ACCURACY, SENSITIVITY AND SPECIFICITY OF CONVENTIONAL AND ADVANCED METHODS FOR THE DIAGNOSIS OF TYPHOID FEVER IN MALWA REGION, PUNJAB

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in

CLINICAL MICROBIOLOGY

By

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2025

DECLARATION

I, hereby declared that the presented work in the thesis entitled Accuracy Sensitivity and Specificity of Conventional and Advanced Methods for the Diagnosis of Typhoid Fever In Malwa Region , Punjab in fulfilment of degree of Doctor of Philosophy (Ph.D.) is outcome of research work carried out by me under the supervision **Dr. Naresh Kumar**, working as Associate Professor, in the Department of Medical Laboratory Sciences, School of Allied Medical Sciences, of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Accuracy Sensitivity And Specificity Of Conventional And Advanced Methods for the Diagnosis Of Typhoid Fever In Malwa Region, Punjab" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Clinical Microbiology, is a research work carried out by Rajan Bector (42000077) is Bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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List of Abbreviations

Abbreviations	Full form
%	Percent
*S	Significant
>	Greater then
ANOVA	Analysis of Variance
cm	centimetre
d.f	Degree of Freedom
E.S.S.	Error Sum of Squares
Et al.	And others/and all
F.tab	Tabulated value of F
F.cal	Calculated value of F
Fig.	Figure
g	Gram/s
hr	Hour
i.e.	That is
MESS	Error Mean Sum of Square
mg	Milligram/s
NS	Non-Significant
C	Celsius
ppm	Part per million
S.E.	Standard Error
S.S.	Sum of Squares
T.S. S	Total Sum of Squares
Viz.	Between

Vol.	Volume
µg	Microgram
IgM	Immunoglobulin M

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ABSTRACT

Background

Typhoid fever and enteric fever are highly significant and public health concern. It is found throughout the world. The infection is prevalent in the western world two centuries ago; however, progress in sanitation and medical care has reduced the incidence in developed countries. Unfortunately, TF continues to be a substantial health concern in developing countries of Asia and Africa. As per the statement of world health organization approximately 20 million people are affected by typhoid fever every year. TF, which is caused by *Salmonella enterica* serotype Typhi (S. typhi), presents difficulties in diagnosis on account of its wide range of symptoms. Blood and bone marrow cultures are considered gold standard procedures; however, more recent techniques such as Typhi IgM offer improved accuracy but not much researches conducted on this specially in India.

The main objective of this study is to conduct the comparison of tests available in market and check the rapidity, accuracy and also cost effectiveness of blood culture, stool culture, Widal and typhi IgM. In developing countries like in India if we take the example of Malwa region Punjab there are issues with typhoid testing like delay in diagnosis, concerns about the accuracy of Widal test which is the second most requested test by doctors after malaria test in the diagnosis of all infectious/fever diseases. In our targeted region maximum doctors were worried due to high false positive cases of typhoid which leads to misdiagnosis. The sensitivity of blood and stool culture ranges is high but before start of antibiotic treatment. As per the statement of three doctors who takes part in this study that many patients initialy takes the treatment from unauthorized practioners, chemist and sometimes self medication offered by google, so to opt culture techniques for such patients is a big challenge. However, the other reason

for conventional techniques which are less utilized in Malwa region of Punjab and other parts of countries is due to their time taking reporting and cost issues.

Material and methods

For the study the samples were collected form three different hospitals including private and government hospitals in Malwa region of Punjab, India. The study comprised of 450 participants, out of which 300 were classified by doctors as suspected typhoid cases and 150 were negative controls. Primarily history, sign and symptoms were recorded in the consent form, after that the blood and stool samples were collected and processed by using the available diagnostic techniques such as the Widal test, Typhi IgG/IgM test, blood culture and stool culture were recorded. The main purpose of the study is to find the accurate test for typhoid fever and to study the disease burden of typhoid on economy.

Result

The total samples of suspected patients were 300 and out of that Typhi IgM technique gave 21 true positive while stool culture and Widal both gave 10 true positive results. When it comes of true negative cases, typhi IgM and stool culture gave 270 and 274 true negative results out of 300 respectively but shockingly Widal test gave 112 true negative results in 300. Typhi IgM gives 9 false positive results while stool culture gave only 5 false positive in total 300 but surprisingly out of 300 Widal gave a huge number of 167 false positive results. Both stool culture and Widal gave 11 false negative results but here not even a single case is given as false negative by Typhi IgM. PPV of Typhi IgM and stool culture have not much difference with 70% and 66.66% values respectively but Widal gave a very low positive predictive value of 5.64%. NPV of Typhi IgM, stool culture and Widal is 100%, 96.14% and 91.05 respectively. Typhi IgM gave 100% true positive rate while both stool culture and Widal gave a very low TPR of 47.61%. True negative rate of Typhi IgM and stool culture is very close values of 96.77% and 98.2% respectively but again Widal has very low TNR of 40.14%. A very high false negative rate value of 52.38% noted for stool culture and Widal test but 0% FNR value noted for Typhi IgM test. False positive rate of Typhi IgM and stool culture is 3.22% and 1.79% respectively but a very high FPR value of 59.85% noted for Widal test. False discovery rate of Typhi IgM is 30% and stool culture is 33.3% but a very high FDR of 94.35% noted for Widal test. 0% false omission rate is noted for Typhi IgM test while stool culture and Widal gave 3.85% and 8.94% FOR respectively. So, in last prevalence threshold value of Typhi IgM and stool culture is 15.18% and 16.16% respectively but Widal gave a poor PT value of 52.83%. Finally, the study reveals the Sensitivity of both stool culture and Widal is 47.61% but a very encouraging 100% Sensitivity result value noted for Typhi IgM test. In the end when it comes to Specificity a very poor value of 40.14% noted for Widal test, Specificity of Typhi IgM and stool culture is 96.77% and 98.20% respectively.

The comparison as per Chi Square Statistics for Stool culture, widal and Typhi IgM when compared with Blood culture the p-Value of both Stool Culture and Typhi IgM is Less than 0.001 which shows significant association between these parameters and Gold Standard test, Less than 0.001 p-value shows that test has some predictive value. The p-value of widal in Chi Square Statistics is 0.286, which shows test does not have significant predictive value and no significant association between widal and blood culture test.

With Kappa Statistic in this study, a measure of agreement between each test's result and true disease status is calculated. Kappa Statistic value of Typhi IgM is 0.809 approx which indicates susbtantial agreement between the two diagnostic tests. Value of stool culture is 0.528 approx, again which indicates the substantial agreement between the stool culture and gold standard test. The Kappa Statistic value of widal is -0.0276 approx. The negative value of widal indicates no agreement between gold standard test and widal test.

Conclusion

As per the statistic of this study the substantial burden of typhoid with a incidence rate of 72.3 per 1000 population per year. The Morbidity of typhoid as per study is 70 per 1000 population per year. But in this study Mortality is 0%, as no death noted in 300 suspected patients included in the study. Mortality rate is 0% because of good quality medical facilities available in Goverment hospitals specifically in mohalla clinics where initial treatment is provided to patients. Mortality rate is 0% in 300 suspected cases as investigations and medicine is totally free in mohalla clinics of punjab so patient comes and meet directly to a MBBS doctor rather than taking medicine from unauthorized practioners in villages and chemists in urban areas. Out of 300 suspected cases, 102 patients were from rural areas and 198 from urban areas. But still typhoid is a big disease burden on country when we calculate the Lost Producivity of India due to typhoid it is 51141.5 crore Indian Rupees anually.

As per the study cost of Blood culture, stool culture, widal and typhi IgG/IgM is Rs 175, Rs 125, Rs 60 and Rs 50 respectively. So when it comes to cost effectiveness Typhi IgG/IgM is best out of these four parameters. As per the study if we pick the best test out of four parameters in rapidity context, Typhi IgG/IgM takes 20 to 30 minutes for reporting the results, blood culture reporting needs seven days, stool culture reporting needs seventy two hours and widal tube method needs one day as the test required overnight incubation. So Typhi IgG/IgM is most rapid test.

The most sensitive method to detect typhoid fever is Bone marrow culture but due to its invasive nature this technique also not used by either doctors and even patients also not agree to give samples Also, not possible for a doctor to wait without initiating treatment to patient as report will take approximately 48 hours.

Typhi IgG/IgM that detects IgM and IgG antibodies in *Salmonella typhi* shows the encouraging results with Sensitivity of 100% and Specificity of 96.77%. This is an immune chromatography assay designed for qualitative detection of *Salmonella typhi* in human serum. Critical success index and Accuracy of Typhi IgG/IgM is 67.44% and 97% respectively. There is a big space for research on Typhi IgM test. Overall study reveals that doctors needs to avoid Widal test as very low sensitivity of 47.61% and specificity of 40.14%. More studies needed to test the credibility of typhi IgM test. To diagnose Typhoid rising titres in widal have great importance but here one more benefit in Chromatographic techniques as it differentiate IgG and IgM infections separately.

CHAPTER-1
INTRODUCTION

1. INTRODUCTION

Enteric fever (EF) and Typhoid fever (TF) is significant causes of illness and death in the western world and other countries [1]. Due to advancements in general medical conditions and sanitation in developed countries the infection is in very much under control but TF is still a fatal illness in poor nations, especially in Asian countries [2]. In developing countries of Asia and Africa where EF is linked to subpar sanitation, contaminated water and food. Paratyphoid fever (PTF) as well as TF continue to be significant cause of disease as well as mortality. TF and PF are both systemic infections brought on by *Salmonella enterica*, which also includes serotypes Paratyphi (*S. paratyphi A, S. paratyphi B etc*) and Typhi (*S typhi*). Highly specialised pathogen that is specific to human is *S. typhi,* has extraordinary mechanisms for survival in its host. As a feco-oral communicable disorder, EF is most found in areas with high rates of untreated water, overcrowding and inadequate sanitation.

The illness is widespread until the first decades of the twentieth century, reaching both the Europe and USA. As per the estimation by the "Global Burden of Disease Study" 586 cases of Typhoid per 100,000 person years in India in 2017. Improvements in sanitation and hygiene were a contributing factor, but there haven't been many community-based studies in India, so these figures were largely approximated from regional data. Sadly, the effectiveness of preventative and control measures for EF has been reduced by the lack of predictions of the disorder's burden at the national level [4,5].

Since the beginning of the twentieth century, effective sewage systems and clean water have significantly reduced the frequency of TF in the United States and Europe [6], but the illness still poses a substantial public health threat in developing nations [7]. TF was once thought to be a serious, sometimes deadly illness.

An earlier 1990s prospective cohort research in the Delhi slums found that there were 980 cases of culture-confirmed TF per 100,000 people annually, with a substantially elevated occurrence of 2,730 cases per 100,000 kids under the age of Five [12]. Research from a metropolitan Bangladesh slum in 2001 found similar results of a high illness burden in young childerns, with an incidence of 1,870 cases per 100,000 pre-school childerns annually [13]. These investigations showed that, despite disparities in estimates from various contexts, TF was a substantial public health concern in India also, particularly among its young childerns. Despite the existence of numerous medications throughout the years for the treatment of TF, unacceptable rates of death and morbidity continue to be observed in underdeveloped nations [14]. Nevertheless, this hypothesise that diagnostic accuracy may vary among both childerns and grownups due to a higher degree of prior exposure to *Salmonella* and other pathogenic organisms in grownups, giving rise to serological cross reactivity. Previously meta-analyses concentrated on all age groups without differentiating performance among kids. If detected early on, TF can be effectively cured with antibiotics [15,16].

TF can present clinically with other infectious illnesses like rickettsia infections, melioidosis, malaria, dengue, and leptospirosis, making a diagnosis difficult to make without laboratory confirmation [17]. Due to the many factors involved, diagnosing and managing diseases is a difficult procedure. The uncertainty and ambiguity brought on by such factors add to its complexity [18,19]. These difficulties in medical practise render the traditional quantitative diagnostic techniques ineffective and need the development of new methodology since the efficacy of a certain disease's therapy is greatly reliant on the degree of accuracy of its diagnosis.

1.1 Microbiology of typhoid fever

Salmon (the pathologists) who initially separated the bacteria from intestines of animal, in his honour was given the name *Salmonella* (Smith & T. H. E. O. B. A.).. These microorganisms belong to the *Enterobacteriaceae* family and are facultatively anaerobic, gram-negative, nonencapsulated, flagellated bacilli. *S. enterica*, *S. choleraesuis*, and *S. typhi* were originally identified as 3 different species. Nevertheless, the categorization scheme was altered in 1983 after DNA tests revealed that *S. enterica* is the only species that covers the majority of *Salmonella* species. After this, the species was split into 7 subgroups based on host range and DNA homology: I, II, IIIa, IIIb, IV, V, and VI. The serotype typhi (subgroup I) is responsible for the majority of human illness [20]. Despite the fact that *S. typhi* is the name given to the organism that causes TF, it is important to understand that typhi refers to the serotype and not the species. Almost 2300 *Salmonella* serotypes have been identified using the Kauffman-White system based on the flagellar H antigens, somatic O antigens, and surface Vi (virulence antigen). The majority of serotypes have been given names based on the place where they were originally discovered [21]. This study substitutes the serotype, typhi, for the species name to keep things simple.

Salmonella can thrive on basic medium, but to avoid overgrowth by other intestinal organisms, selective media like Macconkey, Shigella-Salmonella or XLD agar are used. Nevertheless, agglutination assays may separate organisms into serogroups "A, B, C1, C2, D, and E based on the O antigen", even though most research laboratory do not execute complex serotyping. S. typhi is categorised as group D according to this classification, although this serogroup also contains other *Salmonella* [22]. The evaluation of strains linked to outbreaks can benefit from phage typing and pulsed field gel electrophoresis.

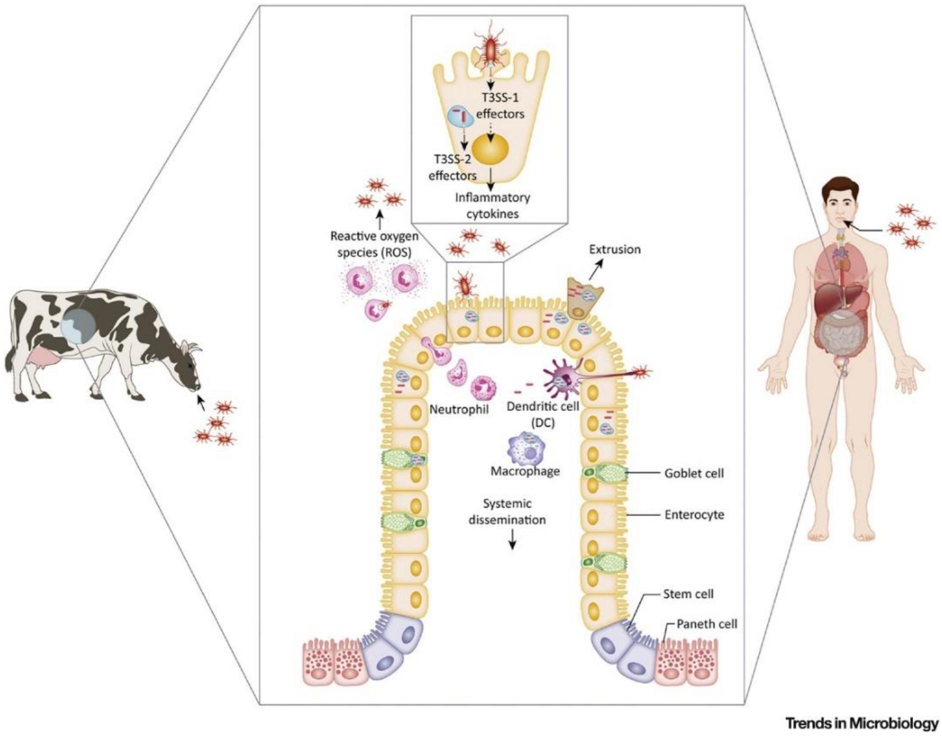


Figure 1. Pathogenesis of *Salmonella Sp.*

1.2 Epidemiology of Typhoid Fever

Salmonella infection in humans results in 2 main categories of clinical symptoms. First, EF, which is mostly brought on by *Salmonella enterica* serovar typhi and Paratyphi (PTF) A, B, or C, is spread by infected water and/or food. The other is that several nontyphoidal Salmonella serovars (NTS) are responsible for a variety of clinical disorders involving diarrheal illness [23].

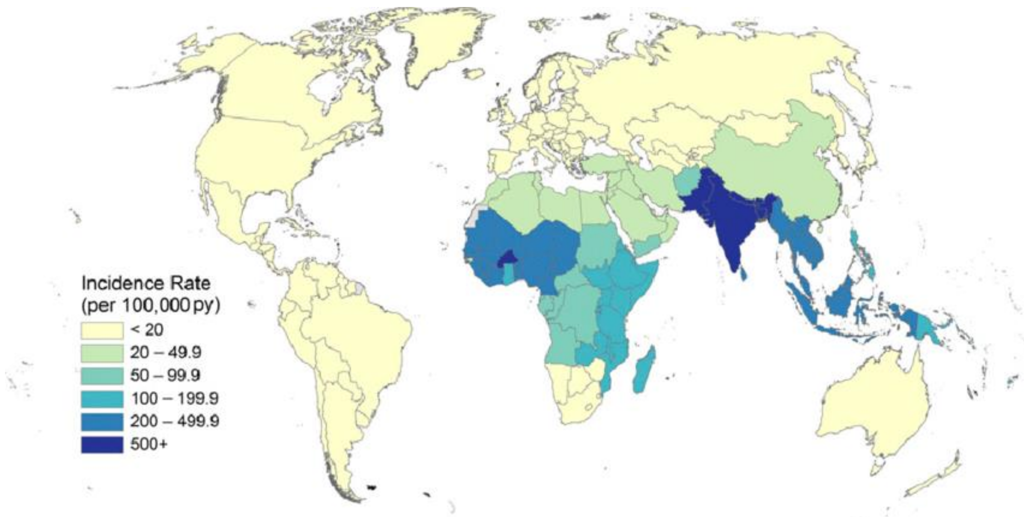


Figure 2. Global estimates of TF and PTF deaths per million in 2015, broken down by nation [24].

In addition to an expected 21 million cases and among 200,000 to 600,000 fatalities each year, *Salmonella typhi* infestations are well acknowledged as a major source of sickness on a worldwide scale [25]. The differential estimates most likely reflect disparities in the techniques employed to calculate the attributable death and age-specific burden of typhoid [26]. Young childerns constitute a cohort with the largest incidence of TF in different parts of the globe, particularly South Asia [27], and may also have hugely disproportionate rates of death and comorbidities.

TF has a modest prevalence in most African nations, with 10 to 100 cases per 100,000 individual years being the most common age group, according to some estimates. The incidence in East Africa was calculated to be 39/100,000 person years. Growing volumes of information on the frequency of various diseases discovered in ill children visiting medical institutions in Africa have recently been published. These findings have significantly increased awareness of non-typhoidal salmonella infections [30]. Although these figures give a general idea of the TF burden, it is still required to take methodologies that separate the risk variations by demographic categories within nations.

1.3 Pathogenesis of typhoid fever

Over 10,000 cells of *S. typhi* have been shown to be required for an active infection among human beings, however this number is variable depending on the environment, host and immune status of the infected. By using tight junction (TJ) components to invade gut tissues or cells by encouraging signalling reactions that facilitate their invasion, enteropathogenic microorganisms degrade the gut epithelia [33,34]. According to reports, *S. Typhi* invasion of the gut epithelia increases TJ permeability [35].

Moreover, investigations have indicated a reduced number of the bacteria [36,37], Microfold cells (M cells), which the pathogen often uses to assault the mucosal lining of the intestines, contribute to generate an undetected bacterial burden in the lack of clinical symptoms and signs, leading to general bacteremia. As a result, although the bacteria can enter the host system, an immediate immunological response from the host is not always triggered. This is an essential characteristic of *S. Typhi* infection, which differs from infections brought on by nontyphoid serovars of *Salmonella spp.* in that the host lacks the major inflammatory response. It has been shown that the potential of bacterial species to penetrate nonphagocytic cells through expression of a "type III secretion system (T3SS), known as T3SS-1", corresponds with their capacity for gut mucosal penetration. As *Salmonella spp*. enter the small intestine, multiple factors, including increased osmolarity and iron content, reduced Oxygen concentration, and neutral pH, stimulate SP-1 expression [38]. Membrane rearrangements and actin polymerization are encouraged by T3SS-1, which also facilitates the direct insertion of effector proteins of bacteria into the host cells. This results in bacterial internalisation. This process, sometimes referred to as the trigger process, is heavily reliant on cells of host [39]. The pathogen's post-infection incubation period may not always be visible as a symptomatic period.

