Evaluation for Immunochromatographic Test Method for Rapid Diagnosis of Typhoid Fever, Jimma, Ethiopia.

By:-

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Abstract

Introduction:*Even thoughdelayed results and the technical complexity makes difficult to routine laboratory practices, Culture remains to be the gold standard in the diagnosis of typhoid fever. Serological methods based on detection of antibodies like the Widal test are unreliable owing to their poor sensitivity and specificity. A reliable and affordable alternative rapid test methods is therefore needed in the diagnosis of typhoid fever.* ***Objective:*** *The aim of this study was to evaluate the reliability of a Rapid Immunochromatographic test method against blood and stool cultures in the diagnosis of typhoid fever in Jimma.* ***Methods:*** *ACross-sectional study was carried out on serum, blood and stool specimens were collected from a total of 275 febrile patients clinically suspected for having typhoid fever. The performances of a Rapid Immunochromatographic test method and the Widal test were evaluated in terms of sensitivity, specificity, positive and negative predictive values against culture as a gold standard. Drug susceptibility pattern were also done for Salmonella isolate. Analysis was carried out using SPSS version 20.0. Kappa test was used to assess the agreement between the tests.****Result:*** *Out of 275 Typhoid suspected cases which participated in the study, 172(62.5%) were males. Among the examined cases, 35(12.7%), 46 (16.7%) and 66(24.0%) were positive for culture, rapid and widal tests, respectively. The sensitivity, specificity, PPV and NPV of rapid test were 83.0%, 92.9%, 63.0% and 97.4%, respectively while that of Widal test were 68.6%, 82.5%, 36.4%, and 94.7% respectively. The Rapid immunochromatographic test showed a very good agreement (kappa= 0.668) but the Widal test was in a fair agreement with culture test (kappa = 0.371). Salmonella species were 100% resistant to Ampicillin and 40.0%, 17. %, 8.6%, 5.7% to Chloramphenicol, Cotrimoxazole, Nalidixic acid, Ceftriaxone and Norfloxacin, respectively.* ***Conclusion:*** *Rapid Typhoid IgG/IgM immunochromatographic test has better sensitivity, specificity and predictive values and has showed very good agreement with culture result as oppose to Widal test.*

Key words:- Typhoid fever, raid test, sensitivity, specificity

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Abbreviations

ATCC	American Type Culture Collection
AUC	Area under Curve
BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards Institute
EBR	Ethiopian Birr
EUCAST	European Committee on Antimicrobial Susceptibility Testing
XLD	Xylose Lysine Deoxycholate
JUMC	Jimma University Medical Center
KIA	Kligler Iron Agar
MAC	MacConkey
MIU	Motility Indole Urea
NPV	Negative predictive value
SPPs	Species
SPSS	Statistical Package for Social Science
TN	True Negative
TP	True positive
PPV	Positive predictive value

CHAPTER I: INTRODUCTION

1.1 Back ground

Typhoid fever is acute generalized infection of reticulo-endothelial-system, enteric lymphatic tissue and bladder caused by *Salmonella enterica serovar* Typhi (*Salmonella.* Typhi) and *Salmonella paratyphi*. It remains a crucial explanation for malady in developing countries like Ethiopia (1). This disease is restricted to human hosts and humans (chronic carriers) represent the reservoir of infection (2).With a continuing thread of multi-drug resistance on the lure and no prospect yet for mass vaccination programs for the most vulnerable populations in endemic areas, the malady probably can stay of utmost medical and public health importance throughout the coming decades. Because clinical signs and symptoms of typhoid fever are nonspecific or could even be absent, the necessity for an easy, rapid, and reasonable pay at point-of-care test for typhoid fever is imperative (3).

Despite the high burden of typhoid fever, the disease has been much neglected in the recent years, partly because of the lack of diagnostic tools. The mainstay of laboratory identification of typhoid fever is blood culture though the gold standard is bone marrow culture. In the low-income countries like Ethiopia where much of typhoid fever occurs, culture may not be routine diagnostic tool. Wherever culture is conducted, bone marrow culture remains uncommon because of the invasiveness and technical issue of the procedure (4).

A broad spectrum of clinical sickness will follow intake of *Salmonella.* Typhi *,* with more severe forms being characterized by uninterrupted high fever, abdominal discomfort, malaise and headache (5, 6). Typhoid fever is transmitted through the faecal oral route by the consumption of contaminated water and food, notably raw or undercooked meat, poultry, eggs and milk (7). The consumption might occur either directly from person-to-person or by intake of food or water contaminated with fecal matter or urine carrier as well as through flies. It is therefore, presumed

that typhoid fever could be a pathological state where safe drinking water and sanitation is insufficient (7). It is estimated that typhoid fever has caused more than 21 million illnesses and more than 216,000 deaths globally. Persons with typhoid fever carry the causative agent *S.* Typhi in their bloodstream and intestinal tract. *S.* Typhi is unambiguously adapt to humans and carriers represent the only real source of these organisms for a short period of time (convalescent carrier) or chronic carriers who shed the organism for extended period of time than one year (8-10).

Isolation of *S.* Typhiis the current gold standard method for confirming a diagnosis of typhoid fever. For the isolation of *S.* Typhiwhere there is no culture available in primary health care settings now-a-days rapid serological tests are commercially available. As the symptoms of typhoid fever are diverse and nonspecific, sometimes the patients having similar symptoms due to other diseases are put on unnecessary antimicrobial treatment or the treatment is delayed in some cases.(11).

S. Typhi can be isolated from blood, urine, stool and bone marrow by culture method, but these tests take 2-3 days. Consequently, the diagnosis is delayed. Serological tests based on antibody detection have been used as an alternative for culture in the diagnosis of typhoid fever (12). Most widely used serological test is Widal test that detects agglutinating antibodies to TO and TH antigen of *S.* Typhi. Some researchers however found false positive and false negative results with this test and processing time of up to 18 hours limits its usefulness (13) .

Conventional Widal agglutination test is a serologic test for diagnosis of typhoid fever by agglutination of cell suspension (14). A killed suspension of *S.* Typhi is used to detect typhoid fever in serum of patients with typhoid fever. *S.* Typhi is treated to retain only 'O' (somatic) or 'H' (flagella) antigens and these antigens are used to detect specific antibodies in blood serum of patients suspected to be affected from typhoid fever (15). The initial serologic response in acute typhoid fever is represented by appearance of IgM Somatic O antibody, while the IgG flagella H antibody develops more slowly but persists for extended period. But is not always true that appositive agglutination test implies the patients were affected by *S.*Typhi as it may arises from cross reaction from other bacteria hence it end up with false positive results leading to misdiagnosis of typhoid fever (16). Having such limitation different health facilities including government and private sector are still using of as the main diagnostic for the isolation and

treatment of *Salmonella*. Gaps exist which need to be addressed especially by use of the most appropriate diagnostic tool hence the importance to evaluate the performance of Typhoid IgG/IgM immunochromatographic Rapid test and the Widal test against blood and stool culture.

To overcome such limitations, several assays and serological tests have been developed. Recent advances in immunology have led to the invention of more sensitive and specific markers of typhoid fever and hence newer serological tests like rapid IgM and IgG immunochromatographic tests are available know (12).

Typhoid IgG/IgM Rapid test (Cassette) plasma or serum is a lateral flow immuno-chromatographic immunoassay. The test cassette consists of: first a deep red colored conjugate pad containing recombinant *S.*typhoid H and O antigens conjugated with colloid gold (typhoid conjugate) and rabbit IgG and IgM-gold conjugate (17).

Therefore; this study aimed at evaluating Typhoid IgG/IgM Rapid test against blood and stool cultures in the diagnosis of typhoid fever, in order that it will help the physician to manage the disease timely and properly which may save the patients time and money and help recover early.

1.2 Statement of the Problem

Typhoid fever caused by *Salmonella*. Typhi is a severe multisystem illness characterized by the classic prolonged fever, and bacterial invasion and multiplication within the mononuclear phagocytic cells with the involvement of different organs of the body like liver, spleen, lymph nodes, and Payer's patches Typhoid fever is potentially fatal if untreated and is caused by *Salmonella* Typhi (18).

Around 22 million new typhoid cases are documented in the world annually. Predominantly young children in poor; resource limited areas, that compose the majority of the new cases and mortality figures (215,000 deaths annually). Most of these deaths are due to *S.* Typhi infection. The South-east Asian countries bear the brunt of the disease, particularly children and young adults. Other areas of prevalence include Africa and South America (1, 12).

Typhoid fever is a global health problem. In most endemic areas, typhoid comprises approximately 75%– 80% of enteric fever. Paratyphoid fever caused by *Salmonella paratyphi* A has emerged in recent years as increasingly common severe infection in developing countries. One global estimate suggested that ~ 21 650 000 cases of typhoid fever (with ~ 216 500 deaths) and ~ 5 410 000 cases of paratyphoid fever in developing countries (19).

