ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
MSc Thesis

A Comparative Study of Blood Culture and Widal test in the Diagnosis of Typhoid Fever in Febrile Patients

BY

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Assurance form

I, the undersigned, declare that this MSc thesis is my original work and has not been presented for a degree in any other university, all information provided with this thesis is up-to-date and accurate and that all sources of materials used for the thesis have been duly acknowledged.

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Acronym

Anti TH	antibody against H antigen of *Salmonella typhi*
Anti TO	antibody against O antigen of *Salmonella typhi*
CSF	Cerebro Spinal Fluid
DCA	Deoxycholate–citrate agar
DMIP	Department Of Microbiology Immunonology and Parasitology
ELISA	Enzyme Linked Immunosorbent Assay
IDH	Infectious Disease Hospital
LDC	Lysin decarboxylase
MDR	Multi Drug Resistance
NAR	Naldixic Acid Resistant
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
SSA	*Salmonella Shigella* Agar
TSI	Triple Sugar Iron Agar
XLD	Xylose–Lysine–Deoxycholate agar

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Abstract

Introduction: Typhoid fever is a major health problem in developing countries. An accurate diagnosis on clinical grounds alone is difficult. In areas of endemicity, such as Ethiopia, bacterial culture facilities, definitive diagnosis for typhoid fever, are often unavailable. So, the Widal test has been in use as the diagnostic assay. However, the value of the test for the diagnosis of typhoid fever has been debated. So evaluating the result of Widal test is necessary for correct interpretation of the result. In addition typhoid fever caused by multidrug resistant strains of *Salmonella typhi* presents a serious problem in many developing countries.

Objective: The main objective of this study is to compare the result of Widal test and blood culture in the diagnosis of typhoid fever in febrile patients and to determine the antimicrobial pattern of isolates.

Methodology: Data was collected from 277 febrile patients with symptoms clinically similar to typhoid fever visiting St. Paul's General Specialized Hospitals from mid December 2010 to March 2011. Blood was inoculated immediately after collection into 45ml of Trypton Soy Broth and further processed for the identification of *S.typhi* and *S.paratyphi*. Antimicrobial susceptibility pattern of *S. typhi* and *S. paratyphi* isolates were determined by the modified Kirby-Bauer disk diffusion technique. Slide agglutination test as screening test and tube agglutination for the determination of antibody titer for reactive slide agglutinations samples have made. An antibody titer of ≥1:80 for anti TO and ≥1:160 for anti TH are taken as a cut of value to indicate recent infection of typhoid fever. Statistical software package for widows (SPSS version 16) was used for analysis of the data and p value ≤0.05 was taken as significance.

Result: A total of 277 febrile patients were recruited for this study, but data from 270 were analysed because the remaining seven patients have no full data to be processed. 186 (68.9 %) were females and 84 (31.1 %) were males. 7 (2.6%) cases of *S. typhi* and 4 (1.5%) cases of *S. paratyphi* were identified with the total prevalence of typhoid fever 4.1 %. The total number of patients who have indicative of recent infection by either of O and H antigens Widal test is 88 (32.6%). The sensitivity, specificity, PPV and NPV of Widal test are 71.4 %, 68.44%, 5.7% and 98.9% respectively. Most (3/7[42.9%]) of the isolated *S.typhi* are highly resistant to amoxicillin. All species are sensitive for norfloxacin and ceftriaxone. *S. paratyphi* isolates show no resistance to gentamycine, tetracycline, norfloxacin and ciprofloxacin. More resistance (3 out of 4) is observed in amoxicillin. One species of *S.typhi* and 2 species of *S. paratyphi* are multi drug resistant.

Conclusion and recommendation: Widal test have a low sensitivity, specificity and PPV, but it has good NPV which indicates that negative Widal test result have a good indication for the absence of the disease. Hence, physicians should not totally depend on Widal test for the diagnosis of typhoid fever and should use other alternative diagnostics such as clinical knowledge to differentiate from other febrile infections. Regarding drug resistance both *S. typhi* and *S. paratyphi* showed high resistance for commonly used drugs against typhoid fever. Therefore, sensitivity test based prescription should be started to prevent the continuous drug resistance development.

Key words: Widal test, blood culture, antimicrobial resistance, sensitivity, specificity, positive predictive value, negative predictive value

1. Introduction

1.1. Background

Typhoid fever is a systemic prolonged febrile illness caused by certain Salmonella serotypes including *Salmonella typhi, S. paratyphi A, S. paratyphi B* and *S. paratyphi C*. It emerged as an important infectious disease in the early 19th century. The illness begins with mounting fever, headache, vague abdominal pain and constipation, which may be followed by appearance of rashes. During the third week, the patient reaches a state of prolonged apathy, toxemia, delirium, disorientation and/or coma followed by diarrhoea. If left untreated, it can lead to complications affecting various organ systems (Fauci et al., 2008). Infection occurs in all age groups with a higher incidence and more variable clinical presentation in children. Since the late 1940s typhoid fever was successfully treated with one of the several antibiotics, chloramphenicol, ampicillin and trimethoptrim-sulphamethoxazole. However, from 1990, multidrug resistant strains to the previously useful antibiotics have emerged, and treatment for such strains requires the use of more expensive quinolone antibiotics such as oral ciprofloxacin or third generation cephalosporins such as ceftriaxone (WHO, 2003). Human beings are the only reservoir and host for typhoid fever, and the disease is transmitted by faecally contaminated water and food in endemic areas especially by carriers handling food. The World Health Organization (WHO) estimates for annual global incidence of typhoid fever, about 21 million cases with >600,000 deaths. Cases are more likely to be seen in areas like India, South and Central America, and Africa with rapid population growth, increased urbanization, and limited safe water, infrastructure, and health systems (Willke et al., 2002, John et al., 2004).

Typhoid fever is a major health problem in developing countries where safe water supplies and adequate sewage disposal are often lacking. Epidemiologic data on typhoid fever in endemic countries is lacking or incomplete. Case identification may be based on clinical, bacteriological or serologic diagnosis; or typhoid fever may be clumped with other diseases or conditions such as fever of unknown origin (Abucejo et al., 2001).

Typhoid fever has important socioeconomic impact because, most of the time, several months are necessary for a patient to recover and be able to work again. So accurate diagnosis of typhoid fever at an early stage is important not only for etiological diagnosis, but also to identify individuals that may serve as a potential carrier, who may be responsible for acute typhoid fever outbreaks (Gopalakrishnan et al., 2002). Several options exist for diagnosing enteric fever: clinical signs and symptoms; serological markers; bacterial culture; antigen detection; and DNA amplification. The clinical diagnosis of typhoid fever is difficult because the manifestations of

the disease are diverse and there are many causes of prolonged fever in typhoid endemic regions. Signs such as relative bradychardia or leucopoenia may be useful but give a low specificity. The culture of blood, bone marrow and stool are the most reliable diagnostic methods but these are expensive techniques and the infecting organism may be dead on arrival at the hospital if the patient has taken antibiotics before clinical samples can be taken. Serological diagnosis is predominantly by the Felix-Widal test, first standardised in the 1950s. Although ELISA and immunoblotting suggest possibilities, the commercially available kits for the serodiagnosis of enteric fever have not performed well in large studies (Wain and Hosoglu, 2008).

Prompt administration of appropriate antibiotic therapy prevents severe complications of enteric fever and results in a case-fatality rate of <1%. The initial choice of antibiotics depends on the susceptibility of the *S. typhi* and *S. paratyphi* strains in the area of residence or travel. For treatment of drug-susceptible typhoid fever, fluoroquinolones are the most effective class of agents, with cure rates of ~98% and relapse and fecal carriage rates of <2%. Short-course ofloxacin therapy is similarly successful against infection caused by nalidixic acid–susceptible strains. Patients infected with nalidixic acid–resistant (NAR) *S. Typhi* strains should be treated with ceftriaxone, azithromycin, or high-dose ciprofloxacin. Ceftriaxone, cefotaxime, and (oral) cefixime are effective for treatment of MDR enteric fever, including NAR and fluoroquinolone-resistant strains (Fauci et al., 2008, WHO, 2003).

Theoretically, it is possible to eliminate the salmonellae that cause enteric fever since they survive only in human hosts and are spread by contaminated food and water. However, given the high prevalence of the disease in developing countries that lack adequate sewage disposal and water treatment, elimination is currently unrealistic. Thus, travellers to developing countries should be advised to monitor their food and water intake carefully and to consider vaccination (Fauci et al., 2008).

1.2. Statement of the problem

Typhoid fever remains a major public health problem in the developing world with very poor estimates of the number of cases and deaths annually. Continued research on the epidemiology, ecology, pathogenesis, diagnosis, treatment and prevention of typhoid can most optimally be pursued in the endemic regions which, unfortunately, also suffer from a lack of research capacity, funding support, and institutional infrastructure. Much needs to be done to promote and strengthen typhoid fever and other infectious disease research in these regions if true progress is to be made. Information across sub-Saharan Africa is very scarce and the issues clearly require urgent and rapid action, particularly in East Africa (Ethiopia and Kenya) which seems to have a high burden of typhoid fever (Pang, 2008).

The signs and symptoms of uncomplicated typhoid fever are nonspecific, and an accurate diagnosis on clinical grounds alone is difficult. Many other infections have the same clinical presentation (Nsutebu et al., 2003, Onyekewere, 2007). In many countries including Ethiopia, the Widal test is the most widely used test in typhoid fever diagnosis because it is relatively cheaper, easy to perform and requires minimal training and equipment. There is no doubt as to the endemicity of typhoid fever in Ethiopia, but precise estimates of the prevalence of typhoid fever among febrile patients are unavailable and there is no coordinated epidemiological surveillance (Pang, 2008, Beyene et al., 2008).

Culture of the causative organism remains the most effective diagnostic procedure in suspected typhoid fever (Wain et al., 2008). Stool culture is also an important adjunct for diagnosis. Stools can be collected from acute patients; it may be positive when blood culture is negative (WHO, 2003) and it is especially important for the monitoring of carriage of *S. typhi* after apparent clinical cure, a risk factor for the families of cases (WHO, 2003, Wain and Hosoglu, 2008).