Table 1: TF's pathological alterations [40]		
Sr.No.	**Organ**	**Pathological Changes**
1.	Skin	Skin alterations with the accumulation of bacilli that result in the traditional rose spots
2.	Heart	Fatty degeneration may cause the heart to expand.
3.	Spleen	Spleen enlarges and softens.
4.	Lungs	TF frequently results in bronchitis.
5.	Gall bladder	Infected gall stones that develop in the gall bladder as a result of cholecystitis might be a serious infection vector for TF carriers.
6.	Liver	With fatty alterations, the liver grows larger.
7.	Peyer patches	Peyer patches (PP) can have many conditions, including as open ulceration, typhoid perforation, ulceration, and hyperplasia.
8.	Kidney	Albuminuria may be caused by the hazy swelling that the kidneys exhibit.

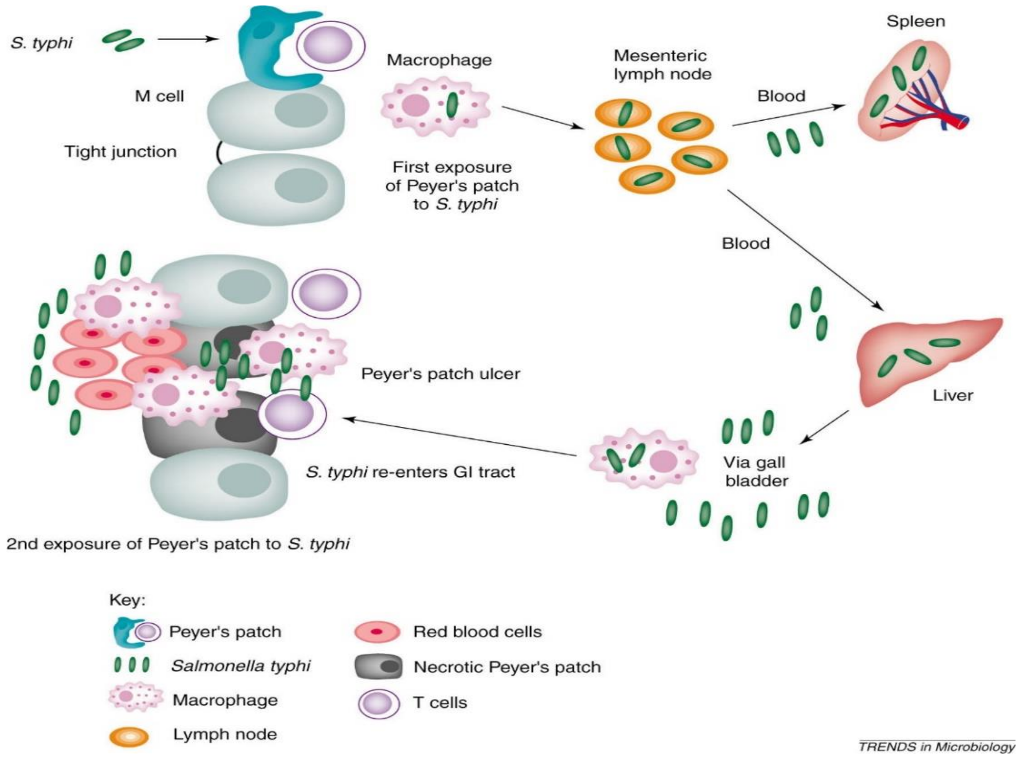


Figure 3. Pathogenesis of Typhoid fever, specifically focusing on the role of Peyer's patches in the small intestine. [41].

1.4 Signs and Symptoms

One of the most prevalent febrile infections in developing nations is TF. Fever and malaise start to appear after the seven to fourteen-day incubation period. Myalgia follow the fever, chills, lethargy, anorexia, headache, nausea, dry cough and unexplained stomach pain. A sensitive stomach, coated tongue, splenomegaly and hepatomegaly follow these [42]. Constipation is common in adults, but new-borns are more likely to have poisoning, diarrhoea, and consequences like disseminated intravascular coagulation (Parry). Neonatal TF is an uncommon but life-threatening and serious illness that may result from vertical intrauterine transfer from an affected mother. TF relapses and re-infections are frequent and happen in fewer than 10 percent of cases. Only molecular typing can differentiate between relapse and reinfection [43].

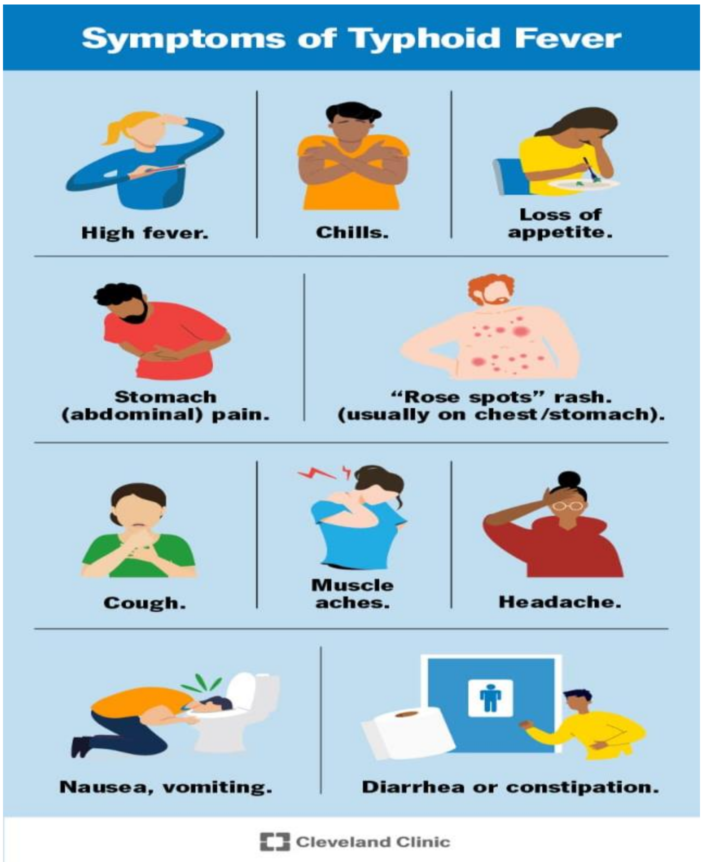


Figure 4. Symptoms of Typhoid.

1.5 Diagnosis of typhoid fever

TF must be accurately diagnosed at an early stage in order to determine the aetiology and to identify those who may be prospective carriers and cause acute typhoid fever epidemics. TF's describing symptoms are varied as well as comparable to those seen with other delirious disorders, making a clinical diagnosis challenging.

The WHO advises *S. typhi* isolation from blood or bone marrow for a TF confirming diagnosis [44]. The gold standard for diagnosis is regarded to be bone marrow culture. Yet, due to its invasive nature, bone marrow aspiration is not frequently performed. Blood culture is the primary method of diagnosis even though its sensitivity ranges from 40 percent to 87 percent [45]. Alternative diagnostic techniques, such as the Widal (81.5 percentage sensitivity; 18.3 percentage specificity) and Typhidot (67 percentage sensitivity; 54 percentage specificity) tests, have low sensitivity and specificity when it comes to identifying TF [46,47,48]. TF patients are often diagnosed in Africa and Asia based on clinical judgement even if the presenting symptoms and signs are hard to differentiate from those of other febrile infections [49].

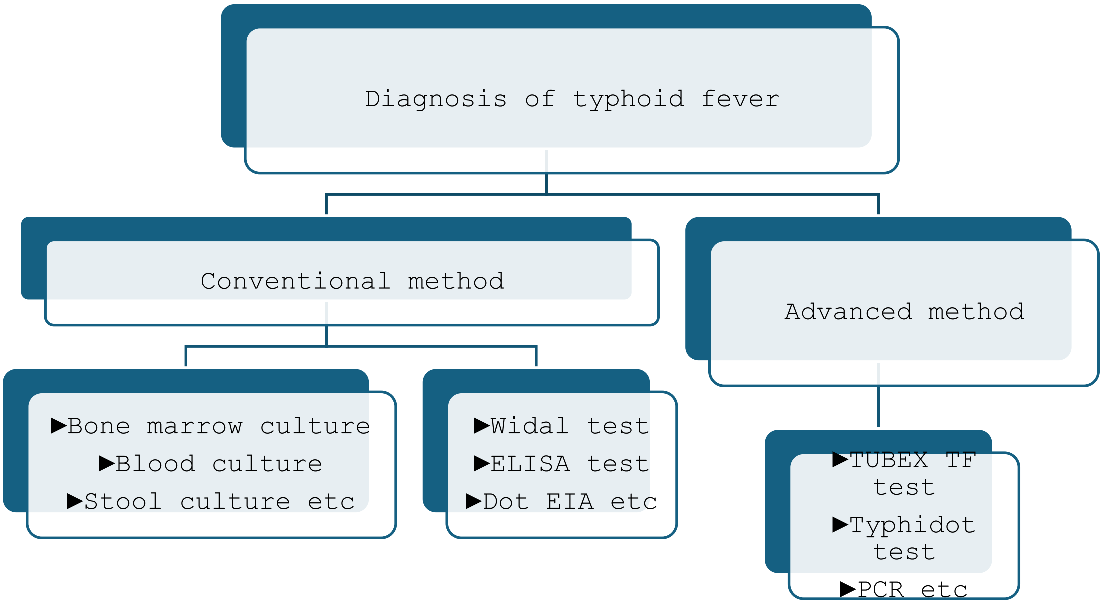


Figure 5. Flowchart showing Different diagnostic methods of Typhoid fever.

1.5.1. Accuracy, sensitivity and specificity of Conventional methods for typhoid diagnosis

The likelihood of a positive test result, supposing the subject is indeed positive, is known as sensitivity (true positive rate). The likelihood of a negative test result, supposing the subject is indeed negative, is known as specificity (true negative rate). TF is still now diagnosed using the Widal test for antibody detection in combination with culture. *Salmonella typhi* isolation has continued to be the gold standard, with bone marrow aspirate culture or a mix of specimens from stool, urine or blood as backups. Yet, it is well acknowledged that cultural amenities are scarce or limited in many places. The culture approach may be specific, but it is also slow and lacks sensitivity. TF is well recognised, although if the culture is negative, findings take 2–7 days to appear [51]. Self-medication practices are notably widespread among patients in India, often leading to delayed hospital presentation, particularly in the early stages of infection.

Blood culture exhibits heightened sensitivity during the first week of infection, contrasting with the non-specific reactivity characteristic of the Widal test, which underscores the diagnostic preference for culture-based methods in early typhoid detection.

1.5.2. Blood culture technique

Collect the samples of blood in aseptic manner before any antibiotic treatment [55]. 10ml blood is needed by venipuncture which is inoculated into a culture bottle containing 50ml of 0.5% bile broth [56]. Blood culture to detect *Salmonella* is performed using XLD (Xylose lysine deoxycholate) agar[52]. XLD contains bile salts and sodium deoxycholate which inhibit the growth of gram positive bacteria[53]. Pink colonies due to xylose fermentation with black centers due to H2S production appears on XLD[54].

- **Bacterial culture**

TF may be diagnosed using the gold standard diagnostic procedure, which is blood culture [57]. Blood culture (BC) sensitivity is greatest during the initial week of sickness and declines as disease advances [58]. The organisms can be identified in the bloodstream at any point throughout the infection, although they are most frequently discovered in the first seven to ten days and during relapses. The preferred approach is blood culture, which has many advantages over cultures from urine or faeces[59].

The identification and isolation of the organisms in *Salmonella* cultures takes between four and seven days [60]. Culture technique is most trusted techniques by doctors but they often advise widal and other investigations due to urgency as culture is a time taking procedure. According to a study's findings, 50 ml of medium was sufficient for 10 ml of blood, perhaps due to the relatively low levels of bacteraemia in certain individuals [61].

- **Stool culture**

To isolate, cultivate and differentiate *Salmonella typhi*, subculture of enriched sample from the Selenite F broth is used on the surface of XLD agar in accordance with the protocol described by Prescott et al. (2005). The volume of faeces that are cultured determines the sensitivity of the stool test, and the positive rate increases with the length of the sickness. Thirty percent of individuals with acute typhoid fever had positive stool cultures [65]. Urine cultures are sensitive between 0 to 58 percent [66].

1.5.3. Serological Diagnosis

Widal test

A serology blood test called the Widal test helps diagnose TF or EF in the body. Georges Ferdinand Widal initially performed this test in 1896, and it bears his name. The Widal test is a sophisticated method of determining if the body has produced antibodies against the *salmonella* bacterium that causes typhoid illness. With a patient's blood sample, it searches for O (Somatic) and H (Flagellar) antibodies. Sera dilutions that have been doubled are used to assess the levels in big test tubes [67]. This test has a modest sensitivity and specificity while being reliable and easy to use [68]. Its sensitivity and specificity are both claimed to be between 70 and 80 percent and 15 to 45 percent respectively. Because of the antibiotic usage that reduced the antibody response, it may be negative in up to 30 percent of typhoid fever cases that have been confirmed by culture. Furthermore, TF individuals may not mount any measurable antibody responses or see any discernible increases in antibody titres. Interestingly, *S. enterica* serotype *typhi* shares both these cross-reacting epitopes and these antigens with other Enterobacteriaceae serotypes. This can lead to inaccurate positive outcomes. These outcomes may also be present in other clinical diseases, including as typhus, bacteremia, cirrhosis, and malaria brought on by other species. The Widal is probably the test of choice in many underdeveloped nations due to its cheap cost.

1.5.4. Accuracy, Sensitivity and Specificity of Advanced methods for typhoid fever

In order to develop new diagnostic techniques with greater sensitivity and specificity suited for individual circumstances associated with past-infection history and vaccination, we must further our knowledge of pathogen-host interactions, with a focus on the bacteriological antigens involved, and host responses against the pathogens during diverse phases of infection. The current conventional tests lack specificity, speed, and sensitivity. Innovative diagnostic methods and New specific antigen have been used to get around the limitations of the current assays.

Table 2. Recent status diagnosis of TF.		
Test Name	**Advantages**	**Disadvantages**
Bone Marrow Culture	**1.** Most sensitive and specific	**1.** Invasive with medical complications **2.** Require technical expertise **3.** Cost issues **4.** Require specialized equipments and training **5.** Painful
Widal Test	**1.** Cost effective **2.** Timely reporting	**1.** Cross reactivity issues **2.** Low sensitivity and low specificity **3.** Tough to establish baseline titer among healthy individuals **4.** misinterpretation
Typhidot Rapid Chromatography	**1.** Rapid test **2.** Cost effective	**1.** Less tested yet

Stool culture and Blood culture	**1.** High sensitivity and high specificity	**1.** Required expertise technical staff **2.** Cost issues **3.** Not feasible in children's as 2-5ml blood required **4.** Takes 48hrs to 7 days **5.** Sensitivity varies when bacterial levels are low

Objectives of the study

The current study has the following objectives:

1. To clinically evaluate the suspected cases of typhoid fever.
2. Isolation and identification of the typhoid causing bacteria from the samples.
3. To study the rapidity, accuracy and cost of the typhoid fever detection methods.
4. To determine the epidemiology and disease burden of typhoid and paratyphoid fever.

CHAPTER 2
REVIEW OF LITERATURE

REVIEW OF LITERATURE

The Gram-negative, facultative anaerobic bacillus *S. enterica subspecies serovar Typhi* is the cause of typhoid disease (*S. typhi*). The related bacterium *S. enterica subspecies serovar paratyphi* (*S. paratyphi*), divided into *S.* paratyphi A, B, and C, is the cause of typhoid fever. A clinical condition known as enteric fever may result from infection with *S. typhi*, *S. paratyphi*, or both. In contrast to other *S. enterica* serovars, *S. typhi* and *S. paratyphi* are human-specific infections that cause systemic illness in certain persons before transitioning to an asymptomatic permanent carrier state. The disease is specific to humans and is characterized by abdominal pain, fever,hypotension, malaise, hepatic problem, vomiting, headache, transient rash, slow heartbeat, splenomegaly, cough, and leukopenia. The most frequent severe complications are intestinal bleeding and perforation [115].

Typhoid fever is thought to affect 26.9 million people annually worldwide, while exact figures are difficult to determine [116]. This is because many places lack adequate diagnostics, screening procedures are not carried out if the patient is asymptomatic, and the presenting symptoms and indications are not precise. Although the Indian subcontinent is believed to have the highest typhoid fever incidence, the disease is distributed somewhat unevenly [117]. Typhoid incidence in sub-Saharan Africa has historically been underreported [118].

Statistics from the Typhoid Surveillance Program in Africa and other organizations show that typhoid may be as common in African regions as in Asia [119] and that both rural and urban inhabitants are affected [120]. The Global burden of disease 2017 study found that South Asia had the highest age-standardized incidence rate, which is 549 cases per 100,000 persons, and the most cases accounting for 71.8% of all cases worldwide, while sub-Saharan Africa had the second-highest rate, i.e., 12.1%, of all the regions [121].

Typhoid/paratyphoid incidence in India was estimated by the GBD study in 2017 to be 586 cases per 1,00,000 people; however, these figures were mostly extrapolated from regional data because of the lack of population-based studies in India [122]. For Punjab in 2017, the incidence varied between 427 cases per 1,00,000 people [123]. While a

2006 study in Kolkata predicted an incidence of 265 cases per 100 000 people, and a 1996 study in Delhi showed an incidence of 976 cases per 100 000 people in a year [124].

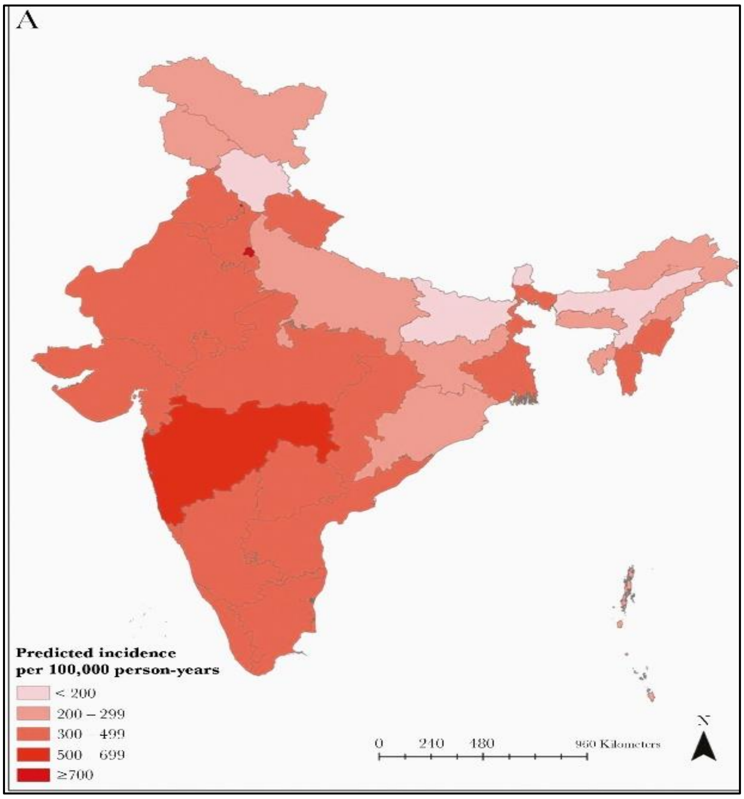


Figure 6: Incidence rates (per 100,000 people) of typhoid and paratyphoid fevers in India (2017) [125].

Consuming contaminated food and/or water may result in the short- or long-cycle transmission of *S. typhi* and *Paratyphi*. Short-cycle transmission is described as contaminating food and water in the nearby environment due to poor sanitation and hygiene practices, either through chronic or acute carriers' shedding. Long-cycle transmission is the term used to describe environmental contamination, such as sewage contamination of water sources or poor piped water treatment. Depending on the epidemiological environment, the proportional contributions of each transmission route may differ between *S. typhi* and *Paratyphi* [126].