Typhoid fever is a multi-systemic illness with a significant morbidity and mortality rate in developing countries including our country Ethiopia (20). Poor sanitation, low standard of living, overcrowding, lack of medical facilities, and indiscriminate use of antibiotics lead to endemicity and prevalence of typhoid fever (21). Emergence of multidrug resistant strains of *Salmonella enterica* serotype Typhihas only added to the burden of the disease(22). Any delay and inappropriate diagnosis and initiating of appropriate therapy only increases the risk of outcome.

The social and economic impact of enteric fever is also high because patients with acute disease and complications may need to be hospitalized (23). This results in loss of work days and, consequently low income. Isolation of *S.* Typhi from blood, bone marrow, or a specific anatomical lesion is the only definitive way of diagnosing enteric fever. The presence of characteristic clinical symptoms or the demonstration of a specific antibody response is suggestive of the disease, but not definitive. However blood culture is gold standard test, its

inaccessibility in resource limited country and low sensitivity of the test, makes it challenging to diagnose typhoid fever in early grounds (2).

The specificity of a blood culture is 100% while its sensitivity is as follows: 1st week – 90%, 2nd week – 75%, 3rd week – 60%, 4th week – 25%. The positivity of blood culture decreases due to the administration of antibiotics; however, the blood culture will continue to test positive in resistant cases. Many times, contaminants like coagulase negative *Staphylococci* in the blood culture may cause a false-negative report. The sensitivity range of blood culture is estimated to be between 40% and 80%. The sensitivity may be low in endemic areas with high rates of antibiotic use, making it difficult to estimate the true specificity (24).

Stool culture can help in detecting typhoid fever. Due to sporadic shedding of the organism in stools potentially compromises the stool culturing approach and becomes positive only after the first week of infection and has a much lower sensitivity than blood culture (30% vs. 40-90%) and carriers may be over represented as acute typhoid fever. Sensitivity is low in developing countries and not routinely used for follow-up (25).

Both blood culture and stool culture are not valuable for early diagnosis and prompt treatment due to delay in procedure followed. So, rapid serologic test is required for initiation of prompt treatment. Several efforts are made to overcome these problems; however, there are a lot of challenges with serologic test due to poor specificity. Now days to minimize such problems, a commercially available Typhoid Rapid IgG/IgM test and rapid serological have place in the diagnosis of typhoid fever and other rapid diagnostic tests (26).

No previous studies have been conducted to evaluate these diagnostic methods in the study area. So, the aim of this study is to determine the sensitivity, specificity, positive and negative predictive values and the reliability Typhoid IgG/ IgM Rapid test as a reliable and feasible alternative method in the diagnosis of typhoid fever in febrile patient.

1.3. Significance of the study

The finding of this study can provide important information regarding evaluation of Typhoid IgG/IgM Rapid test and blood, stool cultures in the diagnosis of typhoid fever. Hence, this study is in part an attempt to determine the sensitivity, specificity and predictive values of Typhoid IgG/IgG Rapid test for the detection of *Salmonella* species and it is important to which extent physician and health care workers have to rely upon diagnostic method. Information regarding the specificity and sensitivity of rapid test will guide clinicians for proper management of cases and information obtained from drug sensitivity test will help and guide health care workers to pick out the most effective antibiotics for treatment and helps to minimize inappropriate use of antibiotics that has been treated through empirical observation.

CHAPTER II: LITERATURE REVIEW

2.1 Overview of Typhoid Fever

Typhoid fever is a potentially severe systemic febrile illness that is caused by *Salmonella enteric serotype* Typhi(*S.* Typhi*) and* very similar but often less severe disease is caused by *Salmonella* serotype *Salmonella paratyphi* A, B and C and remains a significant health problem in many developing countries(27, 28).

In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. In 1997, for example, this age range was reported during an epidemic in Tajikistan. Nevertheless, clinically apparent bacteraemic *S.* Typhiinfection in children aged less than three years has been described in Bangladesh, India, Jordan, Nigeria(29, 30).

In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths. In Indonesia, people aged 3_19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year. A similar situation was reported from Papua New Guinea. When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children aged less than 3 years was low. In Chile, however, single blood cultures for all children aged under 24 months who presented at health centers with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S. Typhi* or *S. paratyphi (31).*

Typhoid fever had not been suspected on clinical grounds in any of the children. In South America the peak incidence occurred in school students aged 5_19 years and in adults aged over 35 years. This kind of study has not been conducted in other areas of endemicity. Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increasing with age and being greater in females than in males. The propensity to become a chronic carrier may have changed with the present availability and selection of antibiotics as well as with the antibiotic resistance of the prevalent strains. The role of chronic carriers as a reservoir of infection was studied in Santiago, Chile, where a crude rate of 694 carriers per 100 000 inhabitants was found (32).

The highest incidence rates of typhoid fever can be found in Asia, particularly in the South-central and South-eastern regions, where an estimated 100 cases/100,000 population occurs each year (33).Typhoid fever is prevalent in areas that are characterized by overcrowding and at the same time have poor access to proper sanitation (34).

Typhoid fever cases and deaths occur among populations without access to drinkable water, adequate sanitation, and hygienic facilities primarily in low-income countries like Africa (35) . Report shows an estimate of 21.5 million infections and 200,000 deaths from typhoid fever globally each year. In Africa, about 4.36 million cases occur out of an estimated population of 427 million and it is often encountered in tropical countries (36).

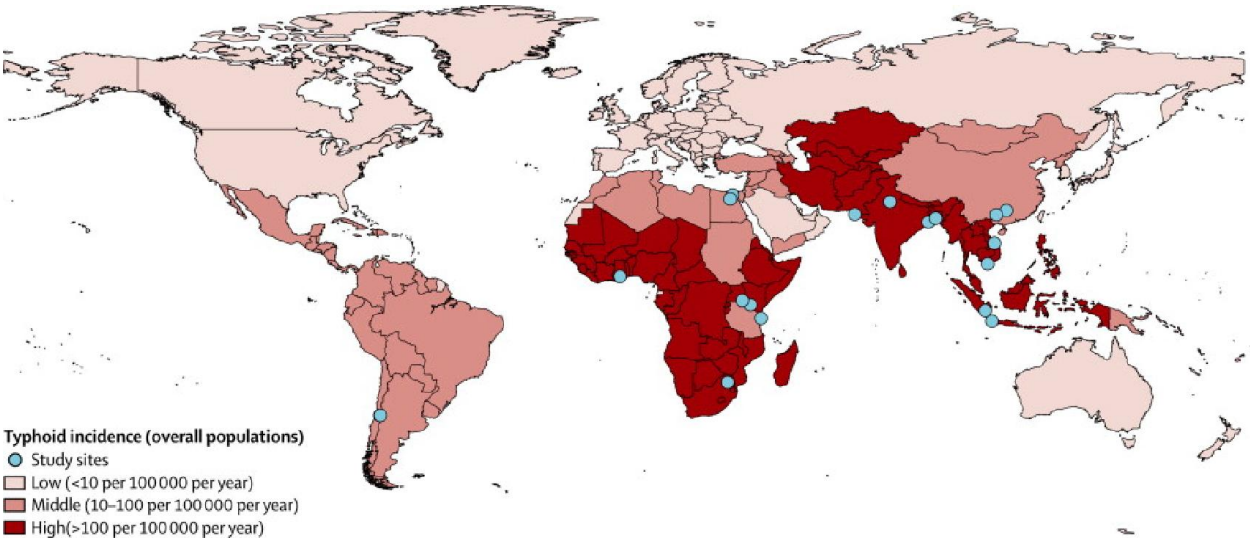


Figure 1: TheGlobal Burden of Typhoid Fever (Adopted from Global Health, 2014)

2.2. Pathogenesis

The earliest pathologic changes are in the stages of bacterial attachment and penetration. Bacteria are firmly attached to intestinal epithelium with an accompanying degeneration of the brush borders. Later as the Salmonellae pass to lymphoid follicles of the intestine, there is diffuse enterocolitis and hypertrophy of Payer's patches (37). This is followed by necrosis of intestinal and mesenteric lymphoid tissues, focal granulomas in the liver and spleen, and characteristic mononuclear inflammatory cells ("typhoid nodules") in many organs. Typhoid nodules are primarily aggregates of altered macrophages ("typhoid cells") that phagocytose bacteria, erythrocytes and degenerated lymphocytes. These nodules also contain plasma cells and lymphocytes, but not typically neutrophils (38). The most common sites for typhoid nodules are the intestine, mesenteric lymph nodes, spleen, liver, and bone marrow (39).