Although a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow, in areas of endemicity, such as Ethiopia, bacterial culture facilities are often unavailable (Wain and Hosoglu, 2008, Parry et al., 1999). Confirmed diagnosis through blood or bone-marrow culture requires expensive and labor-intensive isolation and identification of the organism, which may take up to seven days. A cheap and rapid alternative laboratory test is desirable, especially for developing country settings where typhoid fever is a major public health burden. Various agglutination tests have been developed (Wain and Hosoglu, 2008) of which the Widal method is the oldest and remains the most widely used (Ley et al., 2010). The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever (Olopoenia and king, 2000) and the Widal test is the only rapid diagnostic assay that is available and

affordable (Ley et al., 2010). The value of the test for the diagnosis of typhoid fever has been debated for as many years as it has been available (Olopoenia and king, 2000). It relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in blood culture-confirmed cases. This situation may occur because the acute-phase sample was obtained late in the natural history of the disease, because of high levels of background antibodies in a region of endemicity, or because in some individuals the antibody response is blunted by the early administration of an antibiotic (Olopoenia and king, 2000). Furthermore, patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute-phase sample (Parry et al., 1999). So evaluating the result of single Widal test is necessary for correct interpretation of the result.

In addition to challenges of diagnosis of typhoid fever, drug resistance in typhoid salmonellae is considered as one of the important factors in the morbidity and mortality of the disease. Infections by *S. typhi*, a potentially lethal organism, were successfully managed for many years with chloramphenicol. However, the last two decades have witnessed the emergence and spread of multidrug resistance against conventional antityphoid drugs (ampicillin, chloramphenicol and trimethoprim– sulfamethoxazole) among typhoid salmonellae. Typhoid fever caused by such multidrug resistant (MDR) strains of Salmonella enterica serotype Typhi presents a serious problem in many developing countries (Dimitrov et al., 2007).

In view of the high costs of drugs used for the treatment of typhoid fever and their numerous side-effects, this cross-sectional study was carried out to evaluate the value of a single acute-phase Widal test result for the diagnosis of typhoid fever in febrile patients and to determine the antimicrobial pattern of the isolated species of *Salmonella*.

2. Literature Review

2.1. The organism

Typhoid fever is caused by *Salmonella typhi*, a Gram-negative bacterium. A very similar but often less severe disease is caused by *Salmonella* serotype *paratyphi A*, *paratyphi B*, *paratyphi C* (Murray et al., 2005). The ratio of disease caused by *S. typhi* to that caused by *S. paratyphi* is about 10 to 1 in most of the countries where this matter has been studied (WHO, 2003). But in sub-Saharan Africa, where the burden is the least well characterized, hospital based studies indicates that non-typhi serotype of salmonella particularly *S.enterica* serotype entritides and *S.enerica* serotype typphimurium, greatly outnumber *S.typhi* and *S.paratyphi* as a cause of blood stream infection. (Crump et al., 2010)

2.2. The disease and Symptoms

After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. Then they penetrate the mucous layer of the gut and traverse the intestinal layer through phagocytic microfold (M) cells that reside within Peyer's patches and become phagocytised. Once phagocytosed, Salmonellae disseminate throughout the body in macrophages via the lymphatics and colonize reticuloendothelial tissues (liver, spleen, lymph nodes, and bone marrow), where it resides during the incubation period, usually of 8 to 14 days (WHO, 2003, Murray et al., 2005).

The most prominent symptom is prolonged fever (38.8°–40.5°C; 101.8°–104.9°F), which can continue for up to 4 weeks if untreated. *S. Paratyphi A* is thought to cause milder disease than *S. Typhi*, with predominantly gastrointestinal symptoms. Early physical findings of enteric fever include rash ("rose spots"), hepatosplenomegaly (3–6%), epistaxis, and relative bradycardia at the peak of high fever (Fauci et al., 2008, Buzğan et al., 2007).

The development of severe disease (which occurs in ~10–15% of patients) depends on host factors, strain virulence and inoculum, and choice of antibiotic therapy. Gastrointestinal bleeding (10–20%) and intestinal perforation (1–3%) most commonly occur in the third and fourth weeks of illness and result from hyperplasia, ulceration, and necrosis of the ileocecal Peyer's patches at the initial site of Salmonella infiltration. 1-5% of patients, depending on age, become chronic carriers harbouring *S.typhi* in the gallbladder (Fauci et al., 2008, Parry et al., 1999).

2.3. Contamination and transmission

Humans are the only natural host and reservoir. The infection is transmitted by ingestion of food or water contaminated with faeces. Ice cream is recognized as a significant risk factor for the transmission of typhoid fever. Shellfish taken from contaminated water, and raw fruit and

vegetables fertilized with sewage, have been sources of past outbreaks. The highest incidence occurs where water supplies serving large populations are contaminated with faeces (WHO, 2003, Murray et al., 2005). In developed countries, typhoid is transmitted when chronic carriers contaminate food as a consequence of unsatisfactory food-related hygiene practices (WHO, 2003).

2.4. Magnitude of the problem

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed (WHO, 2003).

There are few established surveillance systems for typhoid in the developing world, especially in community settings; as a result, the true problem is difficult to estimate. WHO estimation in 2004 indicate that there may be at least 21.7 million typhoid cases annually with the global mortality estimates of about 200,000 deaths (John et al., 2004, Bhutta, 2006). The incidence rate of typhoid fever in developed countries is lower than 15 cases per 100,000 populations, with most cases occurring among travellers. In contrast, the incidence varies considerably in the developing world, with estimated incidence rates ranging from 100 to 1000 cases per 100,000 populations (WHO, 2003, John et al., 2004, Bhutta, 2006). In a study investigating typhoid outbreaks in children in Addis Ababa, Ethiopia, the correct clinical impression of typhoid fever was made on 66 (55.6%) of the patients. Blood culture was positive in 34 out of 54 cases with 63% positivity rate (Kariuki, 2008).

2.5. Diagnosis and challenges of diagnosis of typhoid fever

The basis of the diagnosis of typhoid fever is a positive culture from the blood or another anatomical site. Results of blood cultures are positive early in the course of the disease, and stool and urine cultures become positive after the 1st week, but their sensitivity is much lower (Wain et al., 2008, Bhutta, 2006). The stool culture result is also occasionally positive during the incubation period (Wain et al., 2008). However, the sensitivity of blood cultures in diagnosing typhoid fever in many parts of the developing world is limited as widespread antibiotic prescribing may render bacteriological confirmation difficult (Parry et al., 1999). Although bone marrow cultures may increase the likelihood of bacteriological confirmation of typhoid, these are difficult to obtain and relatively invasive. In recent years, the development of sensitive nested PCR diagnostic techniques have made it possible to detect typhoid fever with greater sensitivity than blood cultures (Bhutta, 2006). Other relatively newer diagnostic tests using monoclonal antibodies have been developed, directly detecting *S. typhi*-specific antigens in the

serum or *S. typhi* Vi antigen in the urine. However, only a few have proved sufficiently robust in large-scale evaluations. Despite these new developments in most of the developing world, many laboratories in developing countries including Ethiopia lack adequate infrastructure to perform cultures and many depend on cheaper serological tests to diagnose typhoid, mostly on single Widal test result (Kariuki, 2008).

The interpretation of the Widal test remains problematic to this day, with a great number of articles reporting different cut-offs (Olopoenia and king, 2000) and the test has lost some popularity in recent years as antigenic determinants of both typhoid and non-typhoid Salmonella organisms are now characterised. In interpreting Widal test results, it is important that there should be close communication between the physician requesting the test and the laboratory, since modifications of technique in individual laboratories may affect the Widal titres and some patients with bacteriologically confirmed typhoid fever may fail to develop the usual rise of antibody titres (Olopoenia and king, 2000). In many places, instead of the standard Widal test, a quantitative slide agglutination test is used but this should always be interpreted with reference to clinical data (Olopoenia and king, 2000). False negative results may occur if the blood is collected too early in the disease; therefore, negative results do not rule out typhoid fever and may be best used as a baseline for subsequent comparative titrations. False positive results may be associated with a past history of immunization for typhoid fever, cross-reacting antibodies, or a whole host of infections and conditions (Wain and Hosoglu, 2008; Onyekewere, 2007; Olopoenia and king, 2000; Cheesbrough, 2000).

In a survey conducted in Cameroon, employing a questioner and check list to evaluate the performance of the Widal test, found that most of the visited laboratories (88%) performed the widal slide agglutination test as opposed to the conventional tube agglutination. The results from these rapid slide agglutinations are very subjective with even a poorer specific and high false positive than the tube methods. This may be responsible for the over diagnosis of typhoid fever (Nsutebu et al., 2002). Also false positive Widal tests have been reported for patients with non enteric salmonella infections e.g. malaria, typhus, *C. neoformance* meningitis, immunological disorder, chronic liver disease. The elevated antibody titters in these conditions may be due to cross reacting or anamnestic responses. The consequence is that potentially fatal illness such as malaria, non typhoidal salmonellosis, endocarditis and urinary tract infections are missed. Also the prior use of antibiotics as seen with the widespread use of antibiotics abuse can dampen antibody response giving a low titer in the Widal test even in the face of bacteriologically confirmed typhoid fever resulting in misdiagnosis (Onyekewere, 2007).