The gold standard method for typhoid fever diagnosing infection is *S. typhi* isolation is from blood; however, their sensitivity is limited. Even though bacterial culture from bone marrow is more precise, it is also more invasive and not practicable for everyday use. A serological test called the Widal test can find antibodies that clump together when exposed to the H and O antigens [127]. Due to its simplicity and low cost, it is widely used despite its low sensitivity and the fact that values change substantially depending on the location [128]. Nucleic acid amplification assays, such as RT-PCR, conventional PCR, and nested multiplex, have made it possible to identify *S. typhi* DNA in the blood (PCR). The high expenses of this technology are the principal obstacles preventing its broad application in low-resource environments [129].

Specific commercially available typhoid rapid antibody tests can produce outcomes quickly, enabling quick treatment with antibiotics for those with a positive result. Using pre-dotted antigen strips, immunoglobulin IgG and IgM antibodies to an outer membrane protein are detected in the Typhidot qualitative test.

To detect 99% of the 80 bacteremic events, Washington et al. (1975) found that 20 ml of blood for blood cultures were required three times: the first blood culture produced 64 episodes, the second blood culture had 70 episodes, and the third blood culture made 79 episodes [132].

Weinstein et al. (1983) published the findings from 282 bacteremic adults from whom 17 milliliters of blood were drawn for each culture. The first blood culture produced 258 episodes that could be detected in these people, whereas the second produced 280 episodes [132].

Pang and Puthucheary (1983) evaluated the Widal test's diagnostic value in an endemic area. They tested 300 healthy individuals, 297 nontyphoidal fever cases, and 275 typhoid cases with bacteriological evidence. Agglutinin titers of 1/160 were found in 2% to 5% of 300 healthy individuals. These criteria revealed that considerable H and 0 agglutinin titers of 1/320 or higher were identified in 93% of typhoid cases and only 3% of individuals with nontyphoidal fever. Sera from typhoid infections exhibiting a significant Widal reaction were elevated in both H and 0 agglutinins in 51 of 234 (21.8%). During the first week of the sickness, these sera were collected. Typhoid morbidity and mortality in endemic regions may be decreased by using a Widal test as a diagnostic tool in the early stages of the infection [133].

Comparing the cultures of bone marrow, duodenal strings, blood, and rectal swabs in an Indonesian investigation by **Hoffman et al. (1984)** involving 118 patients yielded positive rates of 86%, 58%, 54%, and 36% respectively. However, the overall diagnosis rate increased to 98% when all four were considered, demonstrating that multiple site cultures can boost diagnostic sensitivity[134].

Rai et al. (1989) studied the indirect fluorescent antibody test using single serum samples and discovered it was more rapid, specific, and sensitive than the Widal test. Serum samples for typhoid fever were collected from 22 healthy individuals, 14 clinically suspected cases, and 41 patients with culture-proven issues. Whereas IFA testing picked up 87.80% of typhoid cases that had been proven culturally, Widal testing only picked up 63.41% of positive cases. The Widal test detected just 57.13% of the individuals with clinically suspected typhoid fever, but the IFA test identified 85.71% of the cases [136].

Kulkarni, M. L. et al. (1994) looked into the effectiveness of a single Widal test for typhoid disease diagnosis. Fifty children with nontyphoidal fevers, 50 children with standard immune systems, and 30 cases of typhoid confirmed by culture underwent the test. Thirty cases of typhoid fever were evaluated, and 21 and 9 of the patients, as opposed to 3 and 1 of the controls, respectively, had "O" and "H" agglutinin titer levels more than or equal to 1:160. These variances were notable. Thirty cases of typhoid fever were examined, and only three had "O" or "H" agglutinin liters more than or equal to 1:160 in contrast to controls. A 1:160 titer for "O" agglutinin was used, with 90% accuracy, 97% specificity, and 70% sensitivity. An H agglutinin's specificity, sensitivity, and accuracy at a titer of 1:160 were 97%, 30%, and 83.1%, respectively. Using the data from the study above, the "O" or "H" titer of 1:160 or greater was considered indicative of typhoid fever. Similarly, to that, when both "O" and "H" liters were considered, they were either suggestive or had a ratio of 1:160 or greater [138].

Typhoid fever was assessed using the Typhidot test by **Jesudason M et al. (2002)**. 39 samples were positive for both IgG and IgM on the Typhidot test, whereas 24 samples positive for IgM and 2 samples only tested positive for IgG. Typhidot was present in 27 of the 30 samples that tested positive for culture. The Typhidot test has a specificity of 80% and a sensitivity of 100% in bacteremic subjects. It will be a valuable additional test to the blood culture and Widal test for the typhoid fever diagnosis [140].

Parry, C. M. et al. (1999) investigated a single-tube Widal test for probable typhoid infection in the acute phase's diagnostic value in 2,000 Vietnamese patients. The test subjects were assumed to have typhoid fever because their blood cultures for *S.typhi* or *paratyphi* A were positive. The Widal test could correctly identify 74% of typhoid fever cases with positive blood cultures[141].

Raza, A. et al. (2001) experimented with comparing the three most current diagnostic methods PCR, Typhidot and widal. PCR, Typhidot and Widal tests were used to analyze blood samples from 20 patients with suspected early typhoid and ten controls. Using polymerase chain reaction, Typhidot and widal test the percentage of patients who tested positive for typhoid was 85%, 65%, and 60%, respectively. For controls, the corresponding numbers were 0, 40%, and 20%. Widal test and Typhidot are superior in specificity, but PCR is superior in sensitivity [142].

To compare the commercial Typhidot and PanBio ELISA kits with the most recent Widal test, **Gopala Krishnan, V. et al. (2002)** Of the fifty sera that tested positive for *Salmonella typhi*, fifty were Widal test positive, but blood culture is negative for typhoid disease. The gold standard for determining the tests' specificity, sensitivity, and efficacy was a positive culture for *S. typhi*. ELISA, Widal and Typhidot M kits' sensitivity, specificity, and test effectiveness were 78%, 76.6%, and 84.0%, respectively. In the field, especially at smaller hospitals with fewer amenities, the Typhidot M appears to be a workable substitute [143].

Ismail, T. F. et al. (2002) assessed the specificity and sensitivity of the dipstick test used by the Royal Tropical Institute of The Netherlands. Just 14 of the 25 people who had typhoid that had been verified by culture had Widal titers less than 1:160. Ten samples were from people whose blood cultures were negative but had serologic evidence by Widal or agglutination that responded to the appropriate dipsticks. As typhoid dipstick techniques depended on IgM detection in acute infections, they could not identify antibodies specific to chronic carriers. It has been recommended that enzyme-linked immunosorbent assays or passive hemagglutination be used to accomplish such discrimination in epidemiological surveys. This test is an easy, quick, and accurate way to identify typhoid illness [145].

Two hundred nine feverish patients presented to a hospital in Makassar, Indonesia, in research by **Pastoor R et al. (2008)**. Of them, *116* (55.5%) had typhoid fever, later confirmed in 54 (24.8%) by blood culture.[151].

To determine the factors that might be responsible for Widal test negativity in an endemic area, **Hosoglu, S. et al. (2008)** conducted research. Of the 166 cases of typhoid fever with a culture included in the study, 56.0% were men. The Widal test was successful in 75 instances. The statistical analysis concluded that no factors contributed significantly to the false negativity of the Widal test. A possible contributing factor for a false negative Widal test was shown to be age (p=0.06). Age and any other compatible clinical signs should be considered when the Widal test is negative [152].

Narayanappa, D. et al. (2010) conducted a study to assess the specificity and sensitivity of the Widal test and Typhidot-M with blood culture for *S. typhi* diagnosis in 105 children who were admitted after having a typhoid fever clinical suspicion. Forty-eight had positive Widal tests for *S. typhi*, 41 had positive blood cultures, and 78 had positive Typhidot-M tests. The findings of the Widal test were 42.8% and 34.1%, but the Typhidot M specificity and sensitivity were 37.5% and 92.6%, respectively. Typhidot IgM was positive in 97.3% of kids with fevers lasting fewer than seven days compared to the Widal test. A rapid and reliable test for detecting typhoid illness in children is Typhidot IgM [156].

Beig, F. K. et al. (2010) compared the Diazo assays and Typhidot M to blood cultures and Widal tests in youngsters. Blood cultures were positive in just 27.3% of the patients. With 100%, 90%, 92.1%, and 100% values, respectively, the Typhidot M test showed the most significant specificity, sensitivity, NPV, and PPV among these culture-positive cases. The Diazo test came in second with results of 85.7%,86.7%, 88.2%, and 83.9%. The total sensitivity of Widal, Diazo, blood culture, and typhidot M in patients with clinically suspected typhoid was 64.6%, 80.9%, 27.3%, and 89.1%, respectively. The most sensitive test during the first week of illness was Typhidot M (86.2%), followed by Widal (41.4%), Diazo (79%), and blood culture (31%). Both Diazo and Typhidot M are reliable screening tests for the identification of typhoid disease [157].

Nakhla, I. et al. (2011) evaluated the diagnostic potency and clinical usefulness of the IgM Lateral Flow tests and Dri-Dot for the serodiagnosis of typhoid disease. For samples taken at the initial diagnosis, IgM Lateral Flow and the Dri-Dot showed a specificity of 86.3% and 71.4% and a sensitivity of 71.4% and 80%, respectively. Paralleling the two tests raised the sensitivity to 84.3% but reduced the specificity to 70.5% [159].

Aziz, T. et al. (2012), In a study looked into the Widal test's effectiveness in detecting typhoid sickness. Widal tests to determine the epidemiology of typhoid fever. The test outcomes were compared to those from standard blood culture assays. The slide agglutination tests and Widal tube used in the study had specificity, sensitivity, negative predictive value, and positive predictive value of 62 percent, 71 percent, 31 percent, and 91 percent, respectively. Since the Widal test is more practical, less expensive, and quicker than other molecular and serological tests, it will continue to be effective as a typhoid fever diagnosis tool [166].

Early *S. typhi* case identification using the Widal test and IgM efficacy was evaluated by **Sultana S. et al. (2012)** evaluate Widal test and IgM in the early detection of instances of typhoid fever. People who were clinically suspected of having *serovar typhi* were 150, and the controls were 50, who were of the same age and gender. Out of 150 samples, only 67 blood samples from the patients proved positive for *S. typhi* by Widal test and 106 by IgM out of the 150 samples. In contrast, 6 of the 50 controls tested positive for Widal and 4 for IgM. For IgM 92.00%, 83.3%, 91.9%, and 83.6% were found to be its specificity, sensitivity, PPV, and NPP. Conversely, the matching Widal test results were 44.4%, 88%, 80%, and 59.5%. Hence, the IgM may replace the Widal test as a faster and more accurate method of diagnosing typhoid illness. The IgM is rapid, easy to use, field-applicable, extremely sensitive, and specific for identifying antibodies in typhoid fever patients [167].

Ramyi, M. S. et al. (2013) evaluated the "Widal" test and stool culture's diagnostic performance in the laboratory for salmonella infection diagnosis in both adults and children. Of the 91 patients, 43 were children, and 48 were adults. Overall, 12 adults tested positive for stool culture, including ten men and two women, while nine children did, including seven men and two women. Thirty subjects passed the Widal test, with 17 men and 13 women. 8 males and five females were positive, for the 13 children who tested positive. According to the statistics, men were generally more impacted than women, and adults were more affected than children. The Widal test was more sensitive, allowing the stool culture to be more specific in children and adults. So, to distinguish salmonella infection from other diseases, medical professionals should use various diagnostic procedures in addition to the Widal test to identify enteric fever [168].

Typhi dot, Widal test, and blood culture's specificity and sensitivity were assessed in research **by Balakrishna TP et al. (2013)** for the typhoid disease diagnosis. Blood cultures for *Salmonella typhi*, Widal and Typhidot were positive in 14%, 21.5%, and 27.5% of the 200 individuals with a clinical typhoid diagnosis. The two methods that are typically used to diagnose typhoid disease are the Widal test and blood culture. A quick serological test that may be performed to diagnose typhoid sickness is the Typhi dot test. The IgM and IgG antibodies against the outer membrane protein of *Salmonella typhi* are sought for by the Typhi Dot Test. In instances when the diagnosis was supported by culture, it has the benefit of being fast and accurate, with a sensitivity of 92% and a specificity of 83% [169].

Sanjeev, H. et al. (2013) assess the specificity and sensitivity of Typhidot in the typhoid disease diagnosis. Blood cultures from 33 of the 50 individuals were positive. Widal tests were favorable for 33 patients, including 26 with promising blood cultures and 7 with unfavorable blood cultures. Thus, in contrast to the standard gold test, the Widal and typhoid tests showed à sensitivity of. 76% and 100%, and specificity of 58.82% and 78.78%, respectively. The results show that Typhidot has a high sensitivity and strong specificity, making it a viable alternative [170].

Danu et al. (2013), undertook an experiment to examine the outcomes of the immunochromatographic assay of the Widal test for Typhi IgG and IgM with the goal of early illness detection and infection treatment. One hundred samples were examined, and 48% passed the Widal test. In 52% of the cases, IgM sample findings were favorable [172].

Lalremruata, R. et al. (2014) investigated the Widal test's efficacy for typhoid fever in children diagnosis. 7 out of every 100 patients had typhoid fever, according to their findings. Applying a cut-off of 100 for H agglutinins or 50 for O agglutinins, the Widal test's specificity was 47.31 percent, sensitivity was 71.43 percent, PPV was 09.25 percent, and NPP was 95.65 percent. The Widal test is simple, affordable, and comparatively non-invasive, but due of a low PPV, it is unreliable in their setup. A more effective fast diagnostic test for typhoid fever is required [173].

Sood, N. K. et al. (2014) investigated an effort to compare the outcomes of semiquantitative slide agglutination with the quantitative tube technique. To identify any notable differences between the two methods, 17 (5.7%) of the 300 samples analyzed for serum samples tested positive using the slide test method, and 25 (8.5%) tested positive using the tube test method. The study shows a slight difference between the two Widal test methods. Since it is quick, simple to apply, inexpensive, and appropriate for use in nations with low resources, the Slide Widal agglutination test is an excellent replacement screening test. Higher sensitivity and specificity in excluding prozone occurrences may be attained using the Tube Widal test compared to the Slide Widal test [180].

Among febrile hospitalized patients, **Maude, R. R. et al. (2015)** investigated typhoid fever from three rapid diagnostic tests and its efficacy. Among 300. feverish patients aged 5–31 years with no evidence of malaria, 34 had confirmed typhoid fever, with 19 having positive blood cultures for *S. typhi* and 15 having negative blood cultures but positive blood PCRs. Patients with typhoid fever were confirmed by PCR and blood culture; the three RDTs' respective specificity and sensitivity were 74% and 59% for the CTK IgG and IgM, 96% and 24% for the SD Bioline RDT IgG and IgM, and 61% and 59% for LifeAssay. The LifeAssay RDT showed a specificity of 91% and a sensitivity of 63% when it was assessed using a Bayesian latent class model and adjusted with a positive cut-off of ≥2 [186].

Ameya, G. et al. (2017), in typhoid-suspected patients attempted to compare stool culture and the Widal test and assess the consistency between test techniques. Ninety-five patients in total took part in the study. Only 19 of the analysed cases exhibited positive stool culture results for *S. typhi*, while 65 had positive slide agglutination Widal test results. Slide agglutination test against stool culture revealed sensitivity, specificity, positive predictive value, and negative predictive value of 84.2%, 35.5%, 24.6%, and 90.0%, respectively. While the tube titration test and stool culture have a decent agreement, the stool culture and slide agglutination test has a poor agreement. Compared to a stool culture, the Widal test offers superior sensitivity and NPV but lower specificity and PPV. Moreover, it does not agree well with stool culture [195].

Udayakumar, S. et al. (2017) examine the diagnostic value of the Widal and Typhidot-M test in the early diagnosis of enteric fever in terms of specificity and sensitivity. From the 270 children who participated in the study, *Salmonella typhi* was isolated from 82 samples (30.4%), whereas blood cultures were negative in 188 samples (69.6%). One hundred seven children (39.6%) tested positive for Widal, and 136 (50.4%) tested positive for Typhidot-M. For the Widal test, the specificity was 79.3%, the sensitivity was 78%, the NPV was 91.4%, and the PPV was 59.8%. The Typhidot-M test has an NPV of 91.4%, a PPV of 69.8%, a specificity of 84.6%, and a sensitivity of 81.7%. A test that provides accurate and early detection of EF is required to properly manage children and reduce morbidity and mortality. Typhidot-M and Widal both tend to correlate less favorably with blood culture [197].

Dinkar, J. K. et al. (2017) found that the Widal test had the highest sensitivity, and the Typhidot-based assay had the highest specificity. Out of 120 people with clinically proven instances of enteric fever. Comparing the Widal test, the Typhidot-based IgG and IgM assay, and blood culture (the gold standard) to diagnose enteric fever, *18* (15%) had blood cultures that showed signs of *Salmonella typhi*, 27 (22.5%) had Widal tests that showed signs of the bacteria, and 36 (30%) had Typhidot test. *S. typhi*dot (IgM & IgG assay) tests are quick, easy screening procedures that help identify present or historical exposure by simultaneously detecting and differentiating between IgM and IgG antibodies to *Salmonella typhi* and *Salmonella paratyphi* generated in response to infection [199].

Subhani et al. (2017) examine the Typhidot test, Widal test, and blood culture to diagnose enteric fever. This study contained 150 samples in total. Blood samples were obtained to conduct the Typhidot, Widal, and culture tests. Of these 150 specimens, 31.33% were positive after blood was cultured; positive findings for the Widal test were 40.76%; and positive results for the Typhidot test were 51.34%. Typhidot-M is a good substitute for the Widal test if done within a week of infection [200].

Salama, R. I. et al. (2019) compared the Typhidot, a quick antibody detection test, and the Widal test, the most popular for typhoid fever diagnosis in Egyptian adults. Blood cultures on 45 of the 140 individuals revealed typhoid illness. *In a study* p. 89.5, Typhidot test results were positive in 42 patients, with diagnostic accuracies of 90.6%, 93.3, 82.3%, and 96.6%, for specificity, sensitivity, PPV, and NPV. The Typhidot test seems more dependable, straightforward, extremely specific, and sensitive for diagnosing typhoid illness when compared to the Widal test [201].

As per the study of **Shahapur PR et al. (2021)**, just 15 samples in the ICT were IgG-positive, as opposed to the 24 samples that tested positive for IgM antibodies. The Widal test revealed 27 instances to have antibodies to the Typhi 'O antigen from *S. enterica*. The Widal test was claimed as being more exact, specific, and sensitive than the ICT. (2021). The diagnostic usefulness and effectiveness of ICT versus the conventional Widal test in the typhoid fever diagnosis were compared [209]

Rani, E. et al. (2021) performed the Widal and Typhidot tests—two widely used fast serological tests for identifying typhoid disease—were evaluated for specificity and sensitivity. Of the 580 blood samples, *223* were Widal positive., while 357 were typhidot positive. Typhidot is a rapid and better test for typhoid illness early detection. It is now commercially available in regions with low resources to diagnose typhoid fever—the specificity and sensitivity of 62.4% and 37%, respectively, for the Widal test. The Typhi dot test yielded 56.5% specificity and 41.4% sensitivity [211].

The specificity and sensitivity of the Typhidot test and the "Widal test" for the diagnosis of enteric fever were evaluated in a **Singh R et al. (2021)**. *Typhidot Test* (35.33%) and slide agglutination test for Widal (24%), employed to diagnose the 150 cases of enteric fever that are clinically suspected in the study, had a favorable rate for *Salmonella typhi* and *Salmonella paratyphi* of 44.83% each. The study emphasizes using Typhidot for early enteric fever diagnosis and completing cross-checks with the blood culture and Widal test for enteric fever diagnosis. Typhidot assays are simple to carry out and can even be completed by unskilled personnel. They were developed to identify IgG and IgM antibodies to the lipopolysaccharide or outer membrane protein antigens of *Salmonella typhi* and *Salmonella. paratyphi*. Using blood culture as the gold standard can be helpful in the early detection of infection during the first few weeks of primary infection, with a specificity and sensitivity of 80% and 90%, respectively [212].