Many other factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the start of appropriate treatment, the choice of antimicrobial, the patient's age and exposure or vaccination history, the virulence of the bacterial strain, the quantity of Inoculum ingested, and several host factors affecting immune status. Recent data from South Asia indicate that the presentation of typhoid may be more dramatic in children younger than 5 years, with higher rates of complications and hospitalization (29, 40). Diarrhea, toxicity, and complications such as disseminated intravascular coagulation are also more common in infancy, with higher mortality (41).

Study conducted in Ethiopia showed patients infected by *Salmonella species*, had abdominal pain (30 (75%)) which was the commonest clinical findings followed by fever 23 (57.5%), tenesmus 20 (50%), vomiting 17 (42.5%) and in 14 (35%) of them frequent feeling of thirsty was observed (42).

2.3. Diagnostic Methods

Typhoid fever is diagnosed by using a combination of the clinical presentation, the isolation of *Salmonella species* from body blood, stool and other clinical specimen (43). Although the conventional wisdom is that *S*. Typhiis obtained from blood during the first week of illness more frequently than from the stool, whereas the reverse applies during the second and third weeks of

the illness, the clinician should be reminded that the organism can be cultured from blood as late as the fifth week of the disease, and the organism may be cultured from the stool throughout the disease (44). Blood culture is considered as the gold standard diagnostic modality and 40-80% diagnostic yield seen if being done in first week of illness (44).

Out of 100 suspected cases of typhoid fever, blood culture positive for *S. typhi* were 16 (16%) (45). More than 80% of patients with typhoid fever have the causative organism in their blood. A failure to isolate the organism may be caused by several factors: a) the limitations of laboratory media b) the time of collection, patients with a history of fever for 7 to 10 days being more likely than others to have a positive blood culture. One study conducted in India in 2017 showed that blood culture is positive in only 16.4% cases of clinically suspected typhoid/paratyphoid patients (46).

Serological tests based on antibody detection have been used as an alternative for blood culture in the diagnosis of typhoid fever. Many commercial tests that detect the presence of *S.* Typhi antigen/antibody have been developed for early diagnosis. Rapid Immunochromatographic is a semi qualitative test based on visual interpretation of the test results. It detects infection specific *S.* Typhi anti O9 IgG/IgM antibodies in patient's serum (47).

Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test is based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella* Typhi. Though the Widal test is extensively used, it cannot give reliable diagnostic results in endemic regions due to difficulty in establishing a steady state baseline titer and cross reactivity with other organisms. Rapid diagnostic methods are required that should be reliable, easy to perform with comparable sensitivity and specificity. Numerous serological kits are available with variable reported sensitivities and specificities in diagnosis of typhoid fever as Typhidot M which is a dot enzyme immunoassay for detection of specific IgM antibodies to *S.* Typhi(48, 49).Typhidot becomes positive as early as in the first week of fever the results can be visually interpreted and is available within one hour (50).

According to Comparative Study of Typhidot and Widal test for Rapid Diagnosis of Typhoid fever in India, blood culture has remained the gold standard for diagnosis of typhoid fever and blood culture positivity among clinically suspected typhoid cases was 28.5 % while Widal test was positive in 68% (48/70) of the patients. Widal test was positive in 9 of the 20-blood culture positive patients and 4 of blood culture negative patients. Thus the test had sensitivity of 45% and specificity of 86% (51).

Similar study conducted in Pakistan to evaluate reliability, sensitivity and specificity of Widal and Typhidot results with blood culture, out of 76 samples 58% were positive for blood culture, 67% positive for Typhidot and 43% were Widal positive. From group II all 15 cases showed no growth on blood culture. About 13% cases were Typhidot reactive while only 27% cases were Widal positive. Amongst 44 culture positive cases from group I, 41 patients were positive for Typhidot and 31 were reactive against Widal, showing sensitivity of 93 % and specificity of 87% while, Widal sensitivity was 70% and specificity was 73% (52).

Study conducted in Nigeria to evaluate simple and rapid dipstick assay for diagnosis of typhoid fever shows that the sensitivity of rapid immunochromatographic assay when compared to blood culture was 86.5% respectively while the specificity was 88.9% (53).

Study conducted in India showed out of total 85 clinically diagnosed typhoid fever cases enrolled 11 cases (13%) were positive by blood culture, 20 cases (23.5%) by Widal test and 22 cases (26%) by Typhidot test. Widal test has a sensitivity of 54%, specificity of 81%, positive predictive value of 30%, negative predictive value of 92% while Typhidot test has a sensitivity of 91%, specificity of 84%, and positive predictive value of 45% and negative predictive value of 98% in comparison with blood culture results (54).

Other study conducted in India, in comparison to the gold standard test i.e. blood culture, the sensitivity and specificity of Typhidot and Widal test was 100% & 76% and 78.78% & 58.82% respectively (55). Other Study conducted in India out of 44 patients positive by Typhidot IgG/M, 26 patients showed positive IgM (either IgM or IgM/IgG both) for typhoid fever (56).

In study conducted in India by, the sensitivity and specificity of Typhidot-M test was 97.8% and 46.3% respectively while the positive and negative predictive value was 60.8% and 46.3%

respectively. The sensitivity and specificity of Widal test were 47.8% and 13% and Positive and Negative predictive values were 31% and 22.6% respectively (57).

Study conducted in Nekemte, Ethiopia showed the mean sensitivity Widal test is 73.5 ±12.6 while the lowest specificity of Widal test was 13.8% and the highest was 98%. The mean PPV of the Widal test was 60% ± 29% and the mean NPV of Widal test was 75.2% ± 24.8% (58).

2.4. Antibiotic resistance pattern

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

A. **Inhibitors of cell wall synthesis**. While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacterial species. A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. Examples: penicillin, cephalosporins, bacitracin and vancomycin.
B. **Inhibitors of cell membrane function**. Cell membranes are important barriers that segregate and regulate the intra and extracellular flow of substances. A disruption or damage to this structure could result in leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can often be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymyxin B and colistin.
C. **Inhibitors of protein synthesis**. Enzymes and cellular structures are primarily made of proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S sub-units of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication.

   Examples: Aminoglycosides, macrolides, streptogramins, chloramphenicol, tetracyclines.

D. **Inhibitors of nucleic acid synthesis**. DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin.

E. **Inhibitors of other metabolic processes**. Other antibiotics act on selected cellular processes essential for the survival of the bacterial pathogens. For example, both sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind to dihydropteroate synthase, trimethoprim inhibit dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans.

Mechanisms of action and resistance to an antibiotic

Resistant to antimicrobials could be by the following ways:

- ✓ Inactivation of the antimicrobial agent
- ✓ Efflux or transport of the antimicrobial
- ✓ Modification of the antimicrobial target site
- ✓ Reduced permeability of the antimicrobial agent

Those drugs that are important in the treatment *of Salmonella* Ciprofloxacin, Cotrimoxazole Nalidixic Acid, Norfloxacin, Cefotaxime and Ceftriaxone could be among the drug of choice. However, some of the drugs used are now increasingly becoming low susceptibility and not effective against *Salmonella* (22).

Antimicrobial resistance continues to emerge in *Salmonella isolate resulting* in loss over time of the value of drugs. Added to the increasing complexity of managing typhoid fever because of antimicrobial resistance, there is a strong case for much greater effort in the treatment *Salmonella* early as possible with appropriate diagnosis. That is with improved sensitivity and specificity. There is need for a strong collaboration between the physicians and the laboratory in the choice of antibiotics for the treatment of typhoid fever (8). Low laboratory standard,

irrational uses of an antibiotic and genetic factor could be among the possible causes for development and spread of drug resistance.

Emergence of multidrug resistance and decreased ciprofloxacin susceptibility (DCS) in *Salmonella* enterica serovar Typhi in South Asia have rendered older drugs, including ampicillin, chloramphenicol, trimethoprim–sulphamethoxazole, ciprofloxacin, and ofloxacin, ineffective or suboptimal for typhoid fever (59). Study conducted in India showed the overall resistance of *Salmonella species* for ampicillin and trimethoprim was 60% each (60).

Study conducted in Addis Ababa showed *Salmonella species* was resistance to ampicillin (59.4%) and sulfamethoxazole (40.6%) (61). Similar study conducted in Jimma showed that *Salmonella* species were resistant to ampicillin (100%) followed by Nalidixic acid (22).

Study conducted in Debre Markos showed high frequency of resistance for *Salmonella* isolates was observed to co-trimoxazole (37.5%) and ampicillin (60%) and 27.5% of *Salmonella* isolates were reported Multi-Drug (42). Other study in Harar reported that all *Salmonella* isolate were resistant to ampicillin (100%) and sensitive to ceftriaxone(62).

CHAPTER III: OBJECTIVES

3.1 General Objective

To evaluate an alternative immunochromatographic Test for the diagnosis of Typhoid Fever, Jimma, Southwest Ethiopia.