In a hospital-based studies conducted in Kenya (at the main referral hospital and at one private hospital in Nairobi) for the Characterization of multidrug resistant typhoid outbreaks, it showed that blood culture-confirmed typhoid cases occur at an incidence of 600-650 per 100,000 blood/CSF from suspected patients per year. In this study, the Widal test method would have missed out 7% of typhoid cases, while at the same time categorize another 18.7% as typhoid cases when they were not (Kariuki, 2008; Kariuki et al., 2004 a). Other studies in endemic areas of Vietnam conducted for the evaluation of commercial serodiagnostic assays for diagnosis of acute serotype Typhi infection with specimens collected in southern Vietnam, it is indicated that neither the Widal test, nor any of the serodiagnostic tests that have since been developed has proven sufficiently sensitive, specific, and practical to be of value in areas where this disease is endemic. In this study, the sensitivity and specificity findings were 64 and 76% for Widal testing in hospitals and 61% and 100% for Widal testing at the Pasteur Institute for the same samples, which also shows that interoperator variability is high in Widal testing (Olsen et al., 2004).

In a retrospective study undertaken to determine the utilization and validity of the Widal test in febrile children, at the Aminu Kano Teaching Hospital, Nigeria ; The single Widal test was positive in 1,803(62.1%) but only 304(10.5%) were confirmed by blood culture. There is therefore, the study recommended that, a need to develop criteria for deciding when to undertake Widal test in febrile children as such large number of children may have non-typhoid febrile illness. It also suggested that clinicians should continue to use clinical skills in deciding which of the febrile children will need Widal test (Adeleke and Nwokedi 2008).

A similar study conducted in Egypt to evaluate blood culture and antibody response with the duration of illness in diagnosis of typhoid fever indicates that a negative result of Widal test would have a good predictive value for the absence of the disease (NPV = 98%), but a positive result would have a very low predictive value for typhoid fever (PPV=32%). However, the sensitivity was highest (89%) in the second week of illness (Youssef et al., 2010). So the results of single step Widal test must be interpreted cautiously because of the low sensitivity of the test (Willke et al., 2002).

2.6. Treatment and drug resistance

Early diagnosis of typhoid fever and prompt institution of appropriate antibiotic treatment are essential for optimal management, especially in children. Although most cases can be managed at home with oral antibiotics and regular follow-up, patients with severe illness, persistent vomiting, severe diarrhoea, and abdominal distension require hospitalisation and parenteral antibiotic treatment (Fauci et al., 2008, Bhutta, 2006).

Appropriate antibiotic treatment (the right drug, dose, and duration) is critical to curing typhoid with minimal complications. Standard treatment with chloramphenicol or amoxicillin is associated with a relapse rate of 5-15% or 4-8% respectively, whereas the newer quinolones and third generation cephalosporins are associated with higher cure rates. Resistance to the traditional first line antimicrobial agents ampicillin, chloraphenicol and trimetoprim-sulphometoxazole defines multi drug resistance (MDR) in *Salmonella enterica* (WHO, 2003, Okonko et al., 2010). The emergence of multidrug resistant typhoid in the 1990s led to widespread use of fluoroquinolones as the treatment of choice for suspected typhoid, especially in areas where the disease was endemic (Dimitrov et al., 2007, Bhutta, 2006). However, the wide spread use of flouroquinolones has also been associated with decreased susceptibility and documented resistance for these class of drugs (Crump et al., 2010). A study conducted in Calcutta, India to provide information about the characteristics of diarrheal stool in multi-drug resistant typhoid fever, indicate that all *S. typhi* strains isolated from blood and stool culture, were resistant to chloramphenicol, ampicillin, amoxicillin, trimethoprim-sulphamethoxazole and tetracycline. However, they were uniformly susceptible to furazolidone, gentamicin, amikacin, norfloxacin, ciprofloxacin and nalidixic acid (House et al., 2001).

In recent years, however, the emergence of resistance to quinolones has placed tremendous pressure on public health systems in developing countries as treatment options are limited (Bhutta, 2006). A retrospective analysis of 135 patients suffering from typhoid fever carried out at the Infectious Diseases Hospital (IDH), in Kuwait, over a period of 4 years (2002–2005) showed that from the 135 *Salmonella enterica* serotypes Typhi and Paratyphi A isolated from patients, 50 (37 %) were multidrug resistant (MDR) and 94 (69.6 %) isolates of both serotypes were nalidixic acid resistant (NAR). Between 90 and 100 % of MDR and NAR strains had decreased susceptibility to ciprofloxacin. Low-level resistance was also detected in 13.8% and 33.3 % of nalidixic acid-susceptible isolates of *S. Typhi* and *S. Paratyphi A*, respectively. All isolates were susceptible to ceftriaxone (Abera et al., 2010). In study conducted to determine the Prevalence of *Salmonella typhi* and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia, *S. typhi* showed high resistances against ampicillin, cotrimoxazole, tetracycline, chloramphenicol, genentamicin and norfloxacillin which indicated that antimicrobial resistance of *S. typhi* is an increasing concern (Abera et al., 2010).

3. Significance of the study

Typhoid fever remains a major public health problem in the developing world with very poor estimates of the number of cases and deaths annually. Information across sub-Saharan Africa is very scarce and the issues clearly require urgent and rapid action, particularly in East Africa (Ethiopia and Kenya) which seems to have a high burden of typhoid fever (Pang, 2008). Many studies on salmonellosis conducted by different investigators in Ethiopia have shown the widespread distribution of *Salmonella* isolates in the community (Beyene et al., 2008). Lack of research capacity, funding support, and institutional infrastructure are problems to promote and strengthen typhoid fever and other infectious disease research in these regions, so true progress in controlling the disease have not been yet occurred (Pang, 2008).

The diagnosis of typhoid fever based on clinical information is difficult because the manifestations of the disease are diverse and there are many causes of prolonged fever in typhoid endemic regions (Wain and Hosoglu, 2008). Blood and bone marrow culture are the most reliable diagnostic methods but these are expensive techniques for developing countries like Ethiopia (Wain and Hosoglu, 2008, Beyene et al., 2008). Due to these reasons, in many developing countries including Ethiopia Widal test remain the diagnostic technique routinely used for diagnosis of typhoid fever. However, the interpretation of the Widal test remains problematic to this day, with a great number of articles reporting different cut-offs in different geographical area and the test has lost its sensitivity and specificity as cross reacting antigens are observed in many other endemic febrile diseases (Ley et al., 2010). Another problem with typhoid fever is that, several serovars, including *S. typhi* were associated with multiple drug resistance to clinically relevant drugs and these drug resistance is increasing from time to time in Ethiopia (Beyene et al., 2008).

To decrease the incidence of typhoid fever in Ethiopia and for appropriate treatment researches are needed for the rational design and evaluation of effective and appropriate diagnostics for enteric fever. Surveillance and follow up of the antimicrobial sensitivity pattern of the causative agent are also mandatory. With these regard, the results of the current study help to develop local recommendations for the interpretation of Widal results in relation to blood culture to help physicians and laboratory personnel interpret the Widal results. Also results of the current study on antimicrobial susceptibility pattern are important for physicians to prescribe the effective drug for treatment of typhoid fever. In addition it indicates the true prevalence of the disease in febrile patients. This study will also serve as a base line data to conduct further related researches in the area.

4. Objective of the study

4.1. General objective

The main objective of this study is to compare the diagnostic performance of Widal test and blood culture in the diagnosis of typhoid fever in febrile patients St. Paul's General Specialized Hospital.

4.2. Specific Objectives

- To provide an estimate of the true prevalence of typhoid fever in the local population of patients being investigated
- To assess the associated risk factors of typhoid fever
- To observe the agreement of slide agglutination and tube agglutination (titration) results of Widal test for the diagnosis of typhoid fever
- To compare result of Widal test with blood culture in diagnosis of typhoid fever from febrile patients
- To isolate species of salmonella responsible for typhoid fever and to examine their antimicrobial susceptibility pattern

5. Materials and methods

5.1. Study design

Cross sectional descriptive type of study was conducted to compare the result of Widal test with blood culture for the diagnosis of typhoid fever.

5.2. Study area and period

The study was conducted from December 2010 to March 2011 in Addis Ababa in St. Paul's general Specialized Hospitals.

5.3. Source population

All patients visiting St. Paul's general Specialized Hospital during the study period

5.4. Study population

All febrile patients with symptoms clinically similar to typhoid fever visiting St. Paul's general Specialized Hospital during the study period.

5.5. Sample size

Over a period of three and half months about 277 febrile patients whose clinical symptoms are similar with typhoid fever and visit the laboratory for Widal test were recruited for this study.

5.6. Inclusion and Exclusion criteria

Febrile patients with clinical symptom compatible with typhoid fever and referred to the laboratory for Widal test and age more than one year were included in the study where as patients who had received antibiotic treatment for their symptom within two weeks before coming to the hospital were excluded from the study.

5.7. Definition of terms

- **Febrile patient:** A patient with a temperature on admission of > 37.5ºC, or history of fever of > 2 days duration, and no identified cause of fever
- **Confirmed case of typhoid fever**

  A febrile patient with a laboratory-confirmed positive blood culture of *S. typhi* or *S.paratyphi.*
- **Positive titer**: an antibody titer of 1:80 and higher for anti TO and 1:160 and higher for anti TH of *Salmonella typhi*.

5.8. Variables: dependant/independent

Independent variables

Sex

Age

Fever of unknown origin

Dependent variables

Widal test result

Culture positive

5.9. Data collection

After patients sign informed consents, information in socio-demographic and risk factors were collected by well structured questionnaire.

Blood sample collection and inoculation: Using a sterile syringe and needle, about 8-10 ml of blood from an adult and about 3-5 ml from a young child were collected. Then **5**-7 ml for adult and 2-3ml of blood from children was dispensed into the culture medium bottle containing 45 ml of Tryptic soy broth (OXOID, England. Without delay the blood was mixed with the broth and then transported to the laboratory and incubated at 37 ºC.

Sub culturing to identify salmonella: after 24 hours incubation sub-culturing was performed from the Typtic Soya broth on XLD agar (OXOID, England) and the agar plate were incubated aerobically at 37°C for 24 hours. After overnight incubation positive cultures were proceed further while Negative broth cultures were incubated until 7 days and sub cultured before reported negative.