Table 3: Various Latest and Similar Comparative studies have been done in relation to Typhoid testing. Some of them are:

Sr. No.	Author	Year	Article Name	Limitations/ Research Gap
1.	Samuel N. Frempong	2019	Early economic evaluation to identify the necessary test characteristics of a new typhoid test to be cost effective in Ghana	1. Widal test compared with a hypothetical parameter. 2. To study the true negative and true positive diagnosis of typhoid 180 days period chosen is a very short period

2.	KarimoOusenu	2021	A cross-sectional comparative study of the performance of the widal test and the typhidot immunoassay for Typhoid Fever diagnosis in the west region of Cameroon	1. Not compared with blood culture method
3.	Sarah Hassan	2021	Comparative study between Widal and Dot Elisa in the diagnosis of Typhoid fever	1. Less sample size 2. Not compared with stool culture results
4.	Abdullahi A. Minjibir	2020	Comparative study of Widal test against Stool culture in diagnosis of Typhoid fever suspected cases in Kano, Northern Nigeria	1. Sample size of 125 patients is less 2. Comparison of only two methods is not enough
5.	Noor Jahan	2021	A comparative evaluation of rapid card with widal slide agglutination tests for rapid diagnosis of Typhoid fever	1. With sample size of 265 only two out of four methods involved in the study
6.	Farjana Akter	2020	Comparative evaluation of rapid Salmonella typhi IgM/IgG and widal test for the diagnosis of enteric fever	1. Sample size of only 71 people 2. Only two methods taken in study with no culture technique
7.	WahdahNorsiah	2020	Evaluation of the diagnosis of Typhoid fever using the widal test and anti-Salmonella typhi IgM Test	1. Sample size of only 42 samples 2. Only two methods taken in

				study with no culture technique
8.	Rasha I. Salama	2019	A Comparative study of Typhidot (Dot-EIA) versus Widal test in diagnosis of Typhoid fever among Egyptian patients	1. Sample size of 140 is very less 2. Stool culture not performed on these 140 patients
9.	Akili Mawazo	2019	Performance of widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam Tanzania	1. Sample size of 158 is less 2. TyphiDot-IgM/IgG not studied in this study
10.	N. Keerthana	2018	Comparative evaluation of performance of qualitative and semi quantitative slide method test versus quantitative tube widal test in diagnosing Enteric Fever	1. Only widal test method is compared by slide and tube method, no other method like TyphiDot and culture were compared
11.	Tej N. Nepal	2018	Relying on Widal test alone could lead to over diagnosis of typhoid fever: Findings from a records review of febrile patients at Damphu hospital, Bhutan, 2011-2012	1. Sample Size of only 70 2. Only two methods out of four is taken

12.	Radhika Ramaraj	2016	Enteric Fever – Over Diagnosis by Slide Widal Method	1. Sample Size of 150 is less 2. Only widal test method is compared by slide and tube method, no other method like TyphiDot and culture were compared
13.	S Udayakumar	2017	Comparative study of Typhidot-M with widal and blood culture in diagnosis of enteric fever	1. Only one age group of children is included 2. Sample Size of 270 is less
14.	HylemariamMihiretieMengist	2017	Diagnostic value of widal test in the diagnosis of Typhoid Fever: A Systematic Review	1. Study of only 16 articles 2. Only PPV and NPV of widal test is studied
15.	G. Ameya	2017	Comparative study of widal test against stool culture for Typhoid suspected cases in Southern Ethiopia	1. Sample Size of Only 95 patients 2. Blood Culture and TyphiDot methods not included

CHAPTER 3

METHODS AND MATERIALS

3.1 Study area

The 450 samples were collected in which 150 samples collected as negative control and 300 suspected patients of typhoid from Ajit Hospital, Government Civil Hospital, and Brar Clinic in the Malwa district region of Punjab, India. The selection of suspected cases is done by MD Medicine doctors on the basis of patients' clinical symptoms. The samples collected were analysed at the Bector Diagnostics. The sample calculation done by scientific calculator for research that is Raosoft available online.

3.2 Duration of study

The present work is a study from 2022 to 2024 covering a period of two years and the testing done from 09.04.2023 to 15.03.2024.

3.3 Source of patients / subjects:

Age, gender, and clinical symptoms (such as fever and duration) of the patients were documented. The blood samples were drawn after the study participants received duly signed informed consent. The total study's participants included 450 individuals, from which 300 suspected cases of typhoid test and 150 cases tested as negative control those who were not having any symptoms of fever, diarrhea, fatigue, headache, body rashes, abdominal pain for the last six months. Blood and stool samples were analyzed using Widal test, Typhi IgG/IgM test, Blood culture and stool culture.

Inclusion Criteria:

(i) Patients of all age group with consent.
(ii) Suspected patient of typhoid fever and testing is advised by registered physician.
(iii) Patient diagnosed with Typhoid fever.
(iv) Patient already having titre of 1:320 or higher in their widal test reports.

Exclusion Criteria:

(i) Patients who already received antibiotic treatment.

3.4 Methods of Laboratory tests

All 450 patients' blood samples were taken in order to investigate the sensitivities and specificities of the Widal test, blood cultures, stool cultures and Typhi IgG/IgM tests. Blood culture, stool culture and Typhi IgG/IgM conducted on day-1 and Typhi

IgG/IgM, stool culture and Widal conducted on day-6. The tests were conducted within one hour of collection of the specimens.

3.5.1. Blood collection and bacterial culture for pathogen isolation

Written consent is taken from all 450 participants. To isolate *S. typhi*, blood samples from patients of various ages were gathered by the Microbiology Laboratory from the Ajit Hospital, Government Civil Hospital, Brar Clinic in the Malwa district of Punjab, India. 5ml blood is collected by venipuncture and inoculated into a culture bottle containing 50ml of 0.5% bile broth. According to the Kauffmann-White system, blood isolates that were culture-proven positive were retested for *Salmonella* by slide agglutination using poly anti-sera (Beacon Company) [219]. Biochemical identification is done by triple sugar iron test and Urease Test. [57]

3.5.2. Media preparation

Preparation of XLD (Xylose Lysine Deoxycholate) agar media is a common procedure used in microbiology laboratories for the isolation and identification of enteric pathogens, particularly Salmonella and Shigella species. Weigh out 5.5 grams of XLD agar powder. Suspend the weighed XLD agar powder in 1 Liter of distilled water in a suitable container. Stir the mixture to ensure proper suspension. Heat the mixture in boiling water, stirring intermittently. Ensure the agar powder is completely dissolved. Unlike other agar media, XLD agar typically does not require autoclaving. The boiling step is sufficient for sterilization. Allow the agar solution to cool to around 50 degrees Celsius. It should still be liquid but cool enough to handle. Mix the solution well to ensure uniformity. Dispense the molten agar into sterile petri plates in an aseptic manner and a sterilized glass chamber which is equipped with UV light. Fill the plates to an appropriate depth for solidification. Once the agar in the plates has solidified, streak the sample onto the surface of the agar using an inoculating loop. Incubate the inoculated plates in an incubator set to 37°C for 24 hours. After incubation, examine the plates for colony growth. Different types of colonies may indicate the presence of specific organisms. Lysine Decarboxylase convert Lysine into amines which are alkaline so pink colonies appeared. Sodium Thiosulphate, ferric ammonium citrate added in media so difference created by H2S indicator system, so black deposits of hydrogen sulphide deposits on colonies. To prevent false reaction Lactose and sucrose added in high amount. Indicator phenol red give pink colonies under alkaline condition and yellow under acidic condition. In identification TSI which has glucose, sucrose and lactose, streak on TSI slant and stab the organism in butt, after 24 hrs in 37°C, pink slant appeared, yellow butt with blackening of slant. In urease test, after 24 hrs in 37°C, negative reaction means no colour change. [57]

To Prepare Selenite F broth, accurately weigh the components according to the formula. Dissolve the ingredients in distilled water. Adjust the pH of the medium to approximately 7.2. Dispense in 10ml quantities in test tubes and sterilized by steaming for 30 minutes. [57]

To prepare Urease agar, accurately measure components. Dissolve ingredients (except urea) in distilled water and adjust the pH 6.8 to 6.9. Autoclave the basal medium at 121°C for 15 minutes. Sterilize urea separately (often by filtration) and add aseptically to the sterilized basal medium cooled to approximately 50°C. Dispense into sterile tubes. Allow the medium to solidify (often prepared as slants). [57]

To Prepare TSI agar, accurately measure components. Dissolve ingredients in distilled water. Autoclave at 121°C for 15 minutes. Dispense into sterile tubes, allowing to solidify as slants. [57]

To prepare blood culture bottles weigh 13grams of Nutrient broth powder (HI MEDIA) and add it to one litre of distilled water in glass flask. Dissolve the ingredients by keeping it boiling water. Add 5grams of Sodium Taurocholate to the nutrient broth kept in the boiling water. Dispense 50ml of this broth to the bottles. Cap the bottles and sterilize at 121°C for 15 minutes.

3.5.3. To Check the accuracy and contamination of Media

An aseptic conditions has been followed during the media preprations, once the media was solidify it was incubated (Blood culture bottles, XLD Media, TSI agar, Urease agar, Selenite F broth) at 37°C overnight to check the contamination of the media. There was no contamination found after overnight incubation.

To check the accuracy of the media, innoculated the known bacteria onto the surface of agar plates and in the broths of blood culture bottles and selenite F tubes, if the media bottles, tubes and plates show the growth characters on the media then the media is satisfactory for testing.

3.5.4. Blood culture test

For blood cultures, the patient's blood was drawn into a prepared culture bottle under stringent aseptic conditions in amounts of 5ml for adults and 3 ml for children [222]. Standard techniques were employed to isolate *S. typhi* from the blood. The inoculated blood culture bottles were incubated at the appropriate temperature (usually 35-37°C) for the recommended duration (typically 24-48 hours) before processing. After incubation, check the broth for motility by hanging drop method. A portion of broth from blood culture bottle was streaked onto the surface of XLD agar plates. The inoculated XLD agar plates were incubated at 37°C for 24 hours. The plates were then examined for colony growth, and the colonies were characterized based on morphology, including color, size, and sheen [57].

3.5 Widal test

- **Quantitative Tube (Widal) Test**

Each test (O, H, AH, BH) needs a collection of nine dry and clean test tubes, measuring 10 x 75 mm. Pipette 1.9 ml of isotonic saline in tube one of all sets. Pipette 1.0ml of isotonic saline in remaining tubes of all sets. Add 0.1ml of serum sample in tube one of each set and mix well. Transfer 1.0ml of diluted sample of tube one to tube two of each set and continue this process till tube seven of each set. Discard 1.0 ml of diluted serum from tube seven of each set. Tube number eight and nine in all sets is for positive and negative control. In all four sets of nine tubes add one drop of respective antigen suspension (O, H, AH and BH) from Widal kit (Beacon Company) and mix well. Dilution of each set is as follows: Tube one (1:20), Tube two (1:40), Tube three (1:80), Tube Four (1:160), Tube five (1:320), Tube six (1:640), Tube seven (1:1280) and Tube eight (Positive Control), Tube Nine (Negative Control). Mix well and let stand at 37°C in serological water bath for 16 to 20 hours to allow amines to clump together. Observe the agglutination by using a concave mirror, the maximum serum dilution exhibiting distinct agglutination is known as the antibody titre [154].

3.6 Stool culture

The patient provided stool samples, which were taken using aseptic methods and sterile containers. Mix approximately one gram of the stool sample in 10ml of Selenite F broth and incubate at 37°C for 12 to 16 hours. To isolate *Salmonella typhi*, subculture the enriched sample from the Selenite F broth onto the surface of XLD agar with the help of inoculating loop (XLD plates dried in incubator at 37°C for 1 to 2 hours) in accordance with the protocol described by Prescott et al. (2005). The inoculated XLD agar plates were placed in an environment with oxygen and kept at a temperature of 37°C for a period of 24 to 48 hours. The morphology of the colonies was utilized to characterize them after incubation, and the plates were examined for the growth of the bacteria and done the biochemical identification [57].

3.7 Typhi IgG/IgM test

An immunoassay using lateral flow chromatography. Rabbit IgG-gold conjugates and recombinant H and O antigen coupled with colloid gold (HO conjugates) are found in the test cassette's burgundy conjugation pad (Medsource Company). A nitrocellulose membrane strip with the control band C, two test bands (the G and M bands). Monoclonal anti-human IgM is pre-coated on the M band to detect IgM against S. typhi, whereas goat anti-rabbit IgG is pre-coated on the C band.

To check the accuracy of Typhoid IgG/IgM Rapid test a positive serum (poly sera) is used. Gather blood by vein puncture into a red top collection tube (the vacutainer shouldn't contain any anticoagulants). Permit the clotting of blood. Transfer the serum gently into a fresh tube that has already been labelled. Maintain the specimens at 2–8 degree Celsius if testing is delayed. As soon as possible following specimen collection, test the samples. Keep the specimen for up to five days at 2–8°C.The samples are kept at -20°C for extended storage. Steer clear of repeated freeze-thaw cycles. Bring the frozen specimen to room temperature gradually before testing, then stir gently. Before testing, specimens with visible specific matter should be centrifuged to remove any remaining material. Use of samples exhibiting severe haemolysis, lipemia, or turbidity should be avoided to prevent influencing the interpretation of the results [248].

Test Procedure Typhi IgG/IgM:

If the specimen is frozen or refrigerated, bring it to room temperature together with the test components. Before the test, thoroughly combine the specimens. When prepared to test, remove the device and open the bag via the notch. Lay the test gadget out on a spotless, level surface. The specimen should be put into the pipette dropper. Make sure there are no air bubbles in the sample well before adding 1 drop (about 30–45 µL) of the specimen while holding the dropper upright. Next, quickly add 1 drop (about 35–50 µL) of assay buffer. Read the outcome in fifteen minutes. In as little as one-minute, positive outcomes may be observed. Check in with the outcomes fifteen minutes later. After evaluating the results, throw away the test gadget to prevent misunderstanding [248].

3.8 Calculations.

Incidence Rate: (Number of new cases during a specific time period) / (Total population at risk during that time period) × (Time Period)

Morbidity Rate: (Number of new cases / population at risk × Time period* 100,000)

Mortality Rate: (Number of Deaths / Population at risk × Time period* 100,000)

Lost Productivity: Number of cases in country × Average Duration of illness * Average Daily wage

From Number of cases in study to Number of cases in country: (Number of Positive patients in study / sample size) × Population of Country

Calculation of number of new cases within a specified period is recorded in this study, to know the disease burdenof typhoid incidence rate, mobidity rate, mortality rate and lost productivity is calculated. Sample size of study is 450 in which suspected cases were 300, time period is 11 month 6 days and average duration of illness is 10.5 days and average daily wage noted is Rs 550 in which number of true positive cases is 21.

Sensitivity: Number of true positives / number of false negative + number of true positives
= number of true positives / Total number of sick individuals in population

Specificity: Number of true negatives / number of false positives + number of true negatives

= Number of true negatives / Total Number of well individuals in population

True Positive Rate (TPR): TP/TP+FN=1-FNR

True Negative Rate (TNR): TN/TN+FN=1-FPR

Positive Predictive Value (PPV): TP/TP+FP=1-FDR

Negative Predictive Value (NPV): TN/TN+FN=1-FOR

False Negative Rate: (FNR):FN/FN+TP=1-TPR

False Positive Rate (FPR): FP/FP+TN=1-TNR

False Discovery Rate (FDR): FP/FP+TP=1-PPV

False Omission Rate (FOR): FN/FN+TN=1-NPV

Prevalence Threshold (PT): $\sqrt{FPR}$ / $\sqrt{TPR}$ + $\sqrt{FPR}$

Critical Success Index (CSI): TP/TP+FN+FP

Accuracy (ACC): TP+TN / TP+TN+FP+FN

One of the objective of the study is to find out the best test among widal, Typhi IgG/IgM and stool culture compared with gold standard blood culture test in the context of sensitivity, specificity and accuracy level. So in this study calculation of all parameters were recorded like true positive rate, true negative rate, false negative rate, false positive rate, false discovery rate, false omission rate, prevalence threshold, accuracy level, critical success index, positive predective value, negative predictive value and senstivity, specificity of selected parameters. For these calculations we first needed data of true positive, true negative , false positive and false negative.

Sick people correctly identified as sick known as true positive while healthy people incorrectly identified as sick is known as false positive.

Healthy people correctlt identified as sick is known as true negative while sick people incorrectly identified as healthy is known as false negative.

3.9 Ethics Statement

This study was approved by the independent research Ethics society of Kolkata on behalf of the lovely professional university. **Approval No. IECCRI/23-24/01**

3.10Statistical analysis

Blood culture was used as the gold standard test to calculate the "sensitivity (Sn), specificity (Sp), positive predictive values (PPV), negative predictive values (NPV), and diagnostic accuracy" of the serological assays. The main conclusion of this research which clarifies the effectiveness of the present diagnostic tests is the positive predictive value. The calculation of the sensitivity, specificity and predictive values was done as previously mentioned [226]. The computer's Microsoft Excel program is used to access all the data. A frequency table created to demonstrate how the distribution of dates and the variables relate. Kappa statistic is used to measure agreement between each test's result and the true disease status. Chi Square is used in comparative studies for significant association between two variables. The SPSS software has been used to find out the significant values.

CHAPTER-4

RESULTS AND DISCUSSION

4.0 Results

a) **Clinical Evaluation:** Consent form all 450 subjects were taken. From which 300 suspected patients and 150 subjects as negative control. Out of 300 suspected patients 162 males and 138 females sent for testing by doctors. All clinical sypmtoms were noted by MD Medicine doctors. Maximum 73 patients were from age group of 20 to 30 year. 58 patients were from 30 to 40 year group. 51 from 40 to 50 year group, 32 from 50 to 60 year group, 39 from 60 to 70 year group and 25 from 18 to 20 year age group.

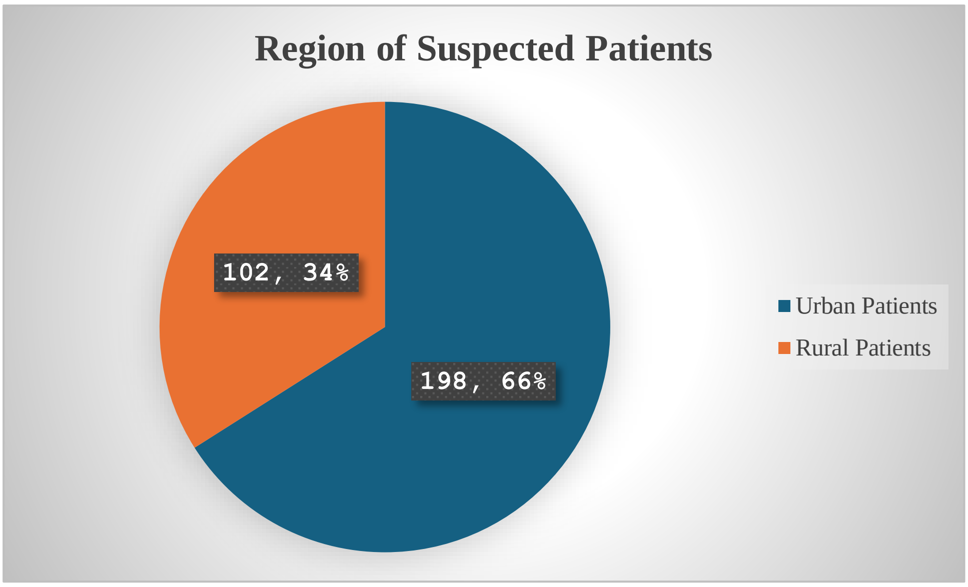


Figure 7: Region of Suspected Patients

Region of suspected patients: As shown in figure, 198 patients were from urban region and 102 patients from rural region. Region of all 300 suspected patients were recorded to calculate where is disease is more prominent, as per this study typhoid is more prominent is urban areas than rural as 66% patients were from urban areas.