3.2 Specific Objectives

- To determine/evaluate the diagnostic value and the sensitivity, specificity, positive and negative predictive value of the Typhoid IgG/IgM Rapid test.
- To evaluate the diagnostic performance of widal test.
- To determine the prevalence of typhoid fever
- Drug susceptibility pattern of *Salmonella* isolates

CHAPTER IV: MATERIALS AND METHODS

4.1 Study Area

Jimma town is located at a distance of 354 km south-west of Addis Ababa. Jimma zone is found in Oromia region with a total population of 2,788,390 according to 2007 E.C population census conducted by the central statistical agency (CSA) of Ethiopia. Jimma University medical center is providing wide varieties of medical both inpatient out patients and other community services for more than 3 million people. It covers an area of 199,316.18 km^2 and an average altitude of about 2180 meters above sea level.

4.2 Study Period

The study was conducted in Jimma University Medical Center from February – October /2017.

4.3 Study Design

A Hospital based cross sectional study design was employed to determine the diagnostic validity of Typhoid IgG/IgM rapid immunochromatographic test method in comparison with a standard reference method among febrile patients with clinically suspected typhoid fever.

4.4 Population

4.4.1 Source of Population

All febrile patients suspected for typhoid fever who visit Jimma University Medical Center during the study period.

4.4.2 Study Population

Febrile patients with high clinical suspicion for typhoid fever who present at Jimma University Medical Center during the study period and who fulfill the inclusion criteria were recruited in the study.

4.5 Inclusion and Exclusion Criteria

4.5.1 Inclusion Criteria

- ✓ Febrile patients with body temperature at presentation of ・38^0C
- ✓ Patients who consent/volunteer to participate in the study

4.5.2 Exclusion Criteria

Febrile patients who have been treated with some antibiotic seven days or less prior to submitting samples and those who refused to participate in the study were excluded.

4.6 Sample size Determination and Sampling Techniques

4.6.1 Sample Size Determination

The sample size for this study was calculated using the formula for estimation of single population proportion.

n= Z^2pq/d2 Where: P= 5.68% Taken from prevalence of human *Salmonellosis* in Ethiopia(63)

Z=value is 95% confidence level which is 1.96; and

n=desired sample size

d=degree of accuracy desired (0.03)

n= Z^2pq/d2

n= $1.96^{2\ p}$ (1-p)/d^2

n=Z2pq/d2 = $(1.96)^2$ * 0.0568(1-0.0568) / $(0.03)^2$ => 0.2058/ 0.0009= 229 and 20% non-respondent rate = 46

229+ 46= **275**

4.6.2 Sampling Technique

Convenience sampling technique was used to select study participants. Patients were consecutively included in the study until the desired sample size was met.

4.7 Study Variable

4.7.1 Dependent Variables

- Prevalence of *Salmonella,*
- Antimicrobial susceptibility pattern
- Sensitivity, Specificity, and Positive and Negative predictive values.

4.7.2 Independent Variable

Socio-demographic variables

- Age
- Sex
- Educational status
- Residence
- Occupational status
- previous history of antibiotic treatment,
- washing before feeding
- type of water used.

4.8 Data Collection Methods and Procedure

4.8.1 Sociodemographic and Clinical Data

Demographic information and clinical data were collected using structured, interviewer administered questionnaires prepared in Afaan Oromo and Amharic (Annex 3). Face to face interview conducted and all patients were interviewed consistently.

4.8.2 Laboratory Methods and Process

4.8.2.1 Culture

For each study participant volunteer to participate in the study, 10ml blood specimen were collected. These samples were inoculated into Tryptic Soy Broth (TSB). Blood culture bottles were incubated aerobically at 37^{o}C and Sub-cultures were made on Xylose Lysine Deoxycholate (XLD) and MacConkey agar (MAC). Then for serological tests poured in to sterile plain tube and the serum was separated for widal and Typhoid IgG/IgM Rapid immunochromatographic test after separating the serum (64) (Annex 4.3).

Patients were provided with clean tight-fitting container and were requested to bring about 2g of (pea-size) fresh stool samples which were then transported to Jimma University microbiology research laboratory within 2 hours for isolation and identification of *Salmonella* species. Stool samples were also cultured on XLD and MacConkey agar and then was incubated at 37^{0}C for 18 – 24 hours.

Isolation and Identification of *Salmonella*:

The isolate was identified based on cultural characteristics, morphology and biochemical test. On MacConkey agar, *Salmonella* produces lactose non-fermenting smooth colonies, while On XLD *Salmonella* appears as red colony with black center) the cell morphology of pure culture was assessed by Gram staining. The morphological study includes cell shape, cell arrangement, presence or absence of endospore, and motility (8, 65). The aseptic condition, purity plate and quality control were maintained throughout the experiment by parallel incubation of control strain (66) (Annex 4.1).

After overnight incubation, culture plates were examined for *Salmonella* like colonies considering Colorless and lactose non-fermenting colonies (2-4 mm) on MAC and Clear to light pink colonies with distinct black centers, and clear to white / pale-red colonies on XLD. These colony was then picked for sub-culturing to non-selective media which could be Nutrient agar.

All isolate of blood and stool cultures were characterized on the basis of morphology, cultural characteristics, biochemical tests, carbohydrate fermentation and decarboxylation of amino acid testing using standard procedures (26, 67)

1) Procedure of Blood Sample for culture

1) Collect under aseptic precautions, 10ml from patients transfer and inoculated into Tryptic Soy Broth (TSB).
2) Blood culture bottles were incubated aerobically at 37^{o}C at least for 24 hours.
3) If growth observed, Sub-cultures were made on XLD and MacConkey agar and incubated at 37^{0}C for 18 – 24 hours on the same selective.
4) Observe the Medias for the growth of specific colony characteristics.

    - ✓ On MacConkey agar, *Salmonella* produces lactose non-fermenting smooth colonies, while On XLD *Salmonella* appears as red colony with black center)

2) Stool sample

1) Instructing the patient or parents or the guardian to collect stool sample the specimen should contain at least 2g of feces.
2) Once the specimen has been placed in the specimen container, the lid should be sealed.
3) Stool was transported to Jimma University microbiology research lab for analysis.
4) Prepare a faecal suspension by suspending approximately 1g of the stool sample in a tube containing 1 ml of sterile saline. If the stool sample is liquid, saline does not need to be added.
5) Inoculate on MAC and XLD and add three or more loops full of faecal suspension.
6) Incubate the plates for the isolation of *Salmonella* at 37 ^{o}C for 24 hrs.
7) Pick *Salmonella* suspected colony and subculture on non-selective media (Nutrient agar)
8) Identified and characterized by standard biochemical testing flow chart.

Biochemical Tests

For biochemical tests, pure colonies obtained by sub-culturing on Nutrient agar and morphologically identical 2-3 colonies of the suspected strains were taken from the agar plates and suspended in nutrient broth. Then the suspensions were inoculated to biochemical testing media and were incubated aerobically at 37°C for overnight. Thus, *Salmonella* species were characterized by series of biochemical tests such as Kligler Iron slant agar (KIA) (Oxoid), Urease test and Oxidase (Oxoid, UK) test and Sulfide-Indole-Motility (SIM) (27).

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the **cytochrome C oxidase** oxidizes the reagent **(tetramethyl-p-phenylenediamine)** to **(indophenols)** purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless(68).

Test requirements for Oxidase test: Moist filter paper with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride), or commercially prepared paper disk, wooden wire or platinum wire.

Expected results of Oxidase test

1. Positive: Development of dark purple color (indophenols) within 10 seconds
2. Negative: Absence of color

Procedure of Oxidase test

1. Take a filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride
2. Moisten the paper with a sterile distilled water
3. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper
4. Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds

Precaution to be taken while performing oxidase test:

1. Do not use Nickel-base alloy wires containing chromium and iron (nichrome) to pick the colony and make smear as this may give false positive results
2. Interpret the results within **10 seconds, timing is critical**

Urease test

This test is used to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink. *Proteus mirabilis* is a rapid hydrolyzer of urea.

Urease test principle

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Many organisms especially those that infect the urinary tract, have urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

Result and Interpretation

Organisms that hydrolyze urea rapidly (e.g. *Proteus* spps) may produce positive reactions within 1 or 2 hours. In routine diagnostic laboratories the urease test result is read within 24 hours.

- If organism produces urease enzyme, the color of the slant changes from light orange to magenta.
- If organism do not produce urease the agar slant and butt remain light orange (medium retains original color)

Triple sugar iron agar (TSI)

Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates.Composition of Triple Sugar Iron Agar (TSI) Lactose, Sucrose and Glucose in the concentration of 10:10:1 (i.e. 10-part Lactose (1%), 10-part Sucrose (1%) and 1-part Glucose (0.1%)). **0.1% Glucose**: If only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red

1.0 % lactose/1.0% sucrose: a large amount of acid turns both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose.