Biochemical identification: Suspected colonies obtained on the above media were screened by means of biochemical tests. Isolates were examined by Triple Sugar Iron agar (TSI) (BBL^{TM}), citrate utilization test, motility (Difco^{TM}), urease test (Himedia ltd. India) and lysine decarboxylation (LDC) [Difco^{TM}] test.

Widal test: The remaining 2-3 ml blood from the total collected blood was dispensed to a test tube for Widal test. Qualitative slide agglutination and semi quantitative tube agglutination (titration) were performed to determine the somatic and flagellar antigens using febrile antigen kits of *Salmonella typhi* (Chromatest Febrile Antigens kits, Linear chemicals, Barcelona, Spain). The slide agglutination test is used as a screening test for the presence of anti TO and anti TH antibodies in the patient's serum. Briefly for the slide agglutination test a drop of *Salmonella typhi* O and H antigens are added on a drop of serum on card and rotated at 100rpm for 1 minute and reported as reactive or non reactive. For those slide agglutinations whose results are reactive and weakly reactive titer was determined. In the tube agglutination test (titration), serum sample

was serially diluted from 1:20 to 1:640 for anti TO and anti TH separately in 12 test tubes. Then a drop of O antigens and H antigens are added in the test tubes, equal amount in all. Based on the manufacturer manual, an antibody titer of 1:80 and higher for anti TO and 1:160 and higher for anti TH antibodies are taken as a cut of value to indicate recent infection of typhoid fever.

Antimicrobial Susceptibility: the antimicrobial sensitivity pattern of the isolates was determined by using modified Kirby–Bauer disk diffusion technique against nine drugs commonly used for the treatment of enteric fever on Mueller Hinton II Agar (BBL ^{TM}). These drugs are Amoxicillin-Clavulanic acid (30µg), Ampicillin (10µg), Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Gentamycin (120µg), Norfloxacin (10µg), Tetracycline (30µg) and Trimethoprim-Sulphometoxazole (25µg) [BBL^{TM} Antimicrobial Susceptibility Test Discs (USA)].

5.10. Quality controls

Standard operational procedures were followed during processing of each sample and all the instruments used for sample processing were checked every morning for proper functioning. *E.coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as a reference strain.

5.11. Data analysis

Statistical software package (SPSS version 16) was used for analysis of the data. Sensitivity: (true positive rate, a/a+c), Specificity: (true negative rate, d/d+b), Positive Predictive Value: (PPV, a/a+b), and Negative Predictive Value: (NPV, d/d+c) were calculated. Where a= Positive culture, and positive Widal test, b= Negative culture, but positive Widal test, c= Positive culture, but negative Widal test, and d= Negative culture, and negative Widal test. P value ≤ 0.05 was used to indicate significant association.

5.12. Ethical considerations

Ethical clearance was obtained from Research Ethical committee of the department of Microbiology, Immunology and Parasitology. Permission was also obtained from the St.Paul's General Specialized Hospitals administration. After the study participants were informed about the study process and its importance to design intervention strategies against the disease, informed consent was obtained from each volunteer and guardian. In addition, confidentiality was kept. Any patient who is not volunteer was not enforced to be included in the study

6. Result

6.1. Socio-demographic data

Total patients involved in this study were 277 febrile patients from St.Paul's General Specialized Hospital. Data of 270 patients were analysed because the remaining sevens missed for the following reasons: 3 missed due to insufficient serum samples to perform Widal test, other three missed due to incomplete sociodemographic data, and one missed due to both insufficient serum sample and incomplete sociodemographic data. Among 270 patients 186 (68.9 %) were females and 84 (31.1 %) were males. Patient's age ranges from 8-80 (mean 35.82 ± 12.4 [SD]). The detail sociodemographic data of the study participants is presented below in table 1.

Table 1. Sociodemographic data of febrile patients suspected of typhoid fever in St. Paul's hospital, December 2010-March 2011.

		Number	%
Sex			
	Male	186	68.9
	Female	84	31.1
Age			
	≤14	7	2.6
	15-29	78	28.9
	30-44	118	43.7
	45-59	56	20.7
	≥60	11	4.1
Occupation			
	Merchant	7	2.6
	Farmer	14	5.2
	Employee	93	34.4
	Daily labourer	25	9.3
	Student	35	13
	House wife	61	22.6
	Others	35	13
Educational background			
	Illiterate	39	14.4
	Only read and write	10	3.7
	Grade 1-4	35	13.0
	Grade 5-8	54	20.0
	Grade 9-10	29	10.7
	Grade 11-12	35	13.0
	Above 12	68	25.2
Residence			
	Urban	251	93.0
	Rular	19	7.0
Region			
	Addis Ababa	229	84.8
	Out of Addis Ababa	41	15.2

Majority of patients (94.3%) are within the age range of 15-59, 34.4 % are government employee, and most (93.0%) are urban residents. More than 48% of all patients have educational background of high school study and above.

6.2. Widal test

6.2.1. Qualitative slide agglutination Widal test

Qualitative slide agglutination Widal test was performed in the hospital laboratory as a primary screening test of serum for presence or absence of the O antigen and H antigens of *S.typhi*. Slide agglutination reaction for O antigen showed that 127 (47.0%) of patients have reactive agglutination result and 72 (26.7%) have reactive agglutination reaction for H antigen (table 2).

Table 2. Qualitative slide agglutination reaction results of Widal test of febrile patients suspected of typhoid fever in St. Paul's hospital, December2010 -March 2011.

	O antigen		H antigen	
Reaction result	**Frequency**	**(%)**	**Frequency**	**(%)**
Reactive	127	(47.)	72	(26.7)
Weakly reactive	33	(12.5)	55	(20.4)
Non reactive	110	(40.7)	143	(53.0)
Total	270	(100.0)	270	(100.0)

143 (53.0%) of patients have non reactive reactin result for H antigen of *Salmomella typhi*.

66 (24.4%) of patients have reactive reaction for both O and H antigens while 61 (22.6 %) have reactive only for O antigen. Only 6 (2.2%) of patients have reactive reaction for H antigen only. Overall 133 (49.3%) of all patients have reactive slide agglutination test by either or both of O and H antigens.

Among the weakly reactive results 26 (9.6%) patients have weakly reactive reaction result for both O and H antigen. Six and one of patients with weakly reactive reaction result for O antigen have non reactive and reactive reaction result respectively for H antigen reaction. From 55 patients with weakly reactive reaction result for H antigen, 25 have reactive reaction result for O antigen while only four have non reactive result for O antigen.

6.2.2. Semiquantitative tube agglutination test (titration)

Antibody titer was determined by diluting the serum sample serially from 1:20 to 1:640 by using fresh 0.95% saline preparation. Titer was performed for those patients whose slide agglutination test result indicates reactive and weakly reactive reactions. From all 270 patients, 160 (59.3%) have reactive and weakly reactive reaction for anti TO antibody and 127 (47.0%) have reactive

and weakly reactive for anti TH antibody. The frequency distribution of titration result is presented in table 3.

Table 3. The frequency distribution of semi quantitative slide agglutination titration test of Widal test in febrile patients suspected of typhoid fever in St. Paul's hospital, December 2010-March 2011.

Titer	O antigen			H antigen		
	Frequency	% (n=160)	% from total(n=270)	Frequency	% (n=127)	% from total(n=270)
No agglutination	40	25.0	14.8	37	29.1	13.7
1:20	32	20.0	11.9	33	26.0	12.2
1:40	15	9.4	5.6	5	3.9	1.9
1:80	42	26.3	15.6	14	11.0	5.2
1:160	21	13.0	7.8	26	20.5	9.6
1:320	6	3.8	2.2	12	9.4	4.4
1:640	4	2.5	1.5	0	0	0
Total	160	100	59.3	127	100	47.0

Serum from 40 (25.0%) patients with reactive (12/40) and weakly reactive (28/40) reaction of slide agglutination for anti TO antibodies have not shown any agglutination in tube agglutination titration test. Similarly 37 (29.1%) of patients with reactive (12/37) and weakly reactive (25/37) reaction for anti TH have not shown any agglutination reaction in tube agglutination test. Among those who have agglutination reaction results, 42 (15.6%) of all patients have titer of 1:80 for O antigen and 33 (26.0%) have titer of 1:20 of H antigen. There is no titer of 1:640 and higher observed for H antigen while there are only 4 (1.5%) patients whose titer of O antigen is 1:640 and higher.

Antibody titer of 1:80 for O antigen and 1:160 for H antigens are taken as cut of values to indicate recent typhoid infection (positive titer). Taking O≥80 as a cut of value 73 (27%) patients have indicative of recent typhoid infection and based on H≥160 as cut of value 37 (13.7%) patients have recent typhoid infection. The total number of patients who have indicative of recent infection by either of O and H antigens is 88 (32.6%). Among these 20 (7.4%) patients have antibody titer indicative of recent infection by both O (≥1:80) and H (≥1:160) antigen tests.

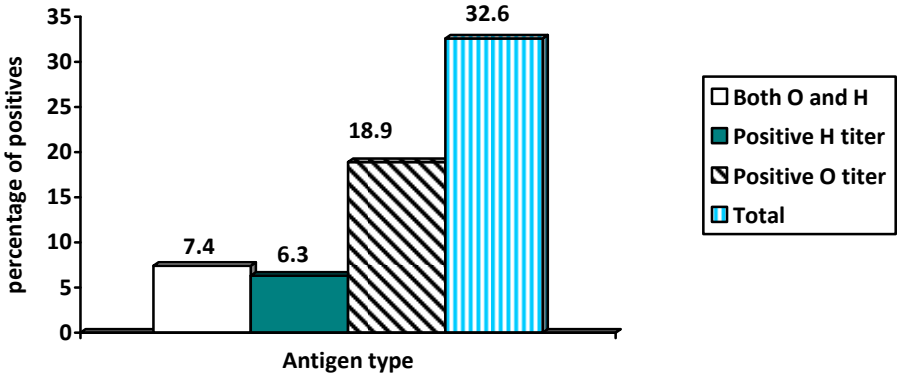


Figure 1: the distribution of positive titers of Widal test by type of agglutinating antibody of febrile patients suspected of typhoid fever in St. Paul's hospital, December 2010-March 2011.