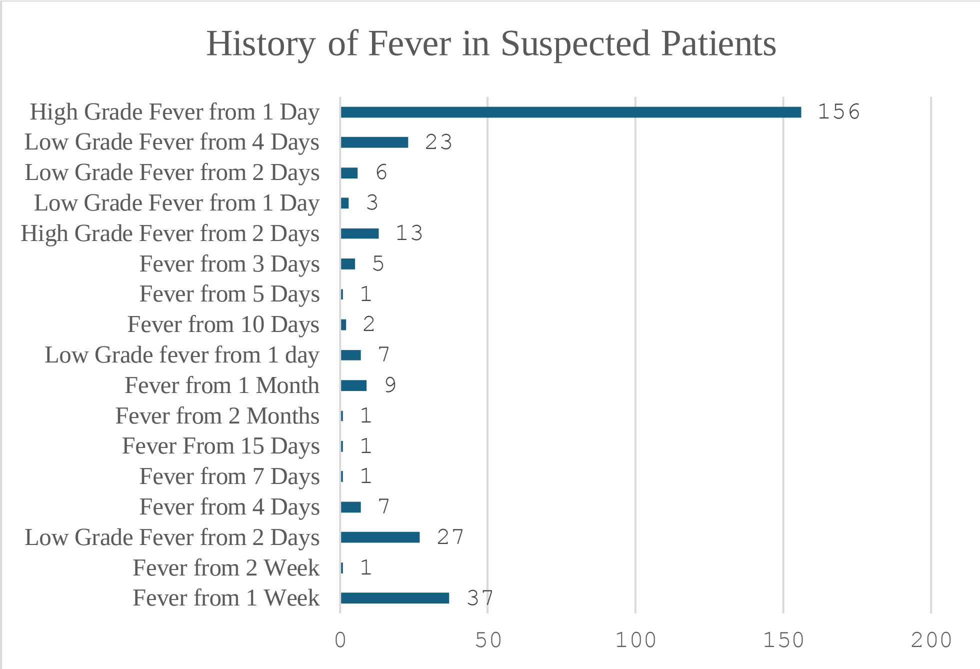


Figure 8: History of Fever in Suspected Cases.

Fever History: As shown in figure 14, Out of 300 suspected patients all 300 patients have fever history, if we sub categorise the fever symptoms then 37 patients have fever for one week. One patient having fever for two weeks, 27 patients having low grade fever from 2 days, 7 patients having fever for 4 days, 1 patient with fever of 7 days, one patient with fever from 15 days, one patient having fever history of 2 months, 9 patients having fever from 1 month, 7 having low grade fever for 1 day, 2 having fever for 10 day, 1 having fever for 5 days, 5 having fever for 3 days, 13 categoried by doctors as high grade fever for 2 days, 3 as low grade fever for 1 day, 6 having low grade fever for 2 days, 23 having low grade fever from 4 days, 156 as high grade fever from 1 day.

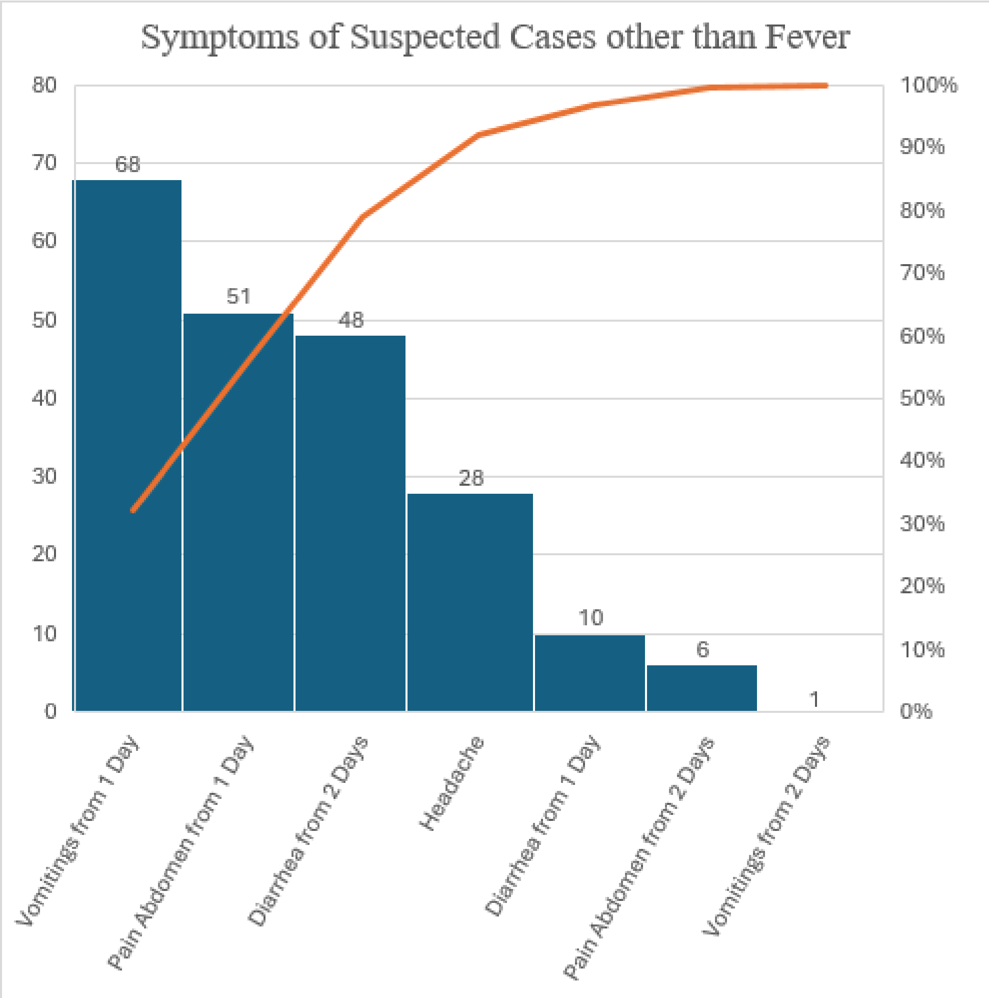
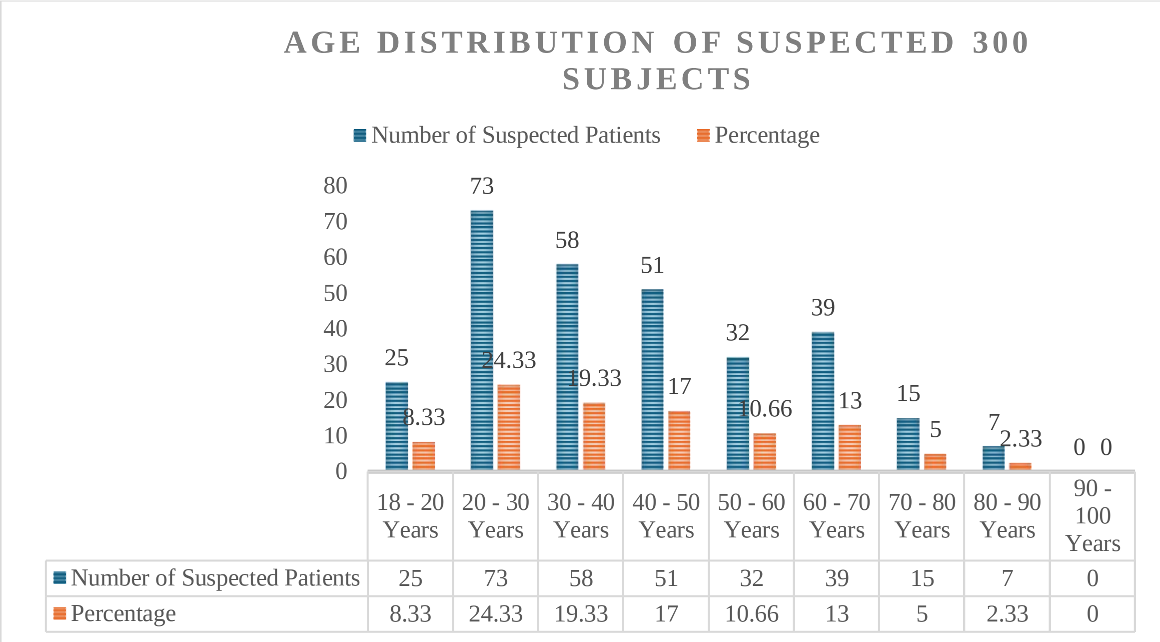


Figure 9: Symptoms of Suspected Cases other than Fever.

Symptoms other than fever: 48 patients having diarrhea sypmtoms from 2 days, 10 patients having diarrhea symptoms from 1 day. 28 patients having sypmtoms of headache, 68 patients having vomiting sypmtoms for 1 day and 1 patient came with vomiting symptoms for 2 days. 51 patients having symptoms of pain abdomen for 1 day, while 6 patients having symptoms of pain abdomen from 2 days (figure 9).



	18 - 20 Years	20 - 30 Years	30 - 40 Years	40 - 50 Years	50 - 60 Years	60 - 70 Years	70 - 80 Years	80 - 90 Years	90 - 100 Years
Number of Suspected Patients	25	73	58	51	32	39	15	7	0
Percentage	8.33	24.33	19.33	17	10.66	13	5	2.33	0

Figure 10: Age distribution of suspected Typhoid patients

Age distribution of suspected patients: Based on the findings (figure 10), the number of individuals suspected to have typhoid within the age range of 18-20 years was observed to be 25, accounting for an average percentage of 8.33%. Within the age range of 20-30 years, the number of suspected cases was found to be 73, representing an average percentage of 24.33%. Similarly, within the age range of 30-40 years, the number of suspected cases was 58, with an average percentage of 19.33%. In the age range of 40-50 years, 51 suspected cases were identified, corresponding to an average percentage of 17%. For individuals aged 50-60 years, the number of suspected cases was 32, accounting for an average percentage of 10.66%. Within the age range of 60-70 years, 39 suspected cases were reported, representing an average percentage of 13%. In the age range of 70-80 years, 15 suspected cases were identified, with an average percentage of 5%. Within the age range of 80-90 years, 7 suspected cases were found, corresponding to an average percentage of 2.33%. No suspected patients were found within the age range of 90-100 years.

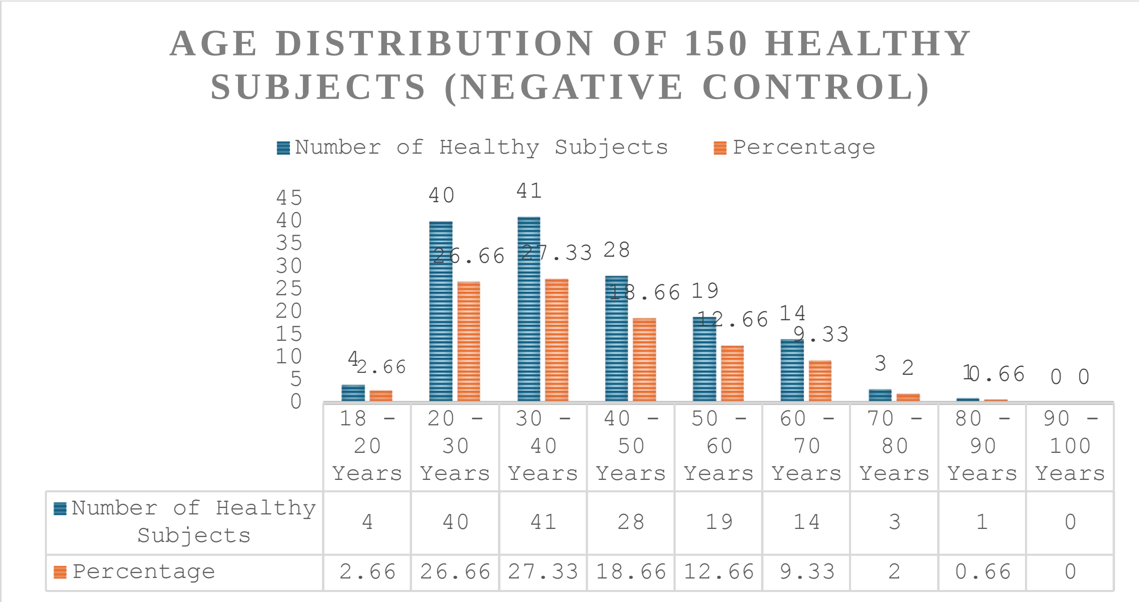


Figure 11: Age distribution of healthy subjects

Age distribution of healthy subjects: As from the findings as shown in figure 17, the number of healthy patients between the age group of 18-20 years was found to be 4 with an average percentage of 2.66 % , between the age group of 20-30 years , the number of healthy subjects was found to be 40 with an average percentage of 26.66 % , between the age group of 30-40 years , the number of healthy patient was found to be 27.33%, between the age group of 40-50 years , the number of healthy patients was found to be 28 with an average percentage of 18.66, between the age group of 50 – 60 years , the number of healthy patients was found to be 19 with an average percentage of 12.66 % , between the age group of 60-70 years , the number of healthy patients was found to be 14 with an average percentage of 9.33 % , between the age group of 70-80 years , the number of healthy patients was found to be 3 with the average percentage of 2, between the age of 80-90 years , the number of healthy patients was found to be 1 with an average percentage of 0.66% and no healthy patients would observed between the age group of 90-100 years.

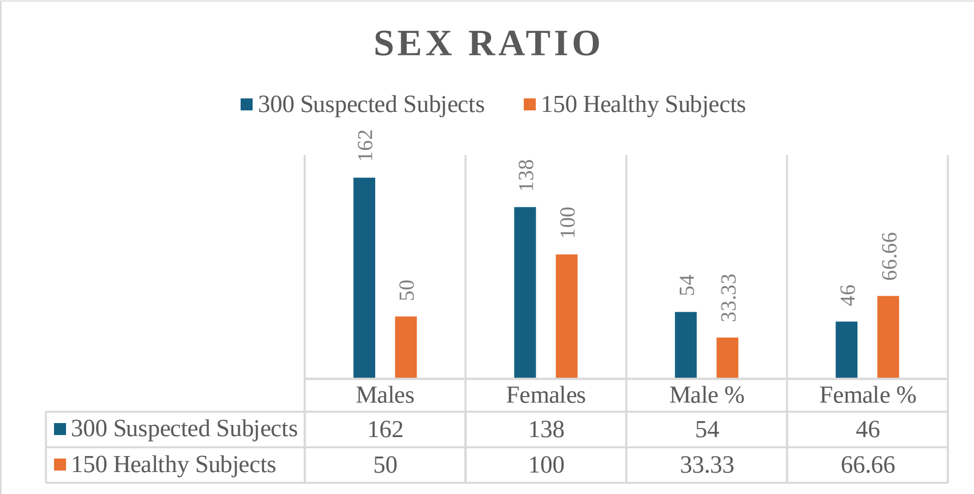


Figure 12: Percentage of sex ratio in suspected patients

Sex Ratio: The graphs (figure 12) indicate the sex ratio between individuals suspected of having typhoid and those who are healthy. Among the suspected patients, there were 162 males, while among the healthy patients, there were 50 males. The average percentage of males among the suspected patients was 54%, whereas among the healthy patients, it was 33.33%. In a similar vein, it was seen that out of a total of 138 girls, a significant number were identified as suspected cases of typhoid, while 100 females were determined to be in good health without any infections. The suspected ladies accounted for an average proportion of 46%, whilst the healthy females constituted 66.66% of the total.

b) **Isolation and Identification**: Out of 300 suspected samples total 21 positive cases detected by blood culture, while stool culture gave 15 positive cases out of 300. So, blood culture has high positive rate than stool culture. Out of 300 suspected patients Widal gave 177 positive results, 100 Positive given by Typhi IgG and 30 Positive Given by Typhi IgM or IgG,IgM both. As rising titre in Widal has great importance but typhi IgG/IgM provided good confidence to rule out the present infection.

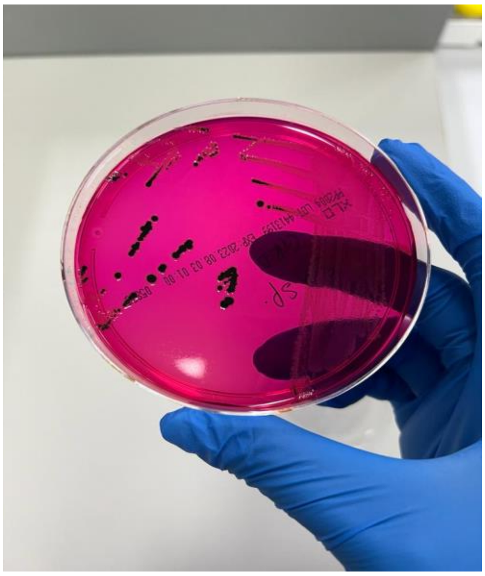


Figure 13: *Salmonella* on XLD agar.

Figure 14: Identification by Triple Sugar Iron Test.

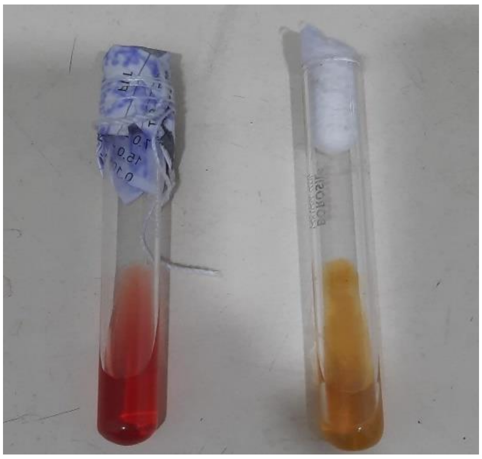


Figure 15: Identification by Urease Test.

Figure 16: Typhi IgG/IgM test.

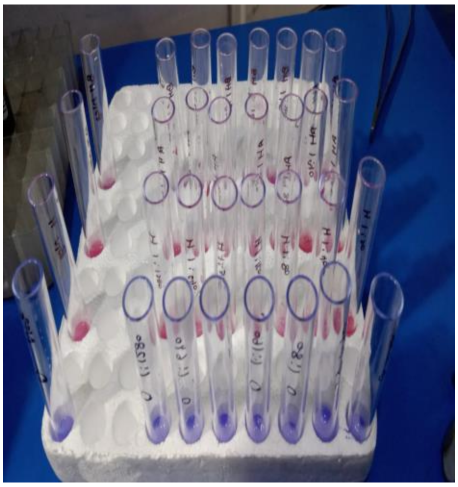


Figure 17: Widal Tube Method.

c) **Accuracy Level**: The comparison as per Chi Square Statistics for Stool culture, widal and Typhi IgM when compared with Blood culture the p-Value of both Stool Culture and Typhi IgM is Less than 0.001 which shows significant association between these parameters and Gold Standard test, less than 0.001 p-value shows that test has some predictive value. The p-value of widal in Chi Square Statistics is 0.286, which shows test does not have significant predictive value and no significant association between widal and blood culture test.

With Kappa Statistic in this study, a measure of agreement between each test's result and true disease status is calculated. Kappa Statistic value of Typhi IgM is 0.809 approx which indicates susbtantial agreement between the two diagnostic tests. Value of stool culture is 0.528 approx, again which indicates the substantial agreement between the stool culture and gold standard test. The Kappa Statistic value of widal is -0.0276 approx. The negative value of widal indicates no agreement between gold standard test and widal test.

Typhi Dot M that detects IgM and IgG antibodies in *Salmonella typhi and Paratyphi* shows the encouraging results with Sensitivity of 100% and Specificity of 96.77%. This is an immune chromatography assay designed for qualitative detection of *Salmonella typhi* and *Paratyphi* in human serum. Critical success index and Accuracy of Typhi IgG/IgM is 67.44% and 97% respectively. There is a big space for research on Typhi IgM test. Overall study reveals that doctors needs to avoid Widal test as very low sensitivity of 47.61% and specificity of 40.14%.

d) **Rapidity**: There is need of advanced laboratory setups to isolate bacteria. Similarly, the most sensitive method to detect typhoid fever is Bone marrow culture but due to its invasive nature this technique also not used by either doctors and even patients also not agree to give samples Also, for blood culture and stool culture, a doctor has to wait without initiating treatment to patient as report will take minimum approximately 48 hours to 72 hours. For widal we need to confirm the slide method results with tube method which will need overnight incubation process. So Typhi IgG/IgM is the most rapid test and also does not need advanced laboratory setup.

e) **Cost-effectiveness**: As per the study cost of Blood culture, stool culture, widal and typhi IgG/IgM is Rs 175, Rs 125, Rs 60 and Rs 50 respectively. So when it comes to cost effectiveness Typhi IgG/IgM is best out of these four parameters.

f) **Epidemiology:** Typhoid is a significant public health concern in malwa region of Punjab. As per the statistic of this study the substantial burden of typhoid with a incidence rate of 72.3 per 1000 population per year.

The Morbidity of typhoid as per study is 70 per 1000 population per year.