Iron: Ferrous sulfate: Indicator of H_2S formation

Phenol red: Indicator of acidification (It is **yellow in acidic condition** and red under alkaline conditions).

It also contains **Peptone** which acts as source of nitrogen which mean that whenever peptone is utilized under aerobic condition ammonia is produced).

Procedure for Triple Sugar Iron Agar (TSI) Test

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony
2. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
3. Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 - 24 hours

Interpretation of Triple Sugar Iron Agar Test

1. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in butt and in the slant. Some organisms generate gases, which produces bubbles/cracks on the medium.

2. If lactose is not fermented but the small amount of glucose is, the oxygen deficient butt will be yellow (remember that butt comparatively have more glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) will be oxidized to carbon dioxide and water by the organism and the slant will be red (alkaline or neutral pH).
3. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids and should be notice that peptone is a major constituent of TSI Agar.
4. If H_2S is produced, the black color of ferrous sulfide is seen.

Principle of Citrate Utilization Test

Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Bacteria capable of growth in this medium use the citrate and convert ammonium phosphate to ammonia and ammonium hydroxide, creating an alkaline pH. The pH change turns the bromothymol blue indicator from green to blue.

Procedure of Citrate Utilization Test

A. Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old. Do not inoculate from a broth culture, because the inoculum will be too heavy.
B. Incubate at 35°-37°C for up to 7 days.
C. Observe for growth and the development of blue color, denoting alkalization.

Result Interpretation of Citrate Utilization Test

Positive: Growth on the medium, with or without a change in the color of the indicator. Growth typically results in the bromothymol blue indicator turning from green to blue. **Negative:** Absence of growth.

Lysine Decarboxylase (LDC)

Lysine Decarboxylase Test (LDC): To assist in the identification of Salmonellae (+ve). Decarboxylases are a group of substrate specific enzymes that are capable of reacting with the carboxyl (COOH) portion of amino acids, forming alkaline-reacting amines and byproduct carbon dioxide. Increased pH of the medium is detected by color change of the pH indicators bromcresol purple and cresol red.

1. Start your Bunsen burner.
2. Select the inoculating loop tool.
3. Flame your inoculating loop to sterilize it.
4. Remove the caps from your test tubes.
5. Flame the mouths of your test tubes.
6. Use the sterile inoculating tool to pick up an inoculum from the culture tube of the unknown bacterium.
7. Immediately transfer the inoculum into the fresh, sterile medium.
8. Flame the mouths of your tubes once again.
9. Replace the caps on the test tubes.
10. Re-flame the inoculating tool.

Procedure

1. Place the inoculated tube into the 35-37 C incubator.

The color was noted at both 24 and 48 hours to determine the result.

2. Retrieve desired incubated culture from the incubator.
3. Observe test result. If the test was followed as described above, the culture will have changed to yellow in the presence of acids or remains purple at neutral p*H* or in the presence of bases/alkali.

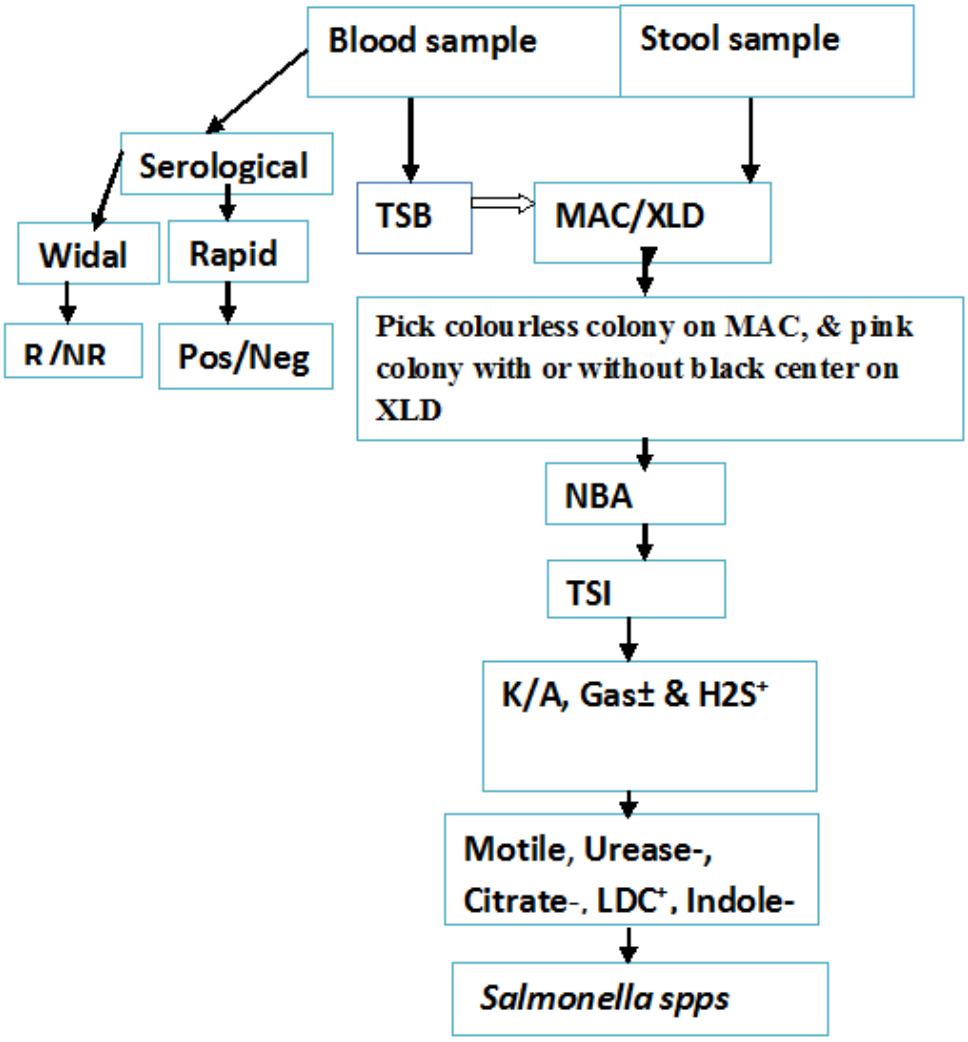


Figure 2: Conceptual frame work for isolation of *Salmonella JUMC, 2017*

NB: TSB- Tryptic Soy Broth, MAC – MacConkey, XLD – Xylose Lysine deoxycholate, NBA – Nutrient Broth Agar, TSI – Triple Sugar Iron Agar, K- Alkaline, H2S – Hydrogen Sulfide, LDC – Lysine decarboxylase, R – Reactive, NR – Non-reactive,

4.8.2.2 Widal tube agglutination test

In this study 2-3 mL of blood samples were collected into a sterile and properly labeled test tube and centrifuged for 5 minutes to separate the serum from the blood. The Widal tube agglutination test was performed for all cases enrolled in the study. According to the manufacturer's instructions (Linear Chemicals, Barcelona, Spain), using saline solution as a diluent added one drop of each antigens specifically for S. typhi serotyping (O:9 and H: d antigens from: Febrile Antigens Kit stained bacterial suspensions, lot: 2199610) was added to each test tube contains patient serum in double folded serial dilution (1/40, 1/80, 1/160, 1/320, and 1/640) in 2 rows one for O antigen and second row for H antigen. After incubation for 24 hours at 37º C, observed for agglutination and then the test was considered positive if O titer ≥ 1/160 and H titer ≥ 320 (69)).

4.8.2.3 Typhoid IgM/IgG Rapid Test-Cassette

Principle: Typhoid IgG/IgM immunochromatographic rapid test on plasma or serum is a lateral flow chromatographic immunoassay (Zhejiang Orient Gene Biotech Co. LTD). The test cassette consists of: 1) a deep red colored conjugate pad containing recombinant S. Typhoid H and O antigens conjugated with colloid gold (typhoid conjugate) and rabbit IgG-gold conjugate, and 2) a nitrocellulose membrane strip containing two test pads (T1 and T2 bands) and control band. Anti-*S.* Typhi IgM if present T1 band became colored, indicating a *S*. Typhi IgM Positive result. Anti-S. typhi IgG if present T2 band became coloured indicating *S.* Typhi IgG Positive test result. Absence of any T bands (T1 and T2) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy coloured band of the immunocomplex of goat ant-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the T bands. Otherwise, the test result was considered invalid (67) (Annex 4.3).

4.8.3 Evaluation of clinical value of serologic tests against culture

Sensitivity, specificity, and predictive values of the test was calculated using the following formula from two by two table.