Most patients (18.9%) have positive titer of O antigen only while only 6.3 % have positive titer of H antigen only**.**

Of the 270 sera tested, agglutination to *S.typhi* in female were observed in 88 of 186 (47.3%) subjects for O antigen and 64 of 186 (34.4%) subjects for H antigen at various dilution, while in the male subjects, 32 of 84 (38.1%) accounts for O and 27 of 84 (32.1%) for H antigen agglutinins. The percentage distributions of positive titers from the total male and female patients are presented in figure 1.

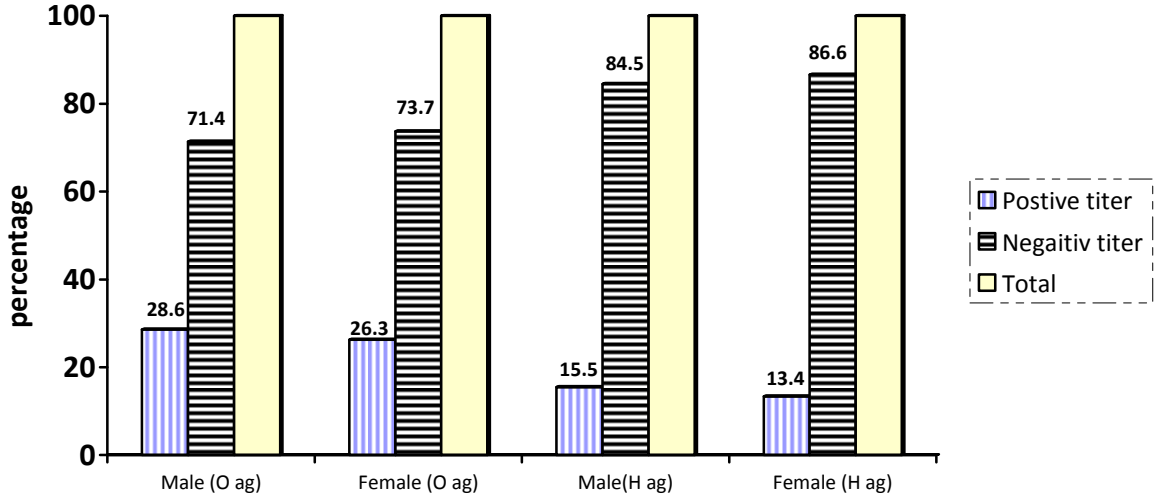


Figure 2. The distribution of positive titers of Widal test by male and female febrile patients suspected of typhoid fever in St. Paul's hospital, December2010-March 2011.

As shown in the above figure more positive titer was observed in male than female for both O and H antigens. Among those who have agglutination at any dilution for H antigen, more male (13/27[48.14%]) have positive titer than females (25/64[39.1%]), similarly 24 of 32 (75%) male

patients and 49 of 88 (55.7%) female patients who have agglutination at any dilution for O antigen have a positive agglutination titer.

6.2.3. Agreement of slide agglutination and tube agglutination (kappa)

The agreement between qualitative slide agglutination and semi quantitative tube agglutination test (titration) to indicate the presence of O and H antigens in patient's serum was determined. In doing these, weakly reactive slide agglutinations reactions are considered as reactive because their titer was determined (Table 4).

Table 4. The agreements of slide agglutination test and semiqualitative tube agglutination test in indicating the presence of O and H antigens from febrile patients suspected of typhoid fever in St. Paul's hospital, December 2010-March 2011.

Slide agglutination test	**Semiquantitative tube agglutination test (titration)**			**kappa**
	Positive titer[a]	Negative titer[b]	Total	
Anti TO				
Reactive	73	87	160	κ=0.406
Non reactive	0	110	110	
Total	73	197	270	
Anti TH				
Reactive	38	89	127	
Non reactive	0	143	143	κ=0.311
Total	38	232	270	

a=Positive titer (O≥1:80, H≥1:160); b= negative titer (O<1:80, H<1:160)

There was a moderate agreement between slide agglutination test and tube agglutination titer for O antigen (Kappa=0.406) and a fair agreement for H antigen slide agglutination and tube agglutination titer (Kappa=0.311).

6.3. Blood culture

Of the total blood cultures only seven (2.6 %) *S. typhi* were isolated from the patients. while *S. paratyphi* were identified from 4 (1.5 %) of patients. 51 (18.9%) patient's blood cultures give positive results of bacteria other than salmonella species (Table 5).

Table 5: the distribution of blood culture results of febrile patients suspected of typhoid fever in St. Paul's hospital, December -March 2011.

Bacteria	Number of isolates (%)
S. typhi	7(2.6)
S. paratyphi	4(1.5)
Non typhoidal salmonella	7 (2.6)
Other bacteria	51(18.9)
Negative blood culture	201(74.4)
Total	270(100.0)

6.4. Comparison of Widal test result and blood culture

Based on the above results of Widal test and blood culture results, an evaluation of Widal titration results for the diagnosis of typhoid fever was performed for O (≥1:80) and H (≥1:160) antigens tube agglutination test results.

Table 6. The distribution of anti TO and anti TH antibody titers among culture positive febrile patients in St. Paul's hospital, December 2010-March 2011

Widal value	*S. typhi* (n=7)	*S. paratyphi* (n= 4)	Non typhoidal salmonella species (n=7)	Other pathogenic bacteria (n=51)	Negative blood culture (n=201)
Positive O titer	5 (71.4%)	2 (50%)	3 (42.9%)	17(33.3%)	46(27.0%)
Positive H titer	2(28.6%)	0 (0%)	2(28.6%)	9(17.6%)	25(12.4%)

Anti TO agglutination titer of 1:80 and higher were detected among 5/7 (71.4%) of culture confirmed typhoid cases by *S.typhi* as compared with 2/4 (50%) of *S. paratyphi* and 3/7 (42.9%) of non typhoidal salmonella.

46 (27%) of patients whose blood culture is a negative result have a positive Widal titer of anti TO while 25 (12.4%) of them have a positive titer of anti TH.

Among seven cases of *S. typhi* 3 (42.8%) have positive titer only for anti TO and 2 (28.6%) have positive titer of both anti TO and anti TH. The remaining two cases have no any positive titer of

anti TO or anti TH. The antibody titer of culture confirmed typhoid fever caused by *S. typhi* is presented below in table 7.

Table 7. The distribution of anti TO and anti TH antibody titers in culture confirmed typhoid fever patients among febrile patients in St. Paul's hospital, December 2010-March 2011

Titer	Anti TO (%)	Anti TH (%)
≥1:80	5/7 (71.4)	3/7(42.9)
≥1:160	2/7(28.6)	2/7(28.6)
≥1:320	1/7(14.3)	1/7(14.3)
≥1:640	0/7(0)	0/7(0)

Among culture confirmed *S.typhi* cases five of them have positive titer for O antigen and only two cases have positive titer for H antigen. There is no antibody titer of 1:640 and higher observed among culture confirmed cases in both O and H antigens.

The overall patients which have positive titer for either or both of O and H antigens, and culture confirmed typhoid fever cases are presented in the following graph.

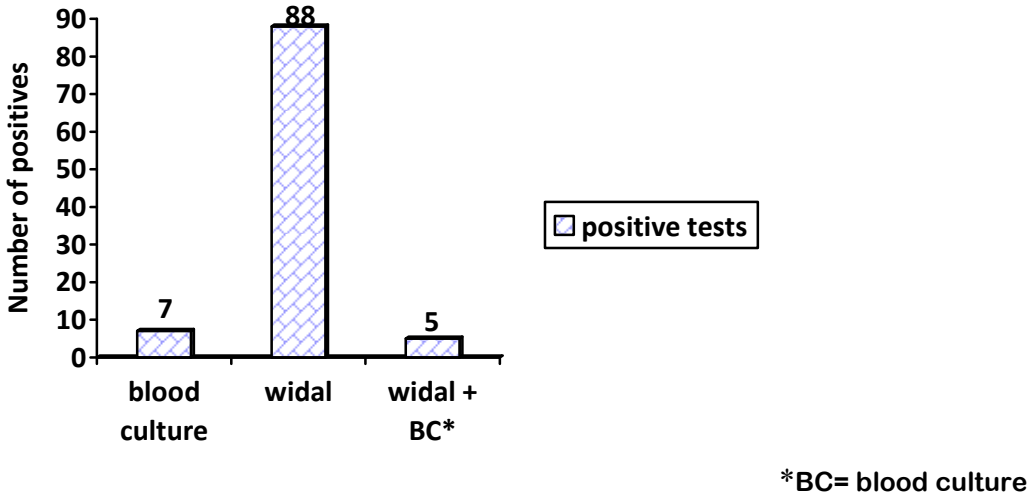


Figure 3. Diagnostic result of typhoid fever by blood culture and Widal titration of febrile patients suspected of typhoid fever in St. Paul's hospital, December 2010-March 2011.

Only five case of typhoid fever are identified by both positive Widal titer and positive blood culture for *S.typhi*.

Table 8. The sensitivity, specificity, PPV, and NPV of titers of anti TO (≥1:80) and anti TH (≥1:160) Widal tests for diagnosis of typhoid fever from febrile patients in St. Paul's hospital, December 2010 -March 2011.

Measurement	O antigen No (%)	H antigen No (%)
Sensitivity	71.4	28.57
Specificity	74.1	86.3
PPV	6.8	5.2
NPV	98.9	97.8

Among typhoid fever cases caused by *S. typhi* the sensitivity of anti TO antibody titer of 1:80 and higher is about 71.4 % while relatively low sensitivity (28.57%) were observed in anti TH titer of 1:160 and higher. Very low positive predictive values were observed in both anti TO and anti TH positive titer, which was 6.8% and 5.2% respectively.