But in this study Mortality is 0%, as no death noted in 300 suspected patients included in the study. Mortality rate is 0% because of good quality medical facilities available in Goverment hospitals specifically in mohalla clinics where initial treatment is provided to patients. Number of new cases in study is 21, patient at risk were 300, time period of study is 11 month 6 days.

g) **Disease Burden:** Typhoid is a big disease burden on country when we calculate the Lost Producivity in study which is 51141.5 crore Indian Rupees anually in India. Here average duration of illness is 10.5 days and average daily wage is Rs 550.

4.1 Determination of efficacy of typhoid diagnostic test

True Positive Data: In the conducted research, blood culture is used as the gold standard test for diagnosing typhoid in suspected patients. Various tests, including Typhi IgG/IgM, Stool Culture and Widal, were done to assess their efficacy in diagnosing the disease. The Typhi/IgM test yielded 21 true positive cases, while the Stool culture and Widal tests identified 10 real positive cases each. Based on the research results, a very surprising outcome has emerged about the Typhoid test, indicating that the Typhi/IgM test is the most recommended diagnostic method for probable cases of typhoid fever.

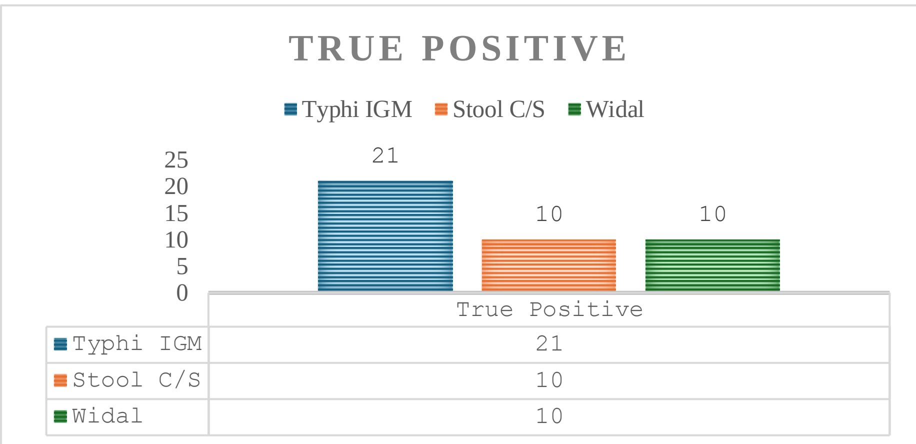


Figure 18: True Positive value of Typhi/IgM, Stool culture and Widal test

True Negative Data: The number of cases correctly identified as negative by these tests were as follows: 270 by Typhi/IgM, 274 by Stool Culture, and 112 by Widal. Based on the research findings, it is noteworthy to mention that the results pertaining to the Typhoid test have shown a very surprising outcome. Specifically, it has been observed that the Stool culture and Typhi IgM test gave more promising results in context to true negative data but Widal gave very poor results as per true negative cases.

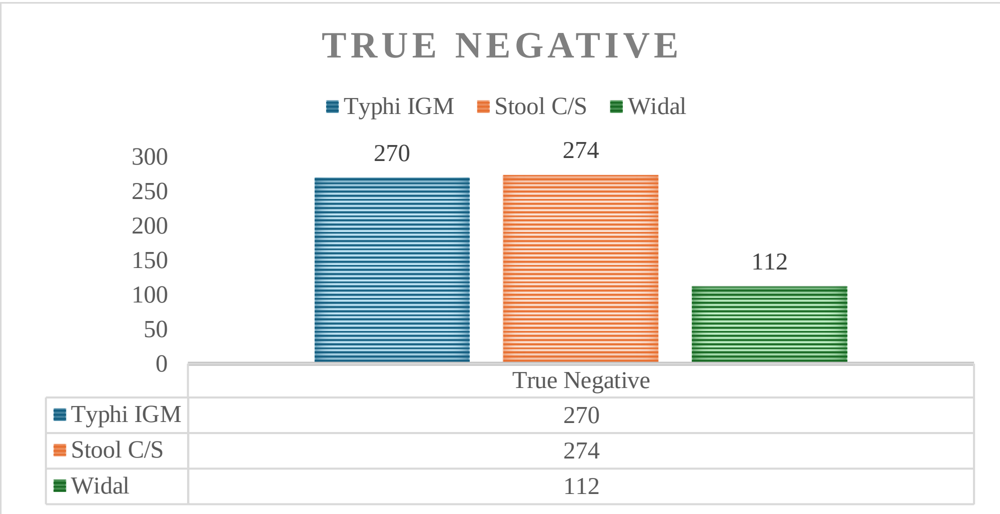


Figure 19: True Negative value of Typhi/IgM, Stool and Widal test

4.2 Comparative analysis of False diagnosis test of typhoid

False Positive Cases: The results indicated that there were 9 false positive cases by Typhi/IgM, 5 false positive cases by Stool Culture, and 167 false positive cases by Widal. Based on the research findings, as per the results pertaining to the Typhoid test are rather unexpected and shocking. Specifically, the Widal test is not recommended in situations when typhoid is suspected.

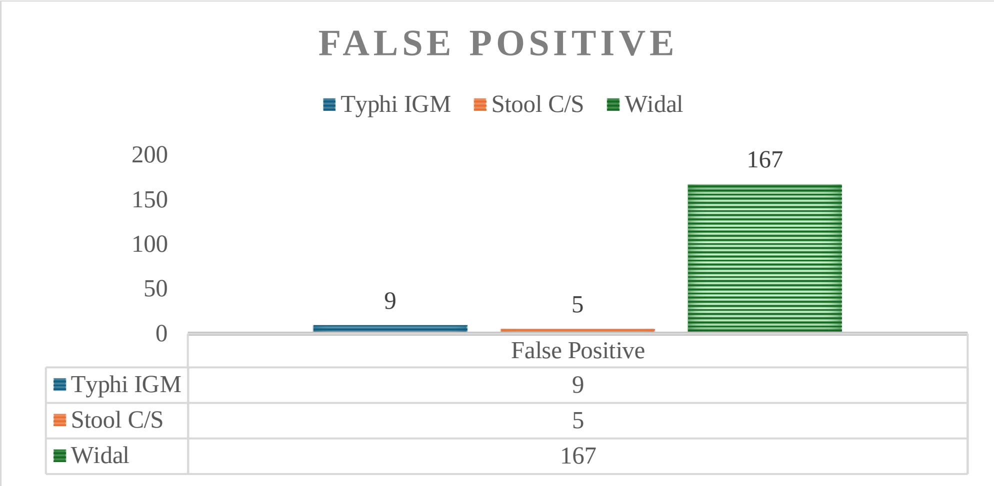


Figure 20: False positive values of Typhi/IgM, Stool and Widal test

False Negative Cases: The results revealed that the Stool culture and Widal tests exhibited 11 false negative instances, however no cases were discovered to be associated with the Typhi/IgM test. According to the results, Typhi IgM is more trustworthy when compared to both Stool culture and Widal tests. A high level of specific IgG antibodies from a previous infection or vaccination can sometimes interfere with IgM antigen binding in the Typhi IgG/IgM rapid test, potentially masking a current infection and giving a false negative IgM result.

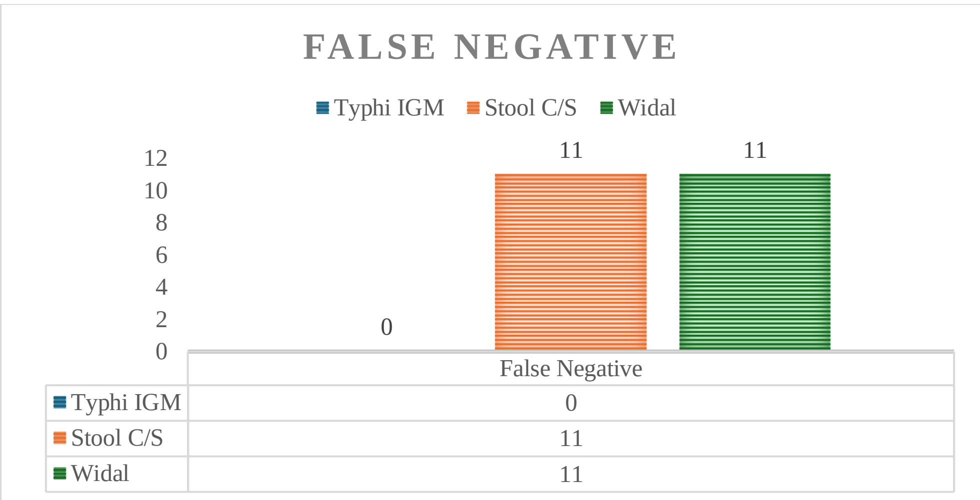


Figure 21: Illustration of False negative value of Typhi/IgM, Stool and Widal test

4.3 Interpretation of PPV and NPV of Typhoid test

Positive Predictive Value (PPV): The research is discovered to evaluate the efficacy of a variety of diagnostic tests by measuring their accuracy. Blood culture is the gold standard for detecting typhoid fever, although there are other tests that have shown promise such as Typhi/IgM, Stool Culture and Widal. Among these tests, Typhi/IgM showed the greatest positive predictive value (PPV) of 70%, indicating its potential as a reliable diagnostic tool. However, both stool culture and Widal exhibited significantly lower PPVs in comparison. The Widal test had a positive predictive value of just 5.64%, but the Stool culture test exhibited a higher positive predictive value of 66.66% than Widal.

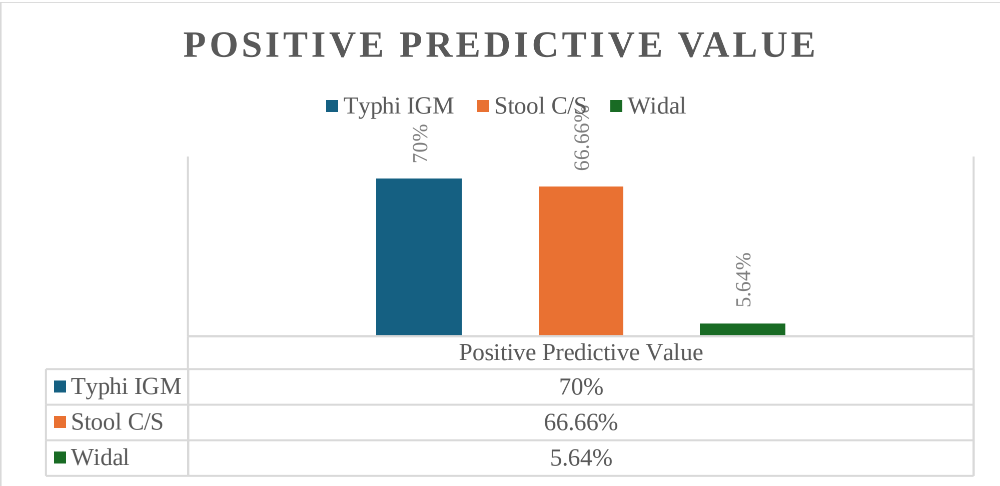


Figure 22: Comparative analysis of PPV of Typhoid tests

Negative Predictive Value (NPV): Findings revealed that Typhi/IgM exhibited the greatest negative predictive value of 100%, indicating its effectiveness in ruling out typhoid fever. However, both stool culture and Widal had a low negative predictive value, suggesting limited reliability in confirming the presence of the disease. The Widal test demonstrated a negative predictive value of 91.05%, however the stool culture test exhibited a higher negative predictive value of 96.14%.

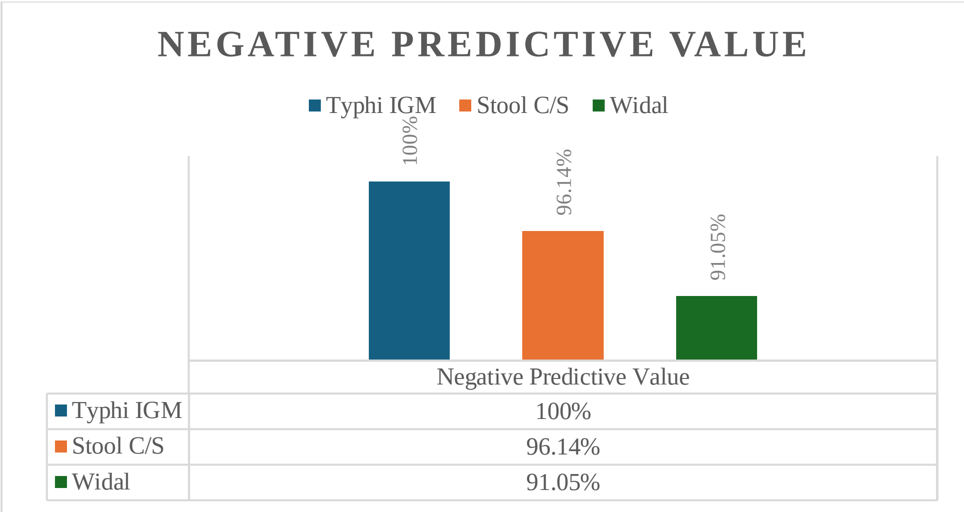


Figure 23: Comparative analysis of NPV diagnosis test of Typhoid

4.4 Determination of accuracy of typhoid tests

True Positive Rate (TPR): The results indicated that Typhi/IgM exhibited the greatest true positive rate of 100%, while both stool culture and Widal showed extremely low true positive rates. Both Widal and Stool culture test exhibited a true positive rate of 47.61%. Typhi IgM measures the proportion of actual positives that are correctly identified.

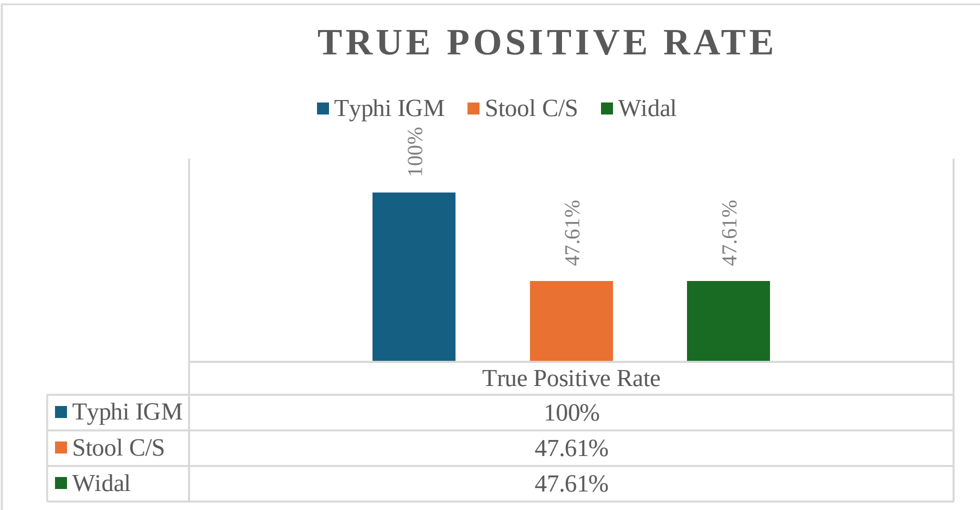


Figure 24: Comparative analysis of True positive rate of Typhoid tests.

True Negative Rate (TNR): As per this study Stool culture exhibited the highest true negative rate of 98.20%, indicating its effectiveness in ruling out the actual negatives of typhoid fever. The Widal test showed a true negative rate of just 40.14%, but the Typhi IgM demonstrated a much higher true negative rate of 96.77%.

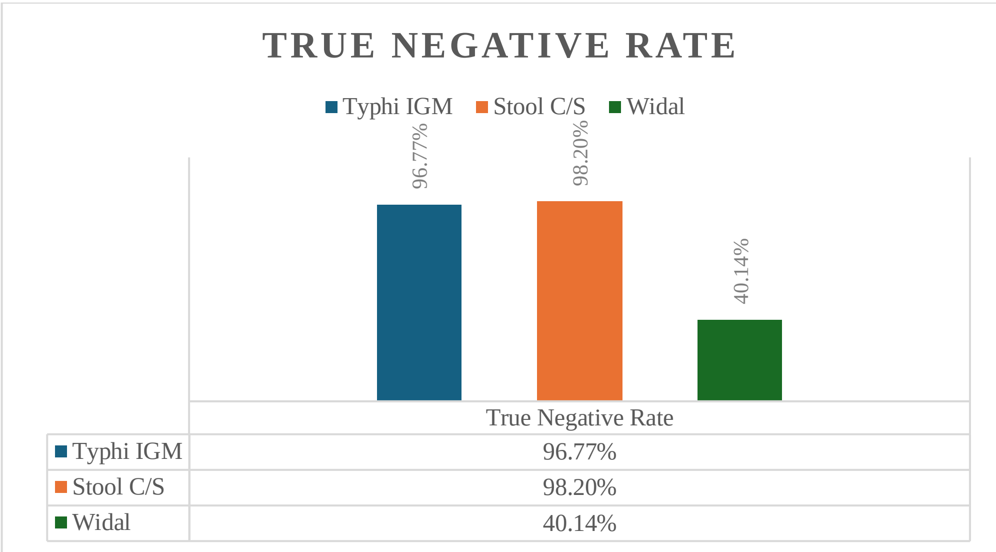


Figure 25: Comparative analysis of True negative Typhoid tests.

4.5 Efficacy of Typhoid diagnosis test

False Positive Rate (FPR): According to present study both Stool culture and Typhi IgM tests has very low false positive rate of 1.79% and 3.22% respectively. Conversely, very high false positive rate of 59.85% found for Widal test which is not good for any diagnostic investigation.

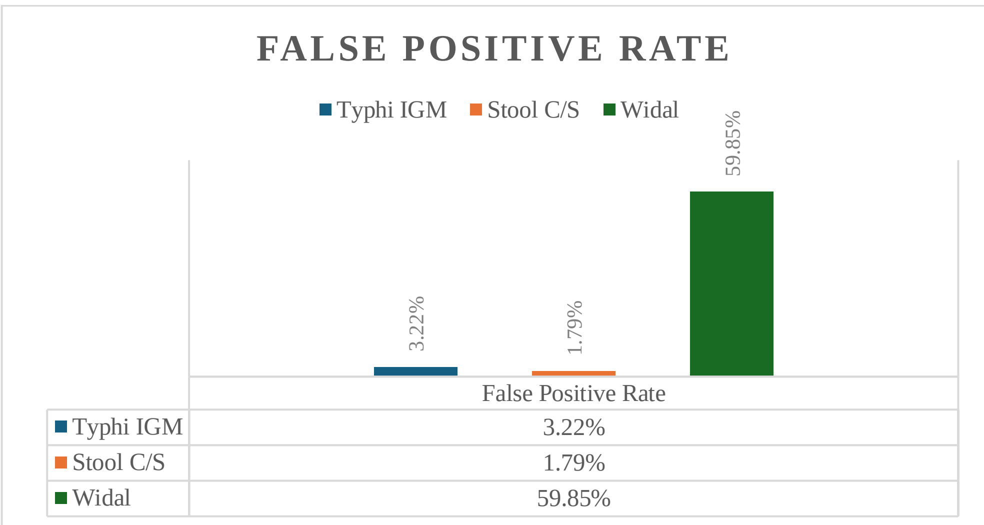


Figure 26: Comparative False positive rate efficacy of Typhoid test

False Negative Rate (FNR): According to present study both Stool culture and widal tests has very high false negative rate of 52.38%. while, 0% false negative rate result by Typhi IgM which is very good for any diagnostic investigation.