Test		**Gold standard (Culture)**		
		Positive	Negative	Total
Test to be evaluated	Positive	TP	FP	TP + FP
	Negative	FN	TN	TN +FN
	Total	TP +FN	TN +FP	TP +FN+TN+FP

Sensitivity = TP / (TP+FN) **PPV =** TP / (TP+FP)

Specificity = TN / (TN+FP) **NPV =** TN / (TN+FN)

Where TP = True Positive FN = False Negative

TN = True Negative FP = False Positive

PPV = Positive predictive value NPV = Negative predictive value

4.8.4 Confidence Interval Calculation

The sensitivity, specificity and accuracy are proportions, thus confidence intervals can be calculated by using standard methods for proportions (70). Two types of 95% confidence intervals are generally constructed around proportions: asymptotic and exact 95% confidence interval. The exact confidence interval is constructed by using binomial distribution to reach an exact estimate. Asymptotic confidence interval is calculated by assuming a normal approximation of the sampling distribution. The choice of these two types of confidence interval depends on whether the sample proportion is a good approximation of normal distribution. If the number of event is very small or if the sample size is very small, the normal assumption cannot be met. Thus, exact confident interval is desired (71, 72) .

4.9 Antibiotic Susceptibility Test.

The antibiotic susceptibility patterns of *Salmonella* species isolated from blood and stool specimen against commonly used antibiotics was done on Mueller Hinton agar (MHA) (Oxoid) and incubated at 37°c; aerobically for 24 hours. Inoculum of direct colony suspension, equivalent to 0.5 McFarland standards is used to adjust the turbidity of the inoculums for the susceptibility testing. The standard disk diffusion technique of modified Kirby-Bauer method is used as recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (73). Following overnight incubation at 37 °C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight-line ruler. and the results were recorded as sensitive (S), Intermediate(I) or resistance (R) based on EUCAST guidelines (73). For the susceptibility testing the following eight antimicrobial drugs and concentrations were used: Chloramphenicol 30 µg, Ampicillin 10 µg, Ciprofloxacin 5µg, Cefotaxime 30µg, Ceftriaxone 30µg, Norfloxacin 10µg, and Trimethoprim-sulfamethoxazole 1.25 / 23.75µg, Nalidixic acid (30µg)**.**The criteria for selection of those antimicrobial agents tested were their activity against *Salmonella* and the fact that some of the listed antibiotics like Ciprofloxacin are used in the treatment in Ethiopia(74)**.**

4.10 Data Quality Assurance

Training was given to laboratory assistant and data collector for two days on the procedures of data collection and handling of collected data. Pre-testing was done and the collected data were checked for completeness at the end of data collection. During laboratory analysis of blood and stool samples standard operating procedures were followed. Culture media were prepared and sterilized based on the manufactures instruction. Then the sterility of culture media is checked by incubating 3–5% of the batch at 37°C overnight and observed for bacterial growth. Finally, those media which showed any sign growth was discarded. The American Type Culture Collection (ATCC) strains *E. coli ATCC 25922* obtained from Ethiopian Public Health Institute was used as a quality control during each samples culture, biochemical test and antimicrobial susceptibility testing(75).

4.11 Data Analysis

The results of demographic, clinical data, laboratory parameter details were compared and analyzed using sensitivity, specificity, positive predictive value, negative predictive value, and the reliability of the tests (kappa, AUC) were compared by IBM SPSS software version 20. Descriptive statistics such as frequency, percentage and cross tabulation were used to present the findings.

4. 12 The Test validity

4.12.1 kappa Test

It is a measure of level of agreement between the Typhoid IgG/IgM Rapid Test and the widal test. Kappa is always less than or equal to 1, it falls within the range (-1 to 1). A value of 1 implies perfect agreement and values less than 1 imply less than perfect agreement. Here is one possible interpretation of Kappa.

- Poor agreement = Less than 0.20
- Fair agreement = 0.20 to 0.40
- Moderate agreement = 0.40 to 0.60
- Good agreement = 0.60 to 0.80
- Very good agreement = 0.80 to 1.00 (76)

4.12.2 The ROC Curve

ROC curve is graphical display of sensitivity (TPR) on y-axis and (1 – specificity) (FPR) on x-axis for varying cut-off points of test values. This is generally depicted in a square box for convenience and it's both axes are from 0 to 1. The area under curve (AUC) is an effective and combined measure of sensitivity and specificity for assessing inherent validity of a diagnostic test. Maximum AUC = 1 and it means diagnostic test is perfect in differentiating diseased with non-diseased subjects. This implies both sensitivity and specificity are one and both errors–false positive and false negative–are zero. This can happen when the distribution of diseased and non-diseased test values does not overlap. This is extremely unlikely to happen in practice. The AUC

closer to 1 indicates better performance of the test. ROC curves can be used to compare the diagnostic performance of two or more laboratory or diagnostic tests (77).

Interpretation for ROC curve

- 0.50 to 0.75 = fair
- 0.75 to 0.80 = good
- 0.80 to 0.90 = very good
- 0.90 to 1.00 = excellent (77)

4.13 Ethical Consideration

Institutional ethical clearance was obtained from Jimma University, Health Research Ethics Review Committee. During data collection each participant were informed about the aim of the study and written consent was obtained before the start of data collection (Annex 2). Participants were informed that they have full right to participate or not. Samples with positive culture result were communicated to physicians in order for patients to get treatment according to drug susceptibility results of isolates.

4.14 Operational Definition

Fever: – Those patients with temperature >38^{0}C and suspected for typhoid cases.

Positive IgM Test: – An antibody detected (*Salmonella* Ab) in the patient's serum in response to acute or recent infection with disease.

Positive IgG test: - An antibody detected (*Salmonella* Ab) in the patient's serum in response to previous exposure or had an infection sometime in the past or may be a carrier for *Salmonella spps.*

Positive widal test: - Considered significant when titer for O**> 1 in 160** and H**> 1 in 320**

Sensitivity: - is the ability of the widal test and the Typhoid IgG/IgM Rapid immunochromatographic tests to quantify correctly and identify subjects with the disease.

Specificity: – is the ability of the widal test and the Typhoid IgG/IgM Rapid immunochromatographic tests to correctly identify subjects without the disease.

CHAPTER V: RESULTS

5.1. Sociodemographic Information

Out of 275 participants 62.5% (172/275) were males and 37.5% (103/275) were females. From a total of 275 patients 51.3% (141/275) of the respondents were from urban areas while 48.7% (134/275) were from rural areas. The mean age of the respondents was 30.37 years and the majority of them (46.7%) were in the age group of 21 – 30 years. The other characteristics of the study subjects are shown in Table 1

Table 1: Sociodemographic characteristics of the study subjects attending Jimma University Medical Center (JUMC), Southwest Ethiopia, 2017

Sociodemographic characteristics		Number	Frequency (%)
Sex	Male	172	62.5
	Female	103	37.5
Residence	Urban	141	51.3
	Rural	134	48.7
Occupational status	Employee	18	6.5
	Farmer	120	43.6
	Student	96	34.9
	Merchant	24	8.7
	Daily laborer	17	6.2
Educational status	Illiterate	94	34.2
	Elementary	59	21.5
	High school	82	29.8
	College & above	40	14.5

5.2 Hygiene and Environmental Related conditions

The source of water for the majority of respondents (77.5%) was tap water (Fig 3). The habits of latrine usage, hand washing before meal, cooking before eating were 96%, 97%, and 97%,

respectively (Table 2). With regard to an antibiotic usage, 65% of the patients buy antibiotics by the order of physicians from drug seller and get consultation from the drug seller how to use and the remaining 35% buy without prescription and few of them decided by their own.

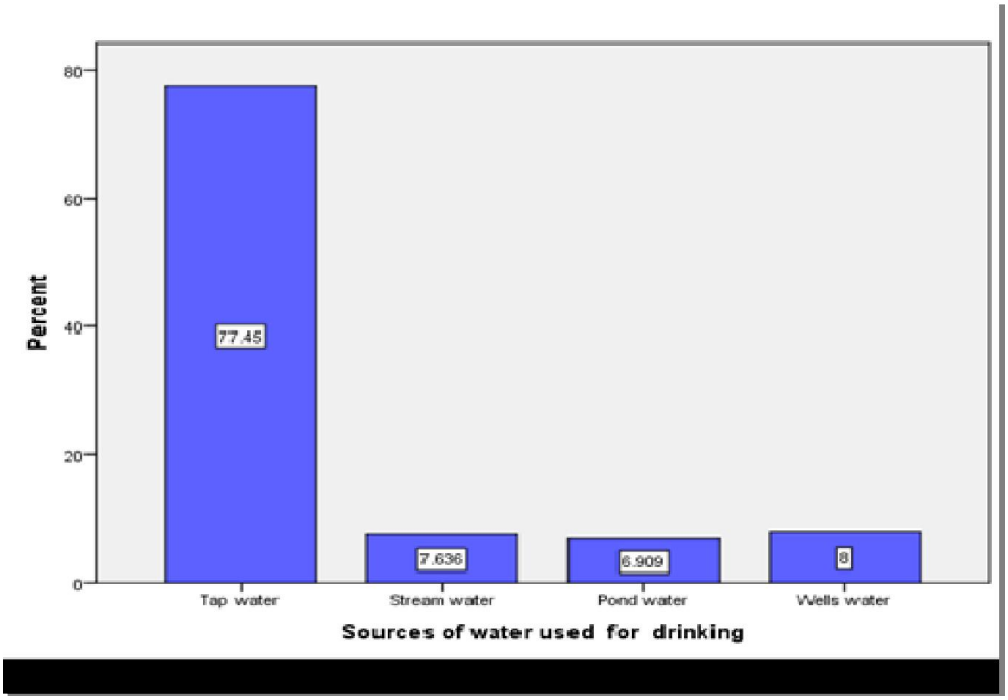


Figure 3: Source of drinking water for respondents in Jimma University Medical Center, 2017