The overall Widal positive titer among culture confirmed cases of *S.typhi* are 5 which all of them have positive titer of anti TO and two of these have also positive titer for anti TH. So the sensitivity, specificity, PPV and NPV of the overall positive titer was similar 71.4%, 68.44%, 5.7% and 98.9% respectively.

6.5. Prevalence of typhoid fever and associated factors

Among the total 270 blood culture tested about 7 (2.6%) cases of *Salmonella typhi* and 4 (1.5%) cases of *Salmonella paratyphi* were identified with the total prevalence of 4.1 % (11/270). Among these eight are females and 3 are males. The age distribution of typhoid fever among study subjects is presented below in the graph.

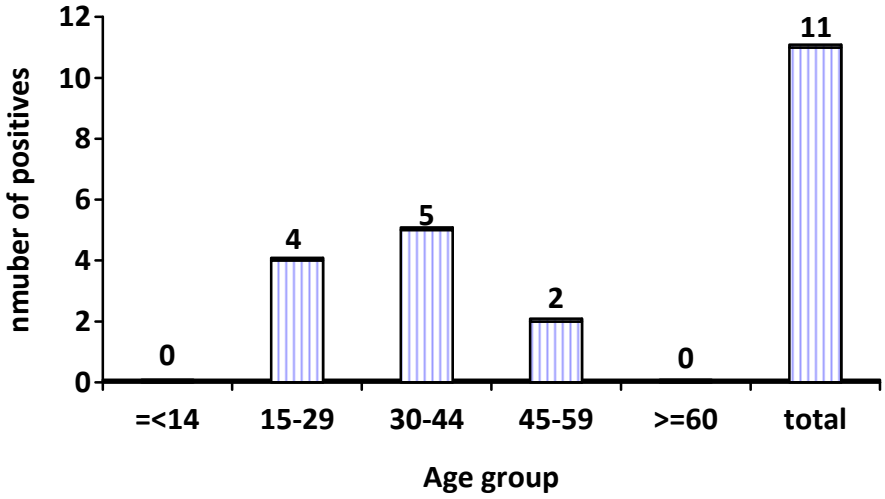


Figure 4. Age distribution of typhoid fever case in febrile patients from St. Paul's hospital, December 2010 -March 2011

Many typhoid fever cases (45.5%) are found under the age of 30-44. All cases are found from age 15-59. There is no typhoid fever infection in young children and older (fewer than 14 and above 60 years) age groups of patients in this study.

From 270 study participants 251 (93%) are urban residents and 19(7%) are rural residents. All case of typhoid fever occurred in urban residents. The distribution of typhoid fever in this study by educational background and occupation is presented below in table 9.

Table 9. Prevalence of typhoid fever by educational background and occupation of febrile patients in St. Paul's hospital, December 2010 -March 2011

Variable	Typhoid fever			
Occupation	**Positive**		Negative	Total No (%)
	No (%)	**OR[95 CI]**	No (%)	
Merchant	0 (0)	-	7(2.6)	7(2.6)
Farmer	0(0)	-	14(5.2)	14(5.2)
Employee	5(1.9)	0.46[0.07-2.86]	88(32.5)	93(34.4)
Daily labourer	2(0.73)	1.08[0.12-9.54]	23(8.47)	25(9).3
Student	0(0)	-	35(13)	35(13)
House wife	2(0.73)	0.58[0.06-5.02]	59(21.87)	61(22.6)
Others	2(0.73)	-	33(12.17)	35(13.0)
Total	11(4.1)		259(95.9)	270(100)
Educational background				
Illiterate	0(0)	-	39(14.4)	39(14.4)
Read & write	1(0.4)	1.3[0.10-17.41]	9(3.3)	10(3.7)
Grade 1-4	1(0.4)	0.039[0.36-4.30]	34(12.6)	35(13.0)
Grade 5-8	1(0.4)	0.25[0.24-2.60]	53(19.6)	54(20.0)
Grade 9-10	0(0)	-	29(10.7)	29(10.7)
Grade 11-12	4(1.5)	2.17[0.45-10.32]	31(11.5)	35(13.0)
Above 12	4(1.5)		64(23.7)	68(25.2)
Total	11(4.1)		259(95.9)	270(100)

Government employee counts most of typhoid fever case 5 (1.9%) while there is no typhoid fever detected from merchants, farmers and students. Above grade 10 educational background are also counts most case (3.0%) of typhoid fever.

Exposure status of study subjects for associated risk factors of typhoid fever were also assessed by this study. Among all study subjects 259 (95.9%) of them have hand washing habit before eating. But 239 (88.5 %) only use soap always during hand washing. All case of typhoid fever is detected in those who have hand washing habit before eating. Only one case of typhoid fever was detected from those who did not use soap during hand washing. But there is no significant association of hand washing with soap and developing typhoid fever (p value= 1.000, 95% CI of OR=0.162-10.597).

250 (92.6%) of patients use piped water for all their water consumption. All 11 case of typhoid fever were detected in those who use piped water source. But there is no significant association of typhoid fever with use of piped water ($p>0.05$).

56 (20.7 %) of study subjects have contacted with typhoid patients within two weeks before coming to the hospital for diagnosis. Only 2 of them have diagnosed for typhoid fever (table 10).

Table 10. Typhoid fever by contact with other typhoid patients in febrile patients of St. Paul's hospital, December 2010-March 2011

Contact with typhoid case	Typhoid		Total	Fischer exact test
	Positive	Negative		
Yes	2 (0.7)	51(18.9)	53(19.6)	P=1.00
No	9(3.4)	208(77.0)	217(80.4)	p>0.05
Total	11(4.1)	259(95.9)	270	OR=0.906 [0.190-4.324]

There is no significant association between typhoid fever and recent contact with other typhoid case (p value= 1.000, 95 % CI for OR [0.9,4.3]). Among 56 (20.7%) individuals who live with recent typhoid case in the house hold only 2 individuals develop typhoid fever. This has also no significance association (p=1, 95% CI for OR [0.2, 4.3]).

102 individuals among all (37.8%) used iced drinks within two weeks of coming to hospital. 5 cases of typhoid fever are detected among those who use iced drinks (Table 10). 74 (27.4%) of them eat foods from street vendors and 3 cases from 11 of typhoid fever are from those who use this foods. There is no statically significant association of eating food from street vendors with developing typhoid fever (p > 0.05).

Table 11. Typhoid fever by Use of iced drinks in febrile patients in St. Paul's hospital, December 2010-March 2011

Use of iced drinks	Typhoid		Total	Fischer exact test
	Positive	Negative		P=0.75
Yes	5	97	102	p>0.05
No	6	162	168	OR=2.16 [0.4,4.7]
Total	11	259	170	

There is no statistically significance of using iced drinks and developing typhoid fever (p>0.05, 95% CI for OR [0.4,4.7])

77 (26.7%) of individuals among all have and use household private toilets and 193 (71.5%) use public toilets. Six cases of typhoid fever are from those who use public toilets. There is no significant association between using public toilet and developing typhoid fever (p value=0.214, 95% CI for OR [0.14, 1.56])

6.6. Antimicrobial Sensitivity pattern of *S. typhi* and *S. paratyphi*

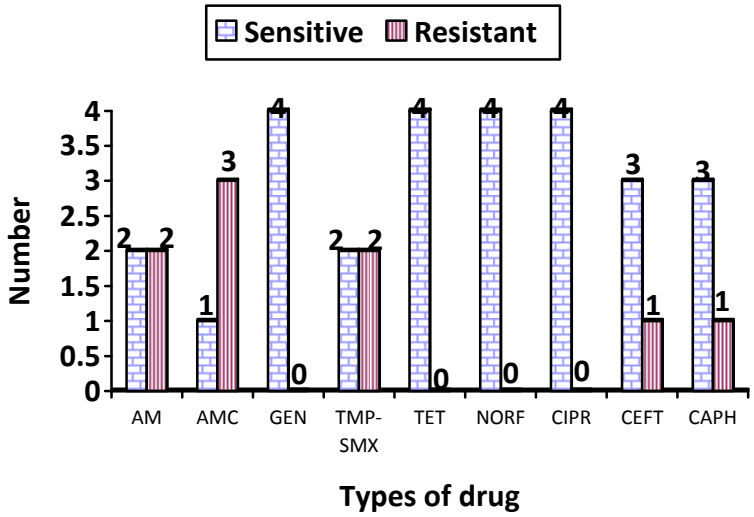
The resistance pattern of *salmonella typhi* and *S.paratyphi* was determined against nine drugs. Most (3/7[42.9%]) of the isolated *S. typhi* were highly resistant to amoxicillin. There was no species which show resistance against norfloxacin and ciprofloxacin. The resistance pattern of the isolates is shown below in the table and graph.

Table 12. Antimicrobial resistance of salmonella typhi (n=7) isolated from blood culture of febrile patients in St.paul's hospital, December 2010-March 2011.

Drug	Sensitive	Intermediate	Resistant
Ampicillin	4 (57.1)	1 (14.3)	2 (28.6)
Amoxicillin	3 (42.9)	1 (14.3)	3 (42.9)
Gentamycin	6 (85.7)	0 (0)	1 (14.3)
TMP-SMX*	6 (85.7)	0 (0)	1 (14.3)
Tetracyclin	4 (57.1)	2 (28.6)	1 (14.3)
Norfloxacin	7 (100)	0 (0)	0 (0)
Ciprofloxacin	7 (100)	0 (0)	0 (0)
Ceftriaxone	5 (71.4)	2 (28.6)	0 (0)
Chloraphenicol	5 (71.4)	1 (14.3)	1 (14.3)

* TMP-SMX=trimethoprim-sulphametoxazole

All species were sensitive for norfloxacin and ciprofloxacin and 85.57 % were sensitive for gentamycin and trimethoprom-sulphometoxazole. 28.6% of isolates have intermediate effect for tetracycline and ceftriaxone.



AM= Ampicillin, AMC=Amoxicillin-clavudin, GEN=Gentamycin, TMP-SMX= Trimethoprim Sulphometoxazol, TET= Tetracycline, NORF=Norfloxacin, CIPR=Ciprofloxacin, CEFT= Ceftriaxone, CAPH=Cloraphenicol.

Figure 5. Antimicrobial resistance of *Salmonella paratyphi* (n=4) isolated from blood culture of febrile patients in St.Paul's hospital, December 2010-March 2011.

Salmonella paratyphi isolates showed no resistance to gentamycine, tetracycline, norfloxacin and ciprofloxacin. More resistance (3 out of 4) was observed in amoxicillin than other drugs. Intermediate drug resistant pattern was not observed in *Salmonella paratyphi* species.

Among the isolated *Salmonella typhi* species only one specie was resistant for more than 3 types of drugs while 2/4 of *salmonella paratyphi* were resistant to more than 3 drugs. Multiple drug resistance (MDR) were observed in these three species. The MDR isolate of *S. typhi* was resistant to ampicillin, amoxicillin, cotrimoxazole and chloramphenicol. The two MDR species of *Salmonella paratyphi* were resistant to ampicillin, amoxicillin, cotrimoxazole and ceftriaxone.

7. Discussion

Research evidences suggests that typhoid fever continue to be a major public health problem in Ethiopia (Beyene et al., 2008). But due to lack of good microbiological diagnostic laboratory facilities the actual burden of enteric fever can't be exactly estimated based on population based survey. But it is estimated that rapid population growth, increased urbanization, inadequate human waste disposal, limited water supply and over burdened health care system have all made the disease control difficult and contribute to endemicity (Beyene et al., 2008, Bhutta, 2006, Aftab et al., 2009).

Widal test remained the most widely used diagnostic method of typhoid fever in developing countries including Ethiopia (Beyene et al., 2008). But the role of Widal tests for the diagnosis of typhoid fever has been debated widely, because first; the sensitivity, specificity, and predictive values of Widal test vary considerably among geographical areas (Bhutta, 2006, Khoharo et al., 2010), second the titer of agglutinins also depend on the level of infections due to other salmonella species and other infectious agent that have cross reacting antigens (Aftab et al., 2009). So evaluating the result of Widal test in the area where it is used is necessary for the correct interpretation of the result.

The sensitivity and specificity of Widal titer of anti TO 1:80 and higher in this study were about 71.4 % and 74.1 % respectively and 28.6 % and 86.3 % for ant TH titer of 1:160 and higher. The overall sensitivity of titer positive Widal test was about 71.4 %, similar with anti TO titer because there was no only anti TH titer positive culture proven typhoid fever. This is similar with the study conducted in the endemic area of Vietnam by Olsen *et al*. for the evaluation of serodiagnostic assay of acute enteric fever. According to Olsen *et al*. the sensitivity and specificity of Widal test were about 64% and 76 % when performed in hospital (Olsen et al., 2004). A similar study in Kenya showed that Widal method gave a lower sensitivity (81.3%) when compared to blood culture (Kariuki et al., 2004). Another study in Kenya has also similar result which showed that Widal testing done on acute phase serum of patients suspected to have typhoid fever had limited diagnostic capability given its low sensitivity in which among all typhoid cases only 26% have diagnostic titer while 53.6% had O and H titer less than 1:40 (Omuse et al., 2009).

More positive Widal titer was observed in male than in female for both anti O and anti H . A study conducted in Nigeria (Udeze et al., 2010) indicates more significant titers were observed in female. But another study (Zailani et al., 2004) indicates that age sex and socioeconomic status of patients have no any effect in Widal titer.

With the cut of value of anti TO ≥1:80 and anti TH≥1:160 Widal titer in this study, Widal test has relatively good NPV (98.9 %), but PPV was very low (5.7%). Positive predictive value is the most important than other measure of clinical diagnostic methods because it gives the proportion of patients with positive test results that are correctly diagnosed but it is highly affected by prevalence of the disease. In this study only 7 (2.5%) out of 270 febrile patients have culture proven febrile typhoid fever. So a negative Widal test result have a good predictive value for the absence of the disease but a positive result would have a low predictive value for the presence of typhoid fever (Ley et al., 2010).

A similar study conducted in Egypt by Youssef *et al.* to compare blood culture and antibody response in the diagnosis of typhoid fever indicates that a negative result of Widal test would have a good predictive value of the disease (NPV=98%) but positive result will have a very low predictive value for typhoid fever (PPV=5.7%) (Youssef et al., 2010). However the sensitivity and specificity of Widal result according to Youssef *et al.* were higher than the findings in this study. This may be because the Egypt study used a second week (convalescent) serum sample to perform Widal test. Low sensitivity for Widal test may also be related to the data collection time. In the current study Widal test was performed just at the admission of the patient in the hospital. The time of sample collection and the test was not too long after the first symptoms and the test was not repeated after blood culture confirmation (Hosoglu et al., 2008).

False positive results of Widal titer were so high in this study (PPV=5.7%). These false positive results may be associated with cross reacting antibodies from serum of febrile patient other than typhoid fever. Elevated antibody titers have been reported from patients with non enteric salmonella infection such as malaria. A study conducted in Cameroon to study the prevalence of typhoid fever of febrile patients with clinically compatible symptom of typhoid fever indicates that 45 % of patients have true diagnosis of malaria but only 2.5 % of patients have culture proven typhoid fever (Nsutebu et al., 2003). A review of scientific studies by Uneke on concurrent malaria and typhoid infection indicates that there were considerably higher rates of concurrent malaria and typhoid fever by Widal test as compared to the bacteriological culture techniques (Uneke 2008).

On the other hand, the presence of Widal agglutination under condition of negative malaria smear, negative *S. typhi* culture and without prior immunization against typhoid suggests that other infections may also share common antigenic determinant with *S. typhi* (Olopoenia and king, 2000). Typhus, *C. neoformance* meningitis, immunological disorder and chronic liver disease are best example for this (Onyekewere, 2007). In a study conducted by Somily *et al* to

detect *S.typhi* agglutination in sera of patients with other febrile illness and healthy individuals, it is found that majority of patients and normal individuals were tested positive for Widal test at dilution of less than 1:40 both for O (62.5%) and H (64.6%) antigen, but a decreasing trend of Widal reactivity was observed with increasing dilution of serum and significant Widal titer is found in 6.4%and 11% of individuals for O and H salmonella antigens respectively (Somily et al., 2011). A similar study conducted in Nigeria in apparently healthy freshman students indicates that a higher significant titer of antibody for anti TO and anti TH antibodies of *S.typhi* (Udeze et al., 2010). This will have two negative outcomes in the patient and also in the community. The first one is, patients are treated (mismanaged) for salmonella having another febrile disease which in turn results in development of drug resistance. The second is the highly fatal disease of febrile illness such as malaria, non typhoidal salmonellosis, endocarditis and urinary tract infection will be missed (Onyekewere, 2007).

False negative results were also found in our study. 2 cases among 7 culture confirmed typhoid fever cases have a negative titer. The false negative Widal test results may be if the blood is collected to early in the disease processes, or if an inadequate inoculation of bacterial antigen to induce the antibody production (Olopoenia and king, 2000). Previous antibiotic treatment may also contribute to negative Widal agglutination test but there was no patient who explained taking antibiotic within two weeks before coming for the diagnosis.

In many hospitals, instead of the standard Widal test, a qualitative slide agglutination test is used (Olopoenia and king, 2000). This may cause over diagnosis of typhoid fever than the tube agglutination (titration) which by itself has many false positive (Olopoenia and king, 2000, Nsutebu et al., 2002). The positivity rate of slide agglutination and tube titration in this study was about 49.3 % and 38 % respectively. So much more false positive results were obtained by slide agglutination reaction. Statistically there was moderate agreement (kappa=0.406) between slide agglutination and tube agglutination titer of anti TO and fair agreement (kappa=0.311) for anti TH. A study conducted in Jimma, south-western Ethiopia, by Mamo *et al*. indicates that there was fair agreement (kappa=0.225) for anti TO and poor agreement (kappa=0.06) for anti TH. The agreement difference with the current study in anti TH and relatively higher agreement observed in current study might because the current study was conducted in febrile patients while Mamo and his colleagues conducted on healthy population (Mamo et al., 2007). But still the agreement of slide agglutination and tube titration was very low.

The slide agglutination test is rapid and is used as a screening procedure. An initial positive screening test requires the determination of the strength of antibody. But in many developing

countries where the disease is endemic the laboratory perform the test, make diagnosis and report as positive or negative (reactive and non reactive) (Olopoenia and king, 2000). This is also the case of St. Paul's hospital where this study was conducted. Normally the result of Widal test should be reported as either of 'no agglutination' or if agglutination is present, in titers (1:20, 1:40...) rather than in reactive or non reactive terms. This type of reporting may be misleading and contribute to the incorrect interpretation of the test result by the physicians (Olopoenia and king, 200).

In this study the overall prevalence of culture confirmed typhoid fever caused by both *S.typhi* and *S.paratyphi* from febrile patients was 4.1 %. Seven (2.6%) were caused by *S. typhi* and 4 (1.5%) are caused by *S. paratyphi*. This result is similar with a study conducted in Embu and Nairobi areas of Kenya which showed the blood culture isolation rate of *S.typhi* for typhoid fever was 3% and 2.2 % respectively for the two areas (Kariuki et al., 2004 **a**). A similar study conducted by Crump *et al* to determine the incidence of febrile disease in all age group febrile patients indicated that *S.typhi* was isolated from 4.2 % of patients (Crump et al., 2003). The current result is also similar with that of Nsutebu *et al.* result in Cameroon which reported that the prevalence of typhoid fever in febrile patients of all age group is about 2.5% (Nsutebu et al., 2003). Low prevalence of typhoid fever may result from improved management of feco-oral transmitted disease and large growing proportion of persons living in both rural and urban areas who have access to piped water (Crump et al., 2003), and increased awareness of people on prevention of disease.