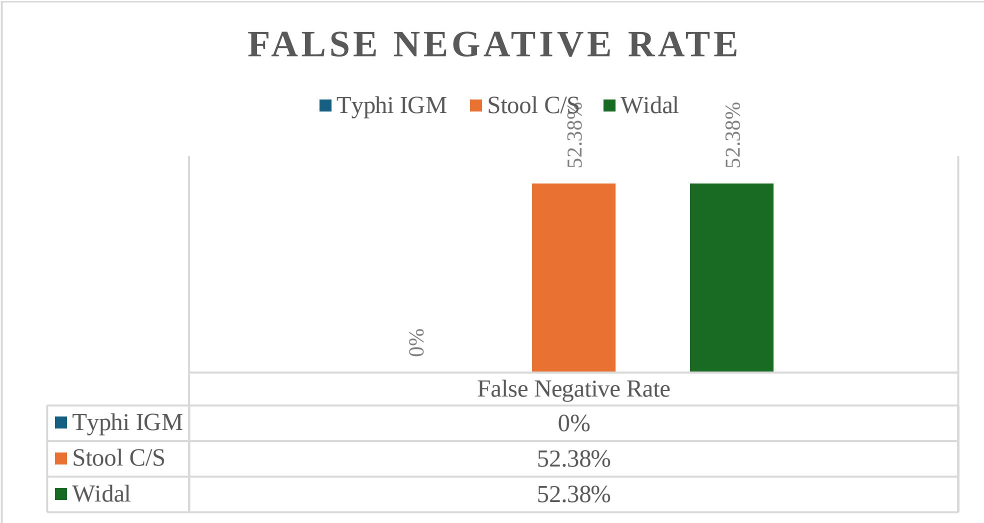


Figure 27: Comparative False negative rate efficacy of Typhoid test

False Discovery Rate (FDR): In this study, various tests, such as Typhi/IgM, Stool Culture and Widal, were done to compare their diagnostic accuracy. The results showed that the Widal test had very high false discovery rate of 94.35%, although both stool culture and Widal test had a very low false discovery rate. The Typhi IgM test demonstrated a false discovery rate of 30%, but the Stool culture test exhibited a false discovery rate of 33.33%. It suggests 94.35% of the positive results from the Widal tests are likely false positives.

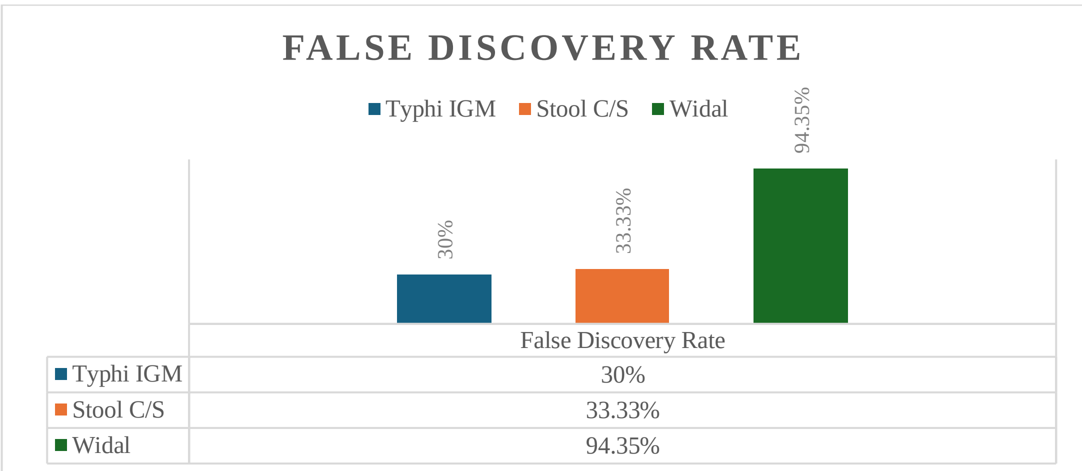


Figure 28: Comparative False discovery efficacy of Typhoid test

False Omission Rate (FOR): The present study revealed that Stool culture test yielded a false omission rate of 3.85%, while the Widal test had the highest false omission rate of 8.94%. Interestingly, 0% False omission rate for Typhi/IgM were found. So as per study 8.94% of actual typhoid cases missed by the Widal test.

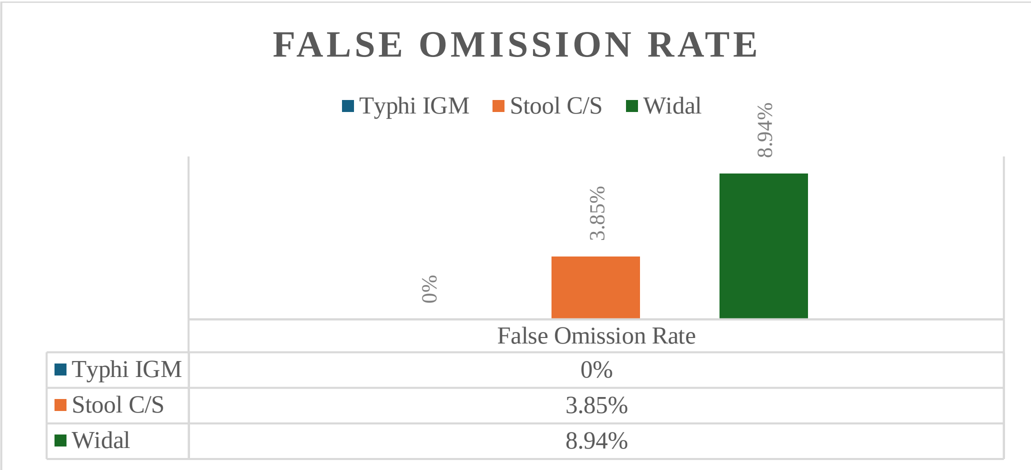


Figure 29: Comparative False Omission efficacy of Typhoid tests

4.6 Determination of CSI / ACC of Typhi/IgM, Stool culture, Widal test

Critical Success Index and Accuracy: The purpose of this study was to evaluate the diagnostic accuracy of several tests, including Typhi/IgM, Stool Culture and Widal, in the detection of typhoid fever cases among persons presenting with symptoms indicative of the illness. Blood culture was used as the gold standard test. The results revealed that Typhi/IgM exhibited a Critical Success Index (CSI) of 67.74% and an accuracy of 97%. The Stool culture test had a Critical Success Index (CSI) of 38.46%. However, it exhibited the highest accuracy rate of 94.66% among the tested methods. The Widal test had a Critical Success Index (CSI) of 5.31% which is very low, indicating a relatively low rate of accurate diagnoses. Again, it exhibited the low accuracy among the tested methods, with a value of 40.66%.

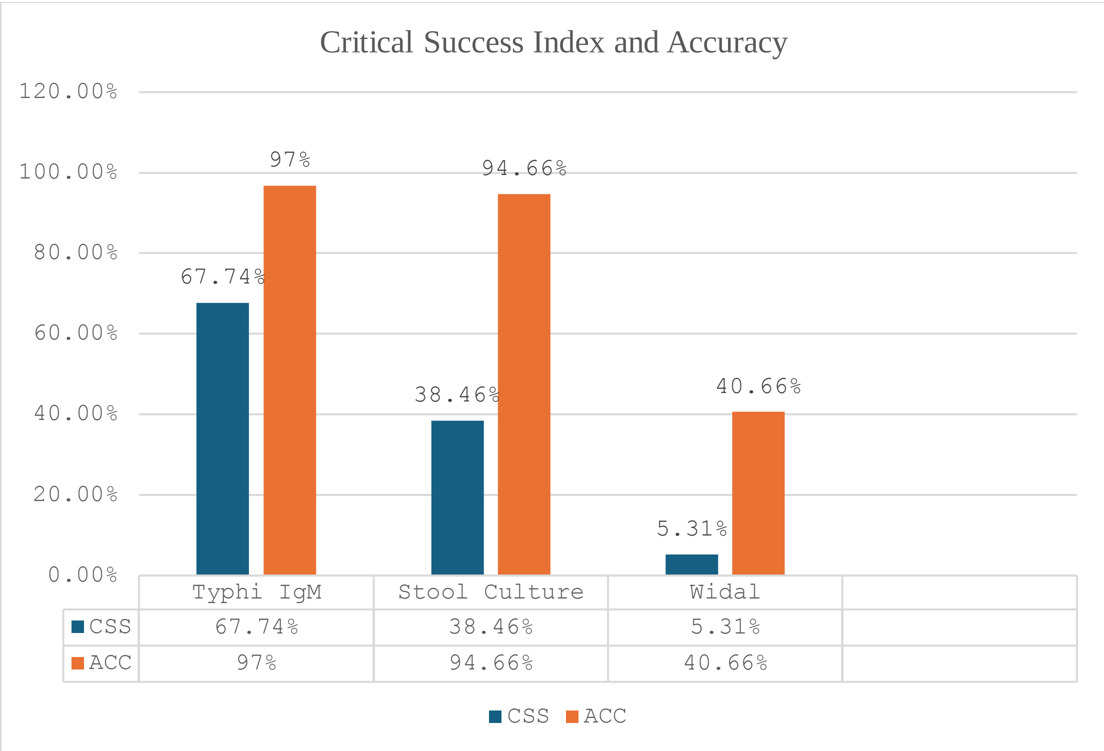
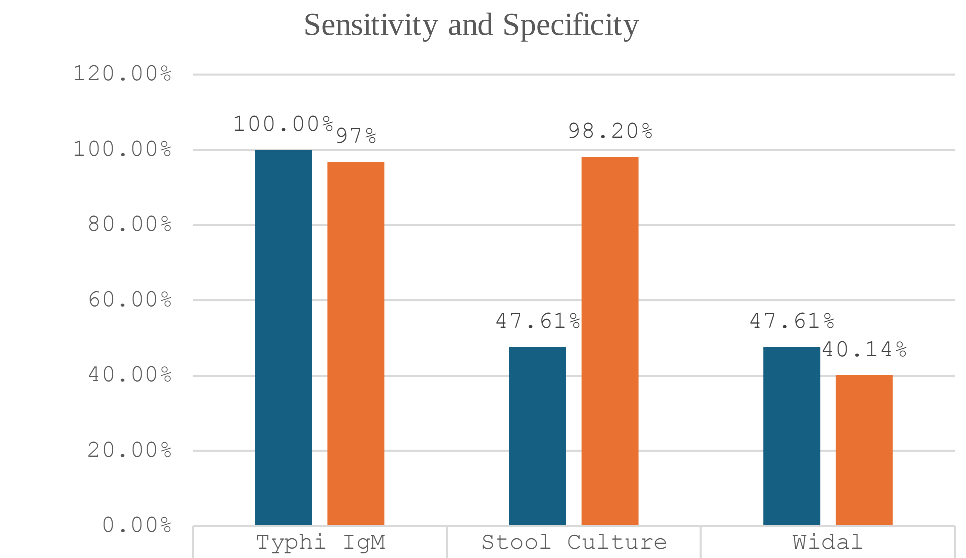


Figure 30: Illustration of CSI and ACC.

4.7 Determination of Sensitivity and Specificity of Typhi/IgM, Stool culture and Widal tests.

Sensitivity and Specificity: As per the main objective of the study, the results indicated that Typhi/IgM exhibited a sensitivity of 100% according to the graph, but its specificity was somewhat lower compare to stool culture at 96.77%. The graph revealed that Stool culture exhibited a very low sensitivity of 47.61%, indicating its inability to correctly identify positive cases. Low sensitivity can be attributed to sporadic shedding of bacteria. Additionally, it demonstrated the greatest specificity of 98.20%, indicating its ability to correctly identify negative cases. Widal test demonstrated a sensitivity of 47.61% and a specificity of 40.14%, as displayed in the graph. In both parameters of sensitivity and specificity Widal shows very discouraging results.



	Typhi IgM	Stool Culture	Widal
Sensitivity	100.00%	47.61%	47.61%
Specificity	97%	98.20%	40.14%

Figure 31: Evaluation of Sensitivity and Specificity.

4.8 Determination of Threshold percentage of Typhi/IgM, Stool culture and Widal test

Prevalence Threshold: prevalence threshold is a critical value used in evaluating the performance of diagnostic tests particularly in disease screening and surveillance. The data revealed that the prevalence threshold of Typhi/IgM is 15.18%. Stool culture test demonstrated a prevalence threshold of 16.16%. Widal test exhibited a prevalence threshold of 52.83%.

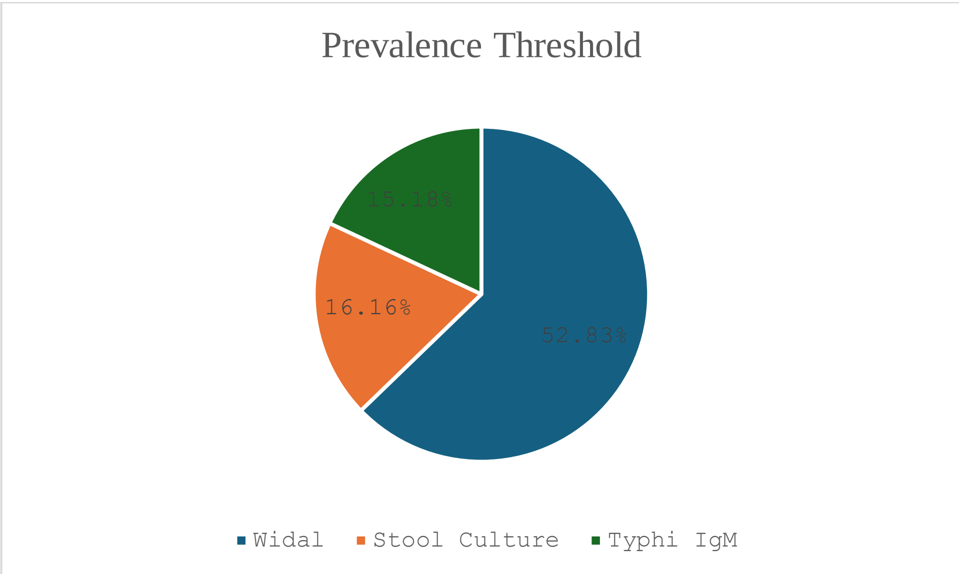


Figure 32: Estimation of Threshold percentage.

DISCUSSION

Typhoid fer is associated with an important cause of disease, as seen by recent estimates indicating 21.6 million cases in 2000 and 26.9 million cases in 2010 [232]. Additionally, the accuracy of current typhoid diagnosis is suboptimal, which further compromises the reliability of these predictions. The blood culture approach is widely recognised as the most often used technique for the identification of typhoid fever [233]. There are continuing endeavours aimed at developing innovative diagnostic assays for typhoid fever, which has the ability to rectify the existing inadequacy in diagnostic capacities. However, a challenge persists in identifying the most appropriate reference test to assess the performance of these new diagnostic methods [234] . Using a reference test with insufficient diagnostic accuracy could exaggerate results, making it difficult to determine whether or not new technologies are improving disease burden estimates. In addition, it is essential to employ a standardised reference test when comparing several index tests statistically [235].

Several investigations have shown that the Typhi/IGM test possesses the greatest sensitivity, making it the most dependable test for identifying true positive cases of typhoid fever. This was substantiated by Hajira et al. (2018), whereby the Typhi/IGM test exhibited comparable sensitivity and specificity values, hence strengthening its diagnostic reliability [236]. Conversely, Omulo et al., (2017) revealed that the Stool/CS test exhibited a significantly decreased sensitivity (47.61%) but the greatest specificity (98.20%), suggesting its efficacy in excluding typhoid fever rather than in its confirmation [237].

Conversely, the Widal test possessed the lowest sensitivity (47.61%) and specificity (40.14%), making it the least effective diagnostic instrument. The research by Muthoni et al., (2016) and Crump et al., (2010) also highlighted the limitations of the Widal test, citing its elevated cross-reactivity with other illnesses, including malaria and dengue [238]. Additional research, including that by Neupane et al., (2021), claim that the Widal test deserves to be discontinued in favor of more dependable diagnostic methods [239].

The positive predictive value (PPV) and negative predictive value (NPV) of a diagnostic test assess its capacity to confirm or rule out an illness. Research conducted by Diwaker et al. (2024) revealed that the Typhi/IGM test had the greatest positive predictive value (PPV) of 70% and a negative predictive value (NPV) of 100%, signifying its exceptional reliability in detecting typhoid fever [240].

In contrast, the Widal test had a significantly lower positive predictive value (5.64%) and a negative predictive value of 91.05%, reflecting its inadequate dependability in the proper diagnosis of typhoid fever. Zhou et al. (2012) revealed same results, indicating that the Widal test should not be used as an independent diagnostic technique [242]. Subsequent research by Watson et al., (2018) indicating that Widal-based diagnoses resulted in excessive antibiotic prescriptions, which could possibly contribute to antimicrobial resistance [243].

The diagnostic tests' accuracy was evaluated against the blood culture approach to ascertain their overall efficacy. Research results revealed that Typhi/IGM exhibited the greatest accuracy at 97% and a CSI of 67.74%, as documented by Ito et al., (2021) [244]. The Stool/CS test achieved an accuracy of 94.66% and a CSI of 38.46%, making it a fairly effective diagnostic instrument.

The Widal test had the lowest accuracy at 40.66% and a CSI of 5.31%, underscoring its unreliability for typhoid diagnosis. Similar findings were reported in research conducted by Ullah et al. (2025), underscoring the requirement to abandon Widal-based diagnosis in endemic areas [245]. Further research in Africa and Southeast Asia (Kim et al., 2020) has shown that dependence on the Culture test results in diagnosis delays and inadequate illness treatment [246].

The rates of false positives and false negatives are essential for assessing the diagnostic accuracy of typhoid tests. Present study reported that the Typhi/IGM test had the lowest false positive rate (9 instances) and no false negative cases, making it the most accurate test. This study demonstrated that the Stool/CS test had a moderate false positive rate of 5 instances and 11 false negative cases, whereas the Widal test had the greatest false positive rate of 167 cases and considerable false negative values.

In present study there is a statistically significant difference among the diagnostic categories (TP, TN, FP, FN), especially driven by the large differences in TN and FP averages.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TP	3	41	13.66667	40.33333
TN	3	656	218.6667	8537.333
FP	3	181	60.33333	8537.333
FN	3	22	7.333333	40.33333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	87587.33	3	29195.78	6.807394	0.013591	4.066181
Within Groups	34310.67	8	4288.833			
Total	121898	11				

Interpretation

- F = 6.81 > F crit = 4.066 → Significant difference between group means.
- P-value = 0.0136 < 0.05 → Statistically significant. We reject the null hypothesis that all group means are equal.

Table 4 - Multivariate Logistic Regression Analysis in present study

Variable	Adjusted Odds Ratio (AOR)	95% Confidence Interval	P-value	Interpretation
Typhi IgM (Positive)	8.5	3.20 – 22.30	<0.001	Strong Independent
Stool Culture (Positive)	3.90	1.80 – 8.10	0.002	Significant Predictor
Widal Test (Positive)	1.10	0.60 – 2.30	0.280	Not Significant

These findings correspond with the research conducted by Shrestha et al., (2018), which advocated for the prioritization of the Typhi/IGM test in regular clinical environments owing to its high reliability and few diagnostic inaccuracies [249]. Moreover, the influence of false positives on healthcare expenditures and patient outcomes has been extensively discussed, with scholars such as Ogolo et al., (2024) highlighting the financial strain of misdiagnosis in resource-constrained environments [250].

Conventional diagnostic technique, such as Widal test, have limits in sensitivity, specificity or erroneous diagnosis. This study examines recent developments and analyzes significant results from studies, including those by Wright et al., (2022), evaluating their clinical value and influence on illness management [251].

CHAPTER-5
CONCLUSION

CONCLUSION

Diagnosing typhoid fever involves a multifaceted approach with culture and immunological testing being the primary methods. Among these the isolation of the causative agent *Salmonella enterica* (*serovar typhi*) from a culture of bone marrow is considered the gold standard offering optimal reliability. However, the effectiveness of this method is hindered by invasive medical procedures and the limited access to culture facilities in rural healthcare centers. Blood culture is another gold standard test which has little limitations to perform the test. Notably, the commonly employed stool culture method while widely used exhibits a relatively low sensitivity range of 40-60% introducing a substantial risk of false-negative outcomes and impacting approximately half of the individuals diagnosed with typhoid fever. The most commonly used test in typhoid fever is Widal test which has low sensitivity as per maximum past studies. Typhi IgG/IgM rapid test is widely used for the diagnosis of enteric fever but needs further studies for its accuracy, efficiency and specificity.

To address these challenges and enhance diagnostic accuracy a comprehensive study was conducted in the selected districts of Malwa region of Punjab, India. This study delved into the diagnostic landscape of typhoid fever shedding light on the prevalence and intricacies associated with this endemic ailment. By evaluating various diagnostic tests employing a diverse array of instruments and laboratory techniques the research aimed to provide a nuanced understanding of the strengths and limitations of different diagnostic approaches. Gathered samples from key healthcare institutions such as Ajit Hospital, Government Civil Hospital and Brar Clinic all three in different cities. The study's duration is eleven months and 6 days allowed for a thorough examination of typhoid fever diagnosis. This research contributes valuable insights into the diagnostic challenges associated with typhoid fever, especially in resource-limited settings. By elucidating the strengths and weaknesses of different diagnostic methods, the study aims to empower healthcare practitioners to make informed decisions, ultimately improving the accuracy and efficiency of typhoid fever diagnosis. This knowledge is pivotal for the development of targeted public health interventions and the mitigation of the impact of typhoid fever in emerging nations.

The study sought to shed light on the diagnostic landscape by rigorously evaluating a range of tests including the Widal test, Typhi IgG/IgM test, blood culture and stool culture with the overarching goal of providing nuanced insights into their efficacy. The gold standard diagnostic method blood culture was acknowledged for its reliability in isolating the causative agent *Salmonella enterica* (serovar typhi). Among the alternative methods evaluated the Widal test, known for its historical use in diagnosing typhoid fever underwent rigorous scrutiny. The Typhi IgG/IgM test, which assesses the presence of specific antibodies indicative of a recent or ongoing infection was also included in the evaluation. These tests which offer potential advantages in terms of accessibility and ease of implementation were subject to meticulous analysis to ascertain their diagnostic accuracy and reliability.

The study explored the utility of stool culture as a diagnostic method. Its evaluation aimed to provide insights into its potential as a diagnostic tool for typhoid fever. The research systematically assessed the strengths and limitations of each diagnostic test considering factors such as sensitivity, specificity.

Notably, the Typhi/IgM test emerged as a promising diagnostic method exhibiting a higher true positive rate (Figure 24) and positive predictive value (Figure 22) compared to other tests. A high level of specific IgG antibodies from a previous infection or vaccination can sometimes interfere with IgM antigen binding in the Typhi IgG/IgM rapid test, potentially masking a current infection and giving a false negative IgM result.

The study delving into demographic patterns of typhoid suspicion extends beyond diagnostics offering a nuanced understanding of the age distribution of cases and variations in prevalence between genders. This exploration is crucial for tailoring effective interventions and refining diagnostic strategies to align with specific demographic nuances. The age distribution of typhoid suspicion uncovered by the study is a pivotal finding, shedding light on the differential vulnerability across age groups. This insight holds significant implications for public health planning and underscores the need for targeted diagnostic efforts. Children and the elderly for instance, may exhibit different clinical manifestations and understanding, these age-specific patterns is vital for accurate and timely diagnosis.

The quest for a standardized reference test is not only a technical challenge but also a critical aspect of ensuring the reliability and credibility of diagnostic findings. The study's exploration beyond diagnostics provides a holistic view of typhoid fever considering demographic patterns, age distribution and gender-specific variations. This broader perspective enriches our understanding of the disease and informs a more targeted and effective approach to both diagnostics and interventions.

The discussion extended to the transmission dynamics of typhoid fever emphasizing the importance of interrupting the fecal-oral transmission cycle. Alternative methods, such as the Widal test and the Typhi IgG/IgM test were explored indicating the ongoing quest for more accessible and cost-effective diagnostic approaches. As per this research work it is likely that Typhi IgG/IgM test will replace the Widal test for its low sensitivity in coming future.

While blood culture consistently emerged as the gold standard, the study recognized the potential of alternative tests like Typhi IgG/IgM and WIDAL. The findings underscored the need for context-specific considerations in selecting diagnostic tools for typhoid fever.

In summary, typhoid fever persists as a significant global health challenge, necessitating precise diagnostics and efficient management. The study's thorough assessment provides crucial perspectives on the diagnostic scenario underscoring the importance of ongoing research and development. While the study carries strengths, it also invites reflection on limitations such as sample size and potential biases, guiding future research endeavors.

Rank wise approach of diagnostic methods according to this study

1. Blood Culture
2. Typhi IgG/IgM
3. Stool Culture
4. Widal Test

The conventional methods delaying the diagnosis but the rapid testing has increased the fast diagnosis with the good sensitivity and accuracy rate.

Future perspectives

For the rapid and accurate diagnosis there is requirement of dual detection technique (antigen – antibody) to improve the diagnostic test at different stages of infection. This technique can help in combating the challenges posed by typhoid fever, a comprehensive and multifaceted strategy is imperative. This strategy must seamlessly integrate technological advancements, public health interventions and collaborative research efforts to effectively address the complex nature of the disease. The promising revelation of the Typhi/IgM test as a diagnostic method underscores the urgency of sustained research endeavors aimed at refining and validating alternative diagnostic approaches. The horizon of future studies should prioritize the development and validation of innovative assays such as the Typhi IgG/IgM test and WIDAL, with a keen focus on their applicability across diverse geographical contexts including regions like the Malwa region of Punjab. Understanding the factors influencing diagnostic accuracy in specific demographic groups is paramount to tailoring effective interventions. Furthermore, overcoming the limitations associated with the gold standard blood culture necessitates efforts to improve accessibility particularly in rural health centers and exploring viable point-of-care alternatives.

The ongoing debate surrounding the efficacy of the Widal test highlights the crucial need for a standardized clinical research system and collaborative initiatives among investigators. Establishing universally applicable diagnostic benchmarks is essential for ensuring consistency and reliability in diagnosing typhoid fever. Ethical considerations, notably obtaining individual consent for data access must be prioritized to uphold the integrity and transparency of future research endeavors. The global burden of typhoid fever as underscored by the World Health Organization's estimates mandates the development of a comprehensive clinical risk stratification strategy. Future research efforts should converge on combining multiple diagnostic techniques to enhance overall accuracy, acknowledging the heterogeneity in diagnostic performance observed across different populations and settings.

Beyond diagnostics, future interventions must grapple with the intricacies of typhoid fever transmission dynamics particularly through carriers and contaminated water sources. Robust diagnostic tools are pivotal for the prompt identification and management of affected individuals thereby contributing to the interruption of the fecal-oral transmission cycle. In essence, the path forward demands a holistic and dynamic approach, targeted interventions and ethical considerations to confront the multifaceted challenges posed by typhoid fever on a global scale.

Limitations

- The study focused on the selected area of Malwa region of Punjab, India, limiting the generalizability of findings to other regions with potentially different healthcare infrastructure and typhoid prevalence patterns.
- The study did not explicitly discuss potential biases, raising questions about the representativeness of the sample. This may impact the external validity of the results.
- While the study evaluated various diagnostic methods it primarily emphasized the gold standard blood culture. This may not fully capture the nuances of other potential diagnostic approaches such as PCR and ELISA.
- Although blood culture is often regarded as the second best method, its drawbacks like low sensitivity and accessibility challenges highlight the necessity for a stronger and more reliable reference standard in diagnosing typhoid fever.

CHAPTER 6

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1. Instruments and apparatus

- Autoclave for sterilisation and steaming purpose.
- Ordinary room temperature table centrifuge.
- Laminar flow.
- Incubator.
- Hot air oven for glass articles.
- Cooling centrifuge (Centriffeeze).
- Gas burner.
- Water bath.
- Refrigerator.
- Gas oven.
- Colony counter.
- Widal rack.
- Distillation plant.
- Nichrome wire with handle.
- Platinum wire with handle.
- Micropipette and tips.
- Glass wares and glass utensils
- Beaker
- Funnel
- Flasks (flat bottom and round bottom.)
- Flasks (conical of different sizes, 100 ml., 200 ml., 500 ml., 1000 ml.)
- Measuring cylinder
- Petri dishes.
- Dryer's tube.
- Blood culture bottle.
- Test tubes ofsize 4"x 1/2", 6" x 3/4".
- Felix tube.
- Measuring pipette.
- Pasteur pipette.

- Glass beads.
- Coverslips.
- Durham’s tubes.
- Glass slides.

2. **XLD Media composition**

Table 3. Composition of Media	
INGREDIENTS	**AMOUNT**
Sodium deoxycholate	1.6 g
Yeast extract	3 g
L-Lysine and NaCl	5 g
Xylose	3.75 g
Lactose and saccharose	7.5 g
Agar	15 g
Distilled water	1 L
Ferric ammonium citrate	0.8 g
Sodium thiosulphate	6.8 g

3. **Selenite F Broth**

Table 4. Composition of Media	
INGREDIENTS	**AMOUNT**
Sodium selenite	4.0 g/L
Peptone	5.0 g/L
Lactose	5.0 g/L
Disodium phosphate	9.5.0 g/L
Monosodium phosphate	0.5 g/L
Distilled water	1 L

4. **Urease Agar**

Table 5. Composition of Media	
INGREDIENTS	**AMOUNT**
Urea	20.0 g/L
Peptone	1.0 g/L
Dextrose	1.0 g/L
Sodium Chloride	5.0 g/L
Dipotassium phosphate	5.0 g/L
Phenol red	0.012 g/L
Agar	20.0 g/L
Distilled water	1 L

5. **Triple Sugar Iron (TSI) Agar**

Table 6. Composition of Media	
INGREDIENTS	**AMOUNT**
Lactose	10.0 g/L
Peptone	20.0 g/L
Sucrose	10.0 g/L
Glucose	1.0 g/L
Sodium Chloride	5.0 g/L
Ferrous sulfate	0.2 g/L
Sodium thiosulfate	0.3 g/L
Phenol red	0.024 g/L
Agar	12.0 g/L
Distilled water	1 L

6. Proforma and Consent Form

DATE		PATIENT ID	
PATIENT NAME		AGE	
FATHER/HUSBAND NAME		SEX	
MARITAL STATUS		OCCUPATION	
ADDRESS			
EDUCATION		Daily Wage	
WEIGHT		HEIGHT	
FEVER HISTORY		Diarrhoea HISTORY	
ABDOMINAL PAIN STATUS		PREVIOUS HISTORY OF TYPHOID	

Patient Consent:

I have provided true and reliable information and fully understand the indication, intended purpose of test.

SIGNATURE OF PATIENT		REFFERING DOCTOR NAME	

Certificate No. 293424

ICRAHS INTERNATIONAL CONFERENCE ON RECENT ADVANCES IN HEALTH SCIENCES

LOVELY PROFESSIONAL UNIVERSITY

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Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Rajan Bector** has successfully participated as "**Delegate and Oral presenter**" in the International Conference on "**Recent Advances in Health Sciences" (ICRAHS-2023)** on the Theme of "Interdisciplinary Research: A key to transform Health care." held on 14th to 15th April, 2023 organized by School of Pharmaceutical Sciences in association with Komar University of Sciences and Technology at Lovely Professional University, Punjab.

Date of Issue : 17-10-2023
Place : Phagwara (Punjab), India

Prepared by	**Dr. Navneet Khurana**	**Prof. Dr. Kawis Aziz Faraj**	**Dr. Monika Gulati**
(Administrative Officer-Records)	**Program Chair**	**Conference Co-Chair**	**Conference Chair**

International Conference on Recent Trends in Biomedical Sciences
(RTBS-2023)

Certificate of Achievement

This is to certify that Prof./Dr./Mr./Ms. Rajan Bector........ of Lovely Professional University....has presented Oral/Poster entitled Conventional and Advanced Methods ...... in Malwa Region, Punjab.......In **International Conference on Recent Trends in Biomedical Sciences (RTBS-2023)** organized by Department of Medical Laboratory Sciences from 6th-7th October 2023 at Lovely Professional University, Punjab, India.

Sandeep Sharma
Prof (Dr.) Sandeep Sharma
Covenor/Organizing Secretary
RTBS2023

Monica Gulati
Prof (Dr.) Monica Gulati
Chairperson
RTBS-2023

Gulam Mohamad Diwana
CIVIL HOSPITAL KHANNA
G T Road, Khanna- 141401
Email: chkhanna123@gmail.com Phone: 01628220102

No. C.H. Khanna/2022/ 1974 Date: 25-11-22

TO WHOM IT MAY CONCERN

This is to certify that Mr. Rajan Bector, PhD student of Lovely Professional University Registration Number 42000077 is conducting research on suspected typhoid fever patients, testing four parameters i.e Widal test, Typhoid IgG/IgM, Blood Culture, Stool Culture on them by taking patient's Blood sample on Day 1 and Blood, Stool sample on Day 6 in Civil Hospital Khanna with patient consent and without taking any charges. There is no risk to the patient in this research and he is authorised to use the data in his doctorate thesis or any publication.

SENIOR MEDICAL OFFICER I/C • CIVIL HOSPITAL • KHANNA

MSBhar

Senior Medical Officer I/c
Civil Hospital Khanna

098142-21760 (Hosp)
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Dr. AJIT SINGH
M.B.B.S; M.D. (Int. Med.)
Ex-Specialist P.G.I. Chandigarh
Regn. No. 10128

MEDICAL SPECIALIST

Dr. IQBAL KAUR
M.B.B.S; M.D. (Ob;Gy)
F.I.C.M.C.H.
P.C.M.S. Class-I (Ex)
Formerly Senior Medical Officer-I
CIVIL HOSPITAL, KHANNA
Ex. Registrar, Rajindra Hospital, Patiala
Regn. No. 15135

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CONSULTING HOURS :
Summer : 9.30 am to 2.00 pm & 4.30 pm to 7.00 pm
Winter : 10.00am to 2.00 pm & 4.00 pm to 6.30 pm

Ref./ Dated________

੧ੴ ||

Dated: 15.10.2021

TO WHOM IT MAY CONCERN

This is to certify that Mr. Rajan Bector, PhD student of Lovely Professionals University Registration Num 42000077 is conducting Research on suspected typhoid fever patients, testing four parameters i.e Widal test, Typhoid IgG/IgM, Blood Culture, Stool Culture on them, by taking patient's Blood Sample on Day 1 and Blood, Stool sample on Day 6 under my guidance in my hospital and in house hospital Clinical Laboratory. There is no risk to the patient in this research and he is authorised to use the data in his doctorate thesis or any publication.

Ajit Singh
DR. AJIT SINGH M.D..
Reg. No. 10128
Ajit Hosp. KHANNA (Pb.)

For AJIT HOSPITAL
G.T. Road, Khanna
Ajit Singh
Prop.

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ਐਮ.ਬੀ. ਬੀ.ਐਸ., ਐਮ.ਡੀ. (ਮੈਡੀਸਨ)

Dr. Navdeep. S. Brar

M.D. Medicine
(Ex. PCMS-1)
Reg. No. 29778

Ref. No. .... Dated ....

Dated: 15.10.2021

TO WHOM IT MAY CONCERN

This is to certify that Mr. Rajan Bector, PhD student of Lovely Professionals University Registration Num 42000077 is conducting Research on suspected typhoid fever patients, testing four parameters i.e Widal test, Typhoid IgG/IgM, Blood Culture, Stool Culture on them, by taking patient's Blood Sample on Day 1 and Blood, Stool sample on Day 6 under my guidance in my hospital and in house hospital Clinical Laboratory. There is no risk to the patient in this research and he is authorised to use the data in his doctorate thesis or any publication.

Dr. N.S. BRAR (MD Medicine)
Ex PCMS-1 Reg.No.29778
Brar Clinic, Amloh Road,
Mandi Gobindgarh (147301)

Consulting Hour:

ਮਿਲਣ ਦਾ ਸਮਾਂ:

Summer (ਗਰਮੀਆਂ):

ਸਵੇਰੇ: 9.30 ਵਜੇ ਤੋਂ 2.00 ਵਜੇ ਤੱਕ
ਸ਼ਾਮ: 5.00 ਵਜੇ ਤੋਂ 8.00 ਵਜੇ ਤੱਕ

Winter (ਸਰਦੀਆਂ)

ਸਵੇਰੇ: 10.00 ਵਜੇ ਤੋਂ 2.00 ਵਜੇ ਤੱਕ
ਸ਼ਾਮ: 5.00 ਵਜੇ ਤੋਂ 8.00 ਵਜੇ ਤੱਕ

ਐਤਵਾਰ 11 ਤੋਂ 2 ਵਜੇ ਤੱਕ

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Independent Research Ethics Society
Regn No.: S/2L 25358 of 2014-15 under W. B. Society Act 1961

Independent Ethics Committee (Clinical Research) India

Regd. Office: 119 Rajdanga Gold Park
Near Tribarna Sangha, RB connector
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PROJECT APPROVAL LETTER

Ethics Committee	**Independent Ethics Committee (Clinical Research) India**		
Address	**119, Rajdanga Gold Park, RB Connector, Kolkata – 700107**		
Date of Approval	**08.04.2023**	**Approval No.**	**IECCRI/23-24/01**

Protocol Title	Accuracy, Sensitivity and Specificity of Conventional and Advanced Methods for The Diagnosis of Typhoid Fever in Malwa Region, Punjab	
Principal Investigator	Mr. Rajan Bector	
The Independent Ethics Committee has reviewed the following document submitted for the above mentioned clinical microbiology study		
Name of documents reviewed	**RV**	**AP**
1. Letter of Candidacy for Ph.D (Lovely Professional University)	✓	✓
2. Recommendations of the Research Advisory Committee (RAC)	✓	✓
3. Permission Letters from Hospitals	✓	✓
4. Research Proposal	✓	✓

RV denotes Reviewed; AP denotes Approved

MEMBERS REVIEWED THE PROJECT

Name of member	Position in ethics committee	Qualification
Dr. S. K. Bandyopadhyay	Chairperson	MD (Pharmacology)
Dr. Pawan Kumar Sharma	Member Secretary	MD (Ayu), MBA
Ms. Moumita Sharma	Member	MBA

The approval is valid from **08.04.2023 till 07.04.2024** and the renewal of this study is subject to review of "Preliminary Study Report" submitted to this Ethics Committee by the Investigator.

The research proponents are here by informed that the Independent Ethics Committee (IEC) will require the following:

- The progress report to be submitted to the IEC at least after 1 month.
- Upon completion of the study, a final study status report needs to be submitted to the IEC.

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OPEN ACCESS

Accuracy, Sensitivity, And Specificity Of Conventional And Advanced Methods For The Diagnosis Of Typhoid Fever In Malwa Region, Punjab

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Abstract

Background: The main objective of this research is to analyze different methods to detect typhoid like Widal tube test, Typhi IgG/IgM Chromatographic technique, Stool Culture and Blood Culture and also record their Sensitivity, specificity, cost effectiveness in Malwa region of Punjab state.

Method: The study is conducted on 450 blood and stool samples of subjects in which 300 were suspected typhoid patients from three different hospitals in which two were private hospitals and one is Government hospital. 150 samples were tested as negative control. Blood culture taken as a Gold Standard test. All the samples were subjected to the Widal test, Typhi IgG/IgM Chromatographic technique, Stool Culture to detect the presence of *Salmonella enterica serovar typhi*. All parameters were evaluated for their sensitivity, specificity, FNR, FPR, FOR, FDR.

Result: When it comes of true negative cases, typhi IgM and stool culture gave 270 and 274 true negative results out of 300 respectively but shockingly Widal test gave 112 true negative results in 300. Typhi IgM gives 9 false positive results while stool culture gave only 5 false positive in total 300 but surprisingly out of 300 Widal gave a huge number of 167 false positive results. Both stool culture and Widal gave 11 false negative results but here not even a single case is given as false negative by Typhi IgM. PPV of Typhi IgM and stool culture have not much difference with 70% and 66.66% values respectively but Widal gave a very low positive predictive value of 5.64%. NPV of Typhi IgM, stool culture and Widal is 100%, 96.14% and 91.05 respectively. Typhi IgM gave 100% true positive rate while both stool culture and Widal gave a very low TPR of 47.61%. True negative rate of Typhi IgM and stool culture is very close values of 96.77% and 98.2% respectively but again Widal has very low TNR of 40.14%. A very high false negative rate value of 52.38% noted for stool culture and Widal test but 0% FNR value noted for Typhi IgM test. False positive rate of Typhi IgM and stool culture is 3.22% and 1.79% respectively but a very high FPR value of 59.85% noted for Widal test. False discovery rate of Typhi IgM is 30% and stool culture is 33.3% but a very high FDR of 94.35% noted for Widal test. 0% false omission rate is noted for Typhi IgM test while stool culture and Widal gave 3.85% and 8.94% FOR respectively.

Conclusion: It can be concluded that the Typhi IgG/IgM Chromatographic technique, quick serological test was found to have high specificity and sensitivity as compared to the cultures and Widal test. Typhi dot test can be used as an alternative to old standard procedures to increase the diagnostic rate and precision.

Keywords: Blood culture, IgM/IgG, *Salmonella enterica*, Typhi Dot, Widal test.

1. Introduction

Salmonella enterica serovars, a causative agent, is responsible for a severe, contagious, and deadly illness called typhoid. Although certain strains are grouped as NTS (nontyphoidal Salmonella) [1], Typhi and Para typhi A, B, and C may all be collectively classified as typhoidal Salmonella. Typhoid fevers may cause mortality, gastrointestinal