Table 2**:**Culture Positive Cases in Relation to Hygienic and Environmental Factors among study subjects attending JUMC, Southwest Ethiopia, 2017

Environmental factors	**Culture result**

		Positive	Negative
Presence of latrine	Yes	33(12.5%)	231(87.5%)
	No	2(18.2%)	9(81.8%)
Habits of hand washing before meal	Yes	31(11.7%)	235(88.3%)
	No	4(44.4%)	5(55.6%)
Cooking vegetables before eating	Yes	29(11.1%)	232(88.9%)
	No	6(42.8%)	8(57.2%)
Cooking animal products before eating	Yes	30(11.2%)	237(88.8%)
	No	5(62.5%)	3(37.5%)

5.3 Clinical Data

Fever was the prominent sign and symptom in all patients suspected for typhoid fever with 100% positive for culture while 60.0% (21/35) and 54.3% (19/35) of the patients who had sign and symptoms of vomiting and abdominal cramp, respectively, were positive for typhoid fever by culture (Table 3).

Table 3: Culture Positive cases in Relation to Clinical Factors of the study subjects attending JUMC, Southwest of Ethiopia, 2017

Clinical factors		**Typhoid fever (culture positive)**
Fever	Yes	35(100)
	No	0 (0)
Abdominal cramps	Yes	19 (54.3)
	No	16 (45.7)
Vomiting	Yes	21(60)
	No	14 (40)

5.4 Prevalence of Typhoid Fever

A total 275 patients clinically diagnosed as typhoid fever were enrolled in the study. The overall prevalence of typhoid fever identified by culture was 12.7% (Table 4). Out of 46 cases reported as positive by Typhoid IgG/IgM Rapid immunochromatographic test, IgG immunoglobulin was detected in 12.4% /while 10.2% IgM immunoglobulin was detected by the test (Table 4).

Table 4: Results of Culture, Rapid IgG/ IgM and Widal test among enrolled cases of JUMC, Southwest Ethiopia, 2017

	Culture	Rapid test		Widal test
		IgG	IgM	
Positive	35 (12.7%)	34(12.4%)	28(10.2%)	66 (24%)
Negative	240 (87%)	241(87.6%)	247(89.8%)	209 (76%)
Total	275 (100%)	275(100%)	275(100%)	275(100)

In this study, typhoid fever was more frequently observed in age group 20- 30 yrs. followed by 31 - 40-year age group (Table 5).

Table 5: Prevalence of Typhoid Fever in Relation to Ages of the Respondents in Jimma University Medical Center, 2017

Age group in years	Number Examined	Number positive using cultures	Number positive using rapid IgG/IgM	Number positive using widal test
≤ 10	7	2 (28.6)	2 (28.6)	2 (28.6)
11 – 20	53	8 (15.1)	10 (18.8)	17 (32)
21 – 30	107	13 (12.1)	15 (14)	22 (20.6)
31 – 40	68	8 (11.8)	14 (20.5)	15 (22)
41 – 50	18	3 (16.7)	4 (22.2)	5 (27.7)
≥ 51	22	1 (4.5)	1 (4.5)	5 (22.7)
Total	275	35(12.7)	46 (16.7)	66 (24.0)

5.5 Evaluation of Rapid Immunochromatographic (Typhoid *IgG/IgM rapid*) test and widal test methods against culture

This study was carried out to study the Typhoid IgG/IgM Rapid test (cassette). We calculated sensitivity, specificity, positive and negative predictive values and the reliability (kappa, ROC curve) tests of Typhoid IgG/IgM Rapid Test in serum and Widal test by keeping culture positive cases from blood and stool samples as gold standard.

Amongst 8 cases positive by blood culture, 6 were positive for IgG while 2 blood culture positive cases falsely negative by IgG and all 8 cases were positive for IgM immunoglobulin (Table 6). Out of 66 cases positive by Widal test, 4 cases were positive by blood culture and 21 cases were positive by stool culture. Both Rapid and Widal test agree on only 30 positive cases (Table 7).

Table 6: Comparison of Results of Serological Tests (Rapid IgG/IgM and Widal) against Blood Culture in enrolled cases of JUMC, Southwest of Ethiopia, 2017

Serological tests		**Blood culture**		
		Positive	**Negative**	**Total**
Widal test	Positive	4	62	66
	Negative	4	205	209
	Total	8	267	275
Results of Rapid IgG	Positive	6	28	34
	Negative	2	239	241
	Total	8	267	275
Results of Rapid IgM	Positive	8	20	28
	Negative	0	247	247
	Total	8	267	275

Table 7: Comparison of Results of Serological Tests (Rapid IgG/IgM and Widal) against Stool Culture in enrolled cases of JUMC, Southwest of Ethiopia, 2017

Serological tests		**Stool culture**		
		Positive	**Negative**	**Total**
Widal test	Positive	21	45	66
	Negative	9	200	209
	Total	30	245	275
Results of Rapid IgG	Positive	28	6	34
	Negative	2	239	241
	Total	30	245	275
Results of Rapid IgM	Positive	23	5	28
	Negative	7	240	247
	Total	30	245	275

5.5 Agreements of Typhoid IgG/IgM Rapid Test and widal Test with culture

This study was carried out to evaluate the diagnostic value of rapid immunochromatographic test (Typhoid IgG/IgM Rapid Test) against blood and stool cultures. The sensitivity and specificity of Typhoid IgG/IgM Rapid Tests against blood culture were (75%, 100%) and (90%, 93%) respectively. While The sensitivity and specificity of Typhoid IgG/IgM Rapid Tests against stool culture were (93.3%, 77%) and (97.6%, 98%) respectively. Typhoid IgG/IgM Rapid Test was statistically significant and substantially agreed with the gold standard ($p<0.001$; $k = 0.668$) (Table 8).

The sensitivity, specificity, PPV and NPV of Widal test against blood culture were 50%, 77%, 6% and 98% respectively. The sensitivity, specificity, PPV and NPV of Widal test against stool culture were 70%, 82%, 32% and 95.7% respectively. P Value for Widal test was <0.001 i.e. statistically significant and fair agreement with the gold standard ($k=0.371$ (Table 8).

Table 8: Sensitivity, Specificity and Predictive values for Serological Test against Blood and Stool Cultures in enrolled cases of JUMC, Southwest of Ethiopia, 2017

Test	**Estimate against Blood culture**			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Widal test	50	77	6	98.1
Rapid IgG	75	90	18	99.2
Rapid IgM	100	93	29	100
	Against Stool culture			
Widal test	70	82	32	95.7
Rapid IgG	93.3	97.6	82.4	99.2
Rapid IgM	77	98	82.1	97.2

Taking the assumption of normal approximation of the sampling distribution the confidence interval for sensitivity, specificity, positive and negative predictive values were shown in the table below (Table 9).

Table 9: The validity of the Typhoid IgG/IgM Rapid Immunochromatographic test and widal test, JUMC, Southwest of Ethiopia, 2017

	Rapid Immunochromatographic tests				**Widal test**	
	Rapid IgG		**Rapid IgM**			
	Estimate	**95% CI**	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Sensitivity	0.75	(0.62, 0.88)	1.00	(0.87, 1.03)	0.5	(0.37, 0.63)
Specificity	0.90	(0.77, 1.03)	0.93	(0.83, 1.06)	0.77	(0.64, 0.90)
PPV	0.18	(0.05, 0.31)	0.29	(0.16, 0.42)	0.06	(0.07, 0.19)
NPV	0.992	(0.862, 1.122)	0.992	(0.87, 1.03)	0.98	(0.85, 1.11)
Sensitivity	0.933	(0.80, 1.06)	0.77	(0.64, 0.90)	0.7	(0.57, 0.83)
Specificity	0.976	(0.846, 1.106)	0.98	(0.85, 1.11)	0.82	(0.69, 0.95)
PPV	0.824	(0.694, 0.954)	0.821	(0.691, 0.951)	0.32	(0.19, 0.45)
NPV	0.992	(0.862, 1.122)	0.972	(0.842, 1.102	0.957	(0.827, 1.087)

5.7 The Diagnostic accuracy of the of Typhoid IgG/IgM Rapid Test and Widal Test

The overall accuracy of the tests can be represented by the area under curve (AUC). The performances or the diagnostic accuracy of the Typhoid IgG/IgM Rapid test and the widal test were calculated using receiver operator characteristics (ROC). Typhoid IgG/IgM Rapid test showed very good agreement (AUC = 0.879) while that of widal test was (AUC = 0.755) keeping the culture result as a gold standard and statistically significant ($p< 0.001$) (Table 10), and the graphical representation of their respective area under the curve (AUC) clearly shown (Figure 4).

Table 10: Comparison of the Diagnostic Accuracy Typhoid IgG/IgM Rapid and Widal Test, JUMC, Southwest of Ethiopia, 2017

Area Under the Curve					
Methods	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IgG/IgM Rapid test	.879	.038	.000	.804t	.954
Widal test	.755	.048	.000	.661	.850

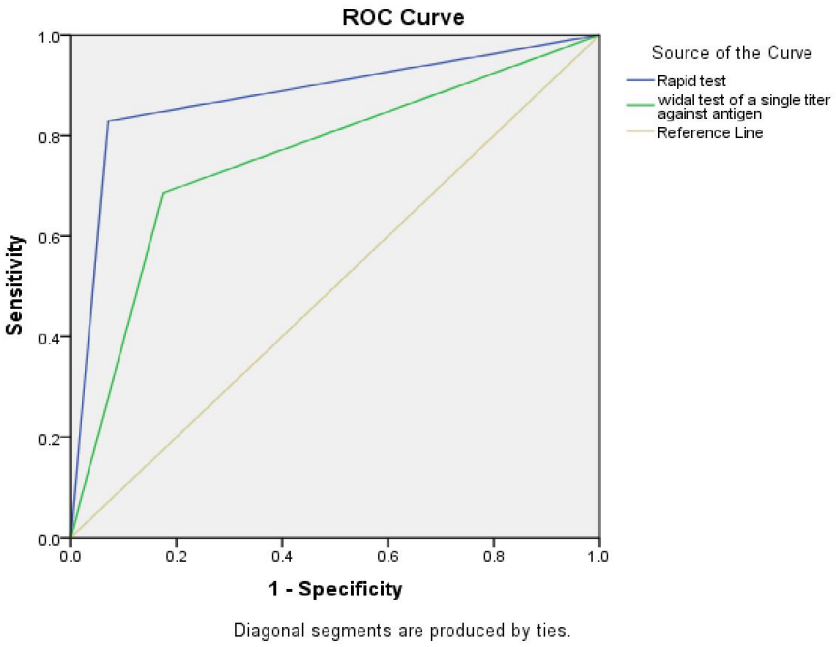


Figure 4: Graphical representation of the area under the curve (AUC) for the Typhoid IgG/IgM Rapid test strip (Blue line) and the Widal test (green line).

5.8 The Antibiotic Susceptibility Pattern

Salmonella species were 100% resistant to Ampicillin and 40.0%, 17.0%, 8.6%, and 5.7% to Chloramphenicol, Cotrimoxazole, Nalidixic acid, Ceftriaxone and Norfloxacin, respectively. The susceptibility patterns of *Salmonella species* were, 97.0% to Cefotaxime, 97.0% to Ciprofloxacin 91.4% to Norfloxacin, 82.9% to Ceftriaxone, respectively (Table 11).

Table 11: Antimicrobial susceptibility patterns of *Salmonella* isolates from patients attending Jimma University Medical Center (JUMC), 2017

	Lists of Antibiotics	Results			S(mm)	I(mm)	R(mm)
		S	I	R			
1	Chloramphenicol (30 µg)	19(54.3%)	2(5.7%)	14(40%)	≥ 18	13 – 17	≤12
2	Ampicillin (10µg)	0(0)	0(0)	100%	≥ 17	14 – 16	≤13
3	Ciprofloxacin (5µg)	34(97%)	1(3%)	0(0)	≥31	21-30	≤20
4	Cotrimoxazole (25µg)	26(74.3%)	3 (8.6%)	6(17.1%)	≥16	11-15	≤10
5	Nalidixic Acid (30µg)	21(60.0%)	11(31.4%)	3(8.6%)	≥19	14 – 18	≤13
6	Norfloxacin (10µg)	32 (91.4%)	1(2.9%)	2(5.7%)	≥17	13 – 16	≤12
7	Cefotaxime (30µg)	34 (97.0%)	1 (3.0%)	0(0)	≥26	23 – 25	≤22
8	Ceftriaxone (30µg)	29(82.9%)	4(11.4%)	2(5.7%)	≥23	20 - 22	≤19

CHAPTER VI. DISCUSSIONS

A total of 275 cases suspected of typhoid feverwere enrolled in this study out of which *Salmonellaspecies* were isolated in 12.7% (35/275) of the patients. The finding is supported by a previous study reported from Nigeria 9.3% (80) and from Ethiopia, Jimma in which *Salmonella species* were isolated in 10.8% (Lambore et al. 2016) of the study subjects. Similarly, the prevalence of *Salmonellaspecies* in this study is lower than study conducted of Arba Minch where prevalence is 20% (78). The difference might arise from local factors like hygiene, sociodemographic factors, immune status and other health related factors.

The findings in the present study suggested that people of all ages are susceptible to infection by *Salmonella.* These results are comparable with the study carried out by Adabara *et al*. (2012) where results showed that both children and adults can get typhoid fever through ingestion of contaminated food and water. Children at early ages are playful, very active curious and such behaviors expose them to risks of contracting typhoid fever than that of older ages. People living in overcrowded areas with poor access to safe drinking water and proper sanitation facilities are prone to infection by typhoid fever (8).

In this study, *Salmonella species* were isolated from individuals with low educational status (34.2%) than individual with educational status of college and above. This finding is comparable with findings of similar studies done in Jimma and Pakistan (22, 79), indicating that education is vital in creating awareness in the community with regard to attitude and practice that are important in preventing the acquisition of typhoid fever and control of other factors that lead to at least minimize *Salmonella* infection (80).

In developing countries, facilities for isolation and culture are often not available, especially in rural areas with health centers and primary hospitals diagnosis relies largely upon clinical signs and symptoms of the disease and rapid agglutination tests like the Widal test that are based on qualitative detection of serum antibodies to *Salmonella typhi and Salmonella paratyphi*. Many studies have documented that these tests lack diagnostic specificity to be used as reliable methods in these settings and are of limited value (81). There is a need for a rapid and reliable diagnostic test to overcome these problems. The present study attempted to suggest a rapid and

reliable serological test that could be used as an alternative in diagnosis of typhoid fever in rural settings with limited laboratory resources.

In the present study, we observed great variation among the tests in the extent of reporting prevalence of typhoid fever 12.7%, 16.7%, 24% by culture, Typhoid IgG/IgM Rapid immunochromatographic test, and Widal test, respectively. This high positive result in Widal test may be associated with cross-reacting antibodies from serum of febrile patients other than typhoid fever. The finding of culture result was lower to the finding previous study conducted in Ethiopia, Arba Minch 20% while low proportion widal test was observed 42% in the same area (78). The differences in the culture results could be arise from the issue of the sample and the sample size. Meanwhile the difference in widal test result might be explained differences in endemicity of typhoid fever, immune status of the community, the way of interpretation (O antigen (≥1:80 titration value) or H antigen (≥1:160 titration value) (81).

Among 30 positive stool culture, 2 of were negative by rapid IgG and among 8 blood culture positive 2 of them were negative by rapid IgG. On the other hand, from 30 stool culture positive cases 7 of them were negative by rapid IgM while none of blood culture positive cases reported negative by rapid IgM. The false-negative rapid immunochromatographic test results might be due to early infection of the disease before production of detectable antibody (86).

The sensitivity of typhoid IgM Rapid test and the Widal test as evaluated against blood culture were 100% and 50% respectively. This indicate that the ability of typhoid IgM Rapid test can detect true positive more than tube titration test to detect true-positive results. The finding is higher than the finding of the study conducted in Egypt where the sensitivity was 86% (82) and lower than the finding of the study conducted in India for the Widal test (93.18%) (83). This difference may be explained due to the immune status of the people.

On the other hand, the specificity of IgM immunochromatographic test against blood and stool culture was 93% and 98%, respectively, while that of the specificity of rapid IgG immunochromatographic test against blood and stool culture was 90% and 97.6%, respectively. This indicate that typhoid immunochromatographic rapid test has the ability to identify patients who actually have the disease and to exclude those who do not have the disease. In similar

situation we clearly observe is that rapid IgG immunochromatographic test against stool culture showed high sensitivity and specificity which IgG immunoglobulin might be detected in previous exposures and carrier for which stool culture could be positive. