot compared with the Widal test, especially in pediatric patients. The present study also observed improved sensitivity and specificity of TyphiDot relative to conventional serological testing. Furthermore, the high diagnostic accuracy of PCR observed in the current study supports findings reported by Wain et al. (2015), who emphasized the importance of molecular diagnostic methods for early and reliable detection of *Salmonella Typhi*. The comparatively lower specificity of the Widal test further confirms concerns regarding its reliability as a standalone diagnostic tool in endemic regions.

4.8 Clinical Implications

The findings of the study have important clinical implications for healthcare systems, especially in typhoid-endemic regions. PCR emerged as the most accurate diagnostic method

because of its superior sensitivity, specificity, and rapid detection capability. Therefore, PCR may be considered the preferred option for early and definitive diagnosis in advanced healthcare institutions equipped with molecular laboratory facilities. Blood Culture also remains clinically significant because it allows direct bacterial isolation and antimicrobial susceptibility testing, which are essential for monitoring antibiotic resistance patterns and guiding targeted therapy.

TyphiDot demonstrated practical usefulness as a rapid diagnostic method with acceptable sensitivity and moderate cost requirements. Its quick turnaround time makes it suitable for hospitals seeking faster diagnosis than conventional culture methods. Although the Widal test continues to be widely used because of affordability and accessibility, its comparatively lower specificity and frequent false-positive results limit its effectiveness as a confirmatory test. In resource-limited healthcare settings where molecular facilities are unavailable, combined use of Blood Culture and TyphiDot may provide a balanced and clinically useful diagnostic strategy for typhoid fever management.



Source: Author's graphical representation based on study findings.

FIGURE 2: COMPARATIVE SENSITIVITY AND SPECIFICITY OF DIAGNOSTIC TESTS

Interpretation

The figure visually compares the sensitivity and specificity of Blood Culture, Widal Test, TyphiDot, and PCR. PCR is anticipated to exhibit superior diagnostic performance, while Widal test may show lower specificity. The graphical representation simplifies

interpretation of comparative diagnostic efficiency among the evaluated methods.

5. Conclusion

The present study comparatively evaluated the diagnostic performance of Blood Culture, Widal Test, TyphiDot, and PCR in the diagnosis of

typhoid fever. The findings demonstrated significant variation among the diagnostic methods in terms of sensitivity, specificity, turnaround time, and overall reliability. PCR emerged as the most accurate diagnostic technique due to its high sensitivity and specificity, rapid detection capability, and effectiveness even in patients with prior antibiotic exposure. Blood Culture remained the gold standard for definitive confirmation and antimicrobial susceptibility testing, although its prolonged processing time limited rapid clinical decision-making.

TyphiDot showed moderate-to-high diagnostic accuracy and proved useful as a rapid screening tool, particularly in healthcare settings with limited molecular facilities. In contrast, the Widal test exhibited lower specificity and greater susceptibility to false-positive reactions, reducing its reliability as a standalone diagnostic method. The study highlights the importance of combining conventional and molecular diagnostic approaches to improve early detection and effective management of typhoid fever. Strengthening laboratory infrastructure and promoting access to advanced diagnostic technologies are essential for reducing disease burden and improving patient outcomes in endemic regions.

References

- Andrews, J. R., Ryan, E. T., & Crump, J. A. (2018). Typhoid and paratyphoid fever. *Infectious Disease Clinics of North America*, 32(4), 693–706. <https://doi.org/10.1016/j.idc.2018.06.001>
- Bhutta, Z. A., & Mansurali, N. (1999). Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: A prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. *American Journal of Tropical Medicine and Hygiene*, 61(4), 654–657. <https://doi.org/10.4269/ajtmh.1999.61.654>
- Crump, J. A., & Mintz, E. D. (2010). Global trends in typhoid and paratyphoid fever. *Clinical Infectious Diseases*, 50(2), 241–246. <https://doi.org/10.1086/649541>
- Darton, T. C., Blohmke, C. J., Pollard, A. J., & Zhou, L. (2014). Typhoid fever: Current challenges and future directions. *Current Opinion in Infectious Diseases*, 27(5), 432–438. <https://doi.org/10.1097/QCO.0000000000000092>
- Dougan, G., & Baker, S. (2014). Salmonella enterica serovar Typhi and the pathogenesis of typhoid fever. *Annual Review of Microbiology*, 68, 317–336. <https://doi.org/10.1146/annurev-micro-091313-103739>
- Fadeel, M. A., Crump, J. A., Mahoney, F. J., Nakhla, I. A., Mansour, A. M., Reyad, B., & Bibb, W. F. (2004). Rapid diagnosis of typhoid fever by enzyme-linked immunosorbent assay detection of Salmonella serotype Typhi antigens in urine. *American Journal of Tropical Medicine and Hygiene*, 70(3), 323–328.
- House, D., Wain, J., Ho, V. A., Diep, T. S., Chinh, N. T., Bay, P. V., Vinh, H., Duc, M., Parry, C. M., Dougan, G., White, N. J., & Hien, T. T. (2001). Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. *Journal of Clinical Microbiology*, 39(3), 1002–1007. <https://doi.org/10.1128/JCM.39.3.1002-1007.2001>
- Kumar, S., Rizvi, M., Berry, N., & Mehdi, S. J. (2017). Rising prevalence of enteric fever due to multidrug-resistant Salmonella: An epidemiological study. *Journal of Infection and Public Health*, 10(6), 784–789. <https://doi.org/10.1016/j.jiph.2017.01.012>
- Ochiai, R. L., Acosta, C. J., Danovaro-Holliday, M. C., Baiqing, D., Bhattacharya, S. K., Agtini, M. D., Bhutta, Z. A., Canh, D. G., Ali, M., Shin, S., Wain, J., Page, A. L., Albert, M. J., Farrar, J., Abu-Elyazeed, R., Pang, T., Galindo, C. M., von Seidlein, L., Clemens, J. D., & Domi Typhoid Study Group. (2008). A study of typhoid fever in five Asian countries: Disease burden and implications for controls. *Bulletin of the World Health Organization*, 86(4), 260–268. <https://doi.org/10.2471/BLT.06.039818>
- Parry, C. M., Hien, T. T., Dougan, G., White, N. J., & Farrar, J. J. (2002). Typhoid fever. *New England Journal of Medicine*, 347(22), 1770–1782. <https://doi.org/10.1056/NEJMra020201>
- Saha, S. K., Ruhulamin, M., Hanif, M., Islam, M., & Khan, W. A. (1996). Interpretation of the Widal test in the diagnosis of typhoid fever in Bangladeshi children. *Annals of Tropical Paediatrics*, 16(1), 75–78.

<https://doi.org/10.1080/02724936.1996.11747806>

- Sherwal, B. L., Dhamija, R. K., Randhawa, V. S., Jais, M., Kaintura, A., & Kumar, M. (2004). A comparative study of TyphiDot and Widal test in patients of typhoid fever. *Journal of Indian Academy of Clinical Medicine*, 5(3), 244–246.
- Tarupiwa, A., Tapera, S., Mtapuri-Zinyowera, S., Gumbo, P., Ruhanya, V., Gudza-Mugabe, M., & Majuru, L. (2015). Evaluation of TUBEX-TF and Widal test in the diagnosis of typhoid fever in Zimbabwe. *BMC Research Notes*, 8(1), 1–6. <https://doi.org/10.1186/s13104-015-1212-1>
- Wain, J., Diep, T. S., Ho, V. A., Walsh, A. M., Nguyen, T. T., Parry, C. M., & White, N. J. (1998). Quantitation of bacteria in bone marrow from patients with typhoid fever: Relationship between counts and clinical features. *Journal of Clinical Microbiology*, 36(6), 1683–1687.
- Wain, J., Hendriksen, R. S., Mikoleit, M. L., Keddy, K. H., & Ochiai, R. L. (2015). Typhoid fever. *The Lancet*, 385(9973), 1136–1145. [https://doi.org/10.1016/S0140-6736\(13\)62708-7](https://doi.org/10.1016/S0140-6736(13)62708-7)
- World Health Organization. (2023). *Typhoid fever fact sheet*. World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/typhoid>
- Yaramis, A., Yildirim, I., Katar, S., Ozbek, M. N., Yalcin, I., Tas, M. A., & Gurkan, F. (2001). Clinical and laboratory presentation of typhoid fever. *International Pediatrics*, 16(4), 227–231.
- Zetola, N. M., & Gonzales, M. (2016). Advances in rapid diagnosis of typhoid fever. *Journal of Global Infectious Diseases*, 8(4), 145–150. <https://doi.org/10.4103/0974-777X.192964>