Most patient with blood culture confirmed typhoid fevers were from young adults (15-29) and above. This is similar to a study in Philippines by Abucejo *et al.* where most culture confirmed cases are under the age group of 15-29 years old. In the current study there was no culture positive case in school age children (≤14). This is contrary to the above study by Abucejo *et al* in Philippines where more than 15 % of typhoid cases were in under school children (WHO, 2003). These differences may be formed because in the current study only 2.6 % of all blood cultures were collected from ≤14 years old age group but in Abucejo and his colleague's study 24% of the blood cultures were collected from only under five children. Most other studies also found that typhoid fever is more common in school age children and in young adults (Kanungo et al., 2008).

Salmonella typhi was by far the most common pathogen isolate from blood culture than that of *S. paratyphi*. Normally the proportion of salmonella typhi and S. *paratyphi* is about 10:1 (WHO, 2003). But many research works showed that there is an increase in *S. paratyphi* cases

(Bajracharya et al., 2006). The current result is also similar in that the proportion of typhoid fever caused by *S.typhi* and *S.paratyphi* is increasing (7:4). This proportion may be changed due to increased urbanization and increased dependence on food purchased from street vendors. In the current study although there is no significant association of typhoid fever and eating food from street vendors ($p>0.05$), 2 out of 4 cases of typhoid fever caused by *S.paratyphi* are isolated in those who used food from street vendors and all cases of typhoid fever were from urban residents.

In addition to *S.typhi* and *S.paratyphi* other bacterias are identified from blood culture of febrile patients who have positive or negative Widal titer. In the current study 7 (2.6%) case of non typhoidal salmonella and 51 (18.8%) other bacteria are identified from blood culture. The result of non typhoidal salmonella (2.6 %) is similar with a study in Tanzania by Ley et al which identified 2.9 % non typhoidal salmonella from blood culture (Feasey et al., 2010).

Positive Widal titers were also seen in cases of nontyphoidal salmonella and in blood culture positive cases for other bacteria. Three out of 7 (42.9%) of nontyphoidal salmonella cases and 17 of 51 (33.3%) of other bacteria positive cultures have a positive titer of anti TO. This results are not similar with the above described study in Tanzania by Ley *et al* which identified only 6.7% positive blood culture for other bacteria, and from this only 14.3% of non typhoidal salmonella but none of other bacteria positive cultures indicate positive titer of anti TO $\geq$1:80 (36). This probably may be because the type of non salmonella bacterial infection that causes febrile illness may be different in different areas.

In this study the in vitro antibiotic sensitivity of *S.typhi* isolates to ciprofloxacin and norfloxacin were highly sensitive. There was no isolate which develop resistance against these drugs (100% sensitive). A study conducted in Calcutta, India to characterise multidrug resistant typhoid fever indicates that all *S.typhi* strains isolated from blood and stool culture were uniformly susceptible to norfloxacin, ciprofloxacin, and naldixic acid. A similar study conducted in kathamundu found that ciprofloxacin was highly sensitive (98.7%) followed by chlorapenicol(98.7%) cotrimoxazole and ampicillin (87.18%) (41). But a study conducted in Bahirdar, Ethiopia by Abera *et al.* among food handlers showed that *S.tyhphi* isolates have highest resistant to norfloxacin, gentamycin, chloraphenicol and cotrimoxazole (Abera et al., 2010).

High level of resistance of *S.typhi* (42.9%) to amoxicillin was found in this study. Only 4 out of 7 (57.1%) isolates of *S.typhi* were sensitive for ampicillin and tetracycline. The above mentioned study in Bahirdar indicates that *S.typhi* isolates were highly resistant against amoxicillin (100 %) and tetracycline (66.7%) (Abera et al., 2010).

In *S.paratyphi* isolates there was no resistance against gentamycin, teteracycline, norfloxacin, and ciprofloxacin and 75% species were sensitive to ceftriaxone and chloraphenicol. A study conducted in kathamandu, Nepal, indicated that salmonella paratyphi were sensitive (100%) to ciprofloxacin, norfloxacin, gentamycin and chloraphenicol (Bajracharya et al., 2006).

Multi drug resistance i.e. resistance to three standard enteric fever antibiotics (chloraphenicol, ampicillin and cotrimoxazole) was found in one species of S.typhi and two species of *S.paratyphi*. Overall, the current study indicated that there is a high level of resistance development in *S. typhi* and *S.paratyphi*. The emergence and continuous development of resistance may be due to improper use of antibiotics.

8. Conclusion and recommendation

Conclusion

- The prevalence of typhoid fever among febrile patients is about 4.1% which indicates that it is still public health concern, but it is not as high prevalent as diagnosed by Widal test and feared by the people.
- The proportion of typhoid fever caused by *S.typhi* and *S.pratyphi*, is increasing than previously expected.
- The qualitative slide agglutination tests have a low agreement with standard tube agglutination test (titration).
- The sensitivity, specificity, PPV and NPV of Widal test are 71.4 %, 68.44%, 5.7% and 98.9% respectively. A high antibody titer development is also seen in nontyphoidal febrile infections.
- Antimicrobial resistance continue to emerge in s.typhi and s.paratyphi, resulting in loss over time of the value of tradiational first line drugs and fluoroquinolones. Based on this study norfloxacin and ciprofloxacin are good drugs for treatment of enteric fever but resistance is developed for ampicillin and amoxicillin.

Recommendation

- Laboratories should perform the standard laboratory procedure of Widal test and report should be in the form of 'no agglutination' or if agglutination occurred it should be by titer instead of in 'reactive' and 'non reactive' terms.
- Physicians should not be totally dependent on laboratory result of Widal test and Interpretation of Widal test for the diagnosis of typhoid fever should be used with their clinical knowledge to differentiate from typhoid fever from other febrile infections.
- Widal test in the labratory should also perform using O and H antigens of *S.paratyphi A, S.paratyphi B* and *S.paratyphi C.*
- Optimum antimicrobial treatment of patients with enteric fever depends on understanding of local patterns of antimicrobial resistance and is enhanced by the result of antimicrobial resistance testing of the salmonella isolated from the individual patients. So appropriate antibiotic indicated by sensitivity test should be employed to prevent development of resistance. The indiscriminate use of drugs in typhoid fever should be avoided.

Limitation of the study

- Small sample size due to short period of data collection
- Widal test for *Salmonella paratyphi A, B* and *C* is not performed due to lack of reagents associated with financial scarcity
- Isolates of *Salmonella* species are not grouped by serological tests

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Annexes

ANNEX I: LABORATOEY PROCEDURES

A. Aseptic blood collection and dispensing technique (adopted from Cheesbrough 200)

Blood for culture must be collected and dispensed with great care to avoid contaminating the specimen and culture medium.

1. Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the venepuncture site.
2. Wearing gloves, thoroughly disinfect the venepuncture site as follows: – Using 70% ethanol, cleanse an area about 50 mm in diameter. Allow to air-dry. – Using 2% tincture of iodine and a circular action, swab the area beginning at the point where the needle will enter the vein. Allow the iodine to dry on the skin.
3. Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol-ether swab.
4. Using a sterile syringe and needle, withdraw about 8-10 ml of blood from an adult or about 3-4 ml from a young child.
5. Insert the needle through the rubber liner of the bottle cap and dispense 5-8 ml of blood into the culture medium bottle containing 45 ml of broth.
6. Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth.

   Important: The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.
7. Clearly label each bottle with the name and number of the patient, and the date and time of collection.
8. As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated.
9. Sub culturing a blood culture broth
   a. Using an ethanol-ether swab, cleanse the top of the bottle. Using a sterile needle and small syringe, insert the needle through the rubber liner in the cap, and withdraw about 1 ml of the broth culture.
   b. Inoculate the broth on selective agar, and incubate the agar plate aerobically overnight.

B. Inoculation and reading of UREA (adopted from Vandepitte et al., 2003)

1. Using an inoculating loop, collect 2–3 non-lactose-fermenting colonies from the primary plates and transfer to tube containing UREA.
2. Incubate the tubes for 2–4 hours at 35 ^{0}C and observe for a change in colour to pink (urease-positive). Discard the urease-positive tubes.
3. Subculture growth from the urease-negative tubes to MIL and to KIA, and incubates all tubes, including the urease-negative tube containing UREA, overnight at 35 ^{0}C in an aerobic incubator.

C. Inoculation and reading of MIL and KIA (adopted from Vandepitte et al., 2003)

1. Inoculate the MIL by inserting a straight inoculating needle to 2mm above the bottom of the tube. Withdraw the needle along the same line.
2. Inoculate the KIA by stabbing the agar butt with a straight inoculating needle and streaking the slant in a zigzag.
3. Label all tubes with the number of the laboratory and incubate overnight at 35 ∞C.
4. Examine the tube of Urease-negative UREA (see above) for delayed urease reaction. Discard the delayed urease-positive cultures.
5. Examine the MIL medium for motility, lysine and indole reaction. Motile organisms will spread out into the medium from the line of inoculation and produce diffuse growth. Non-motile organisms will grow only along the line of inoculation. A positive lysine reaction is indicated by an alkaline reaction (purple colour) at the bottom of the medium, and a negative reaction by an acid reaction (yellow colour) at the bottom of the medium (caused by fermentation of glucose). To test for indole production, add 3–4 drops of Kovacs reagent to the medium. A red to pink colour indicates the presence of indole and the persistence of the bright yellow layer indicates a negative test.
6. Examine the KIA medium. All Enterobacteriaceae ferment glucose, producing acid and gas or acid only, which gives a yellow slant. If gas is produced, bubbles or cracks are seen throughout the medium; the medium may even be pushed up in the tube if a large amount of gas is produced. If lactose is simultaneously fermented, both the agar butt and the slant become acid, i.e. yellow (e.g. in the case of *E. coli*). If lactose is not fermented (e.g. in the case of *Shigella* and *Salmonella* spp.), the agar butt is yellow but the slant becomes alkaline, i.e. red. Blackening along the stab line or throughout the medium indicates the production of hydrogen sulfide.

Identification characteristics of salmonella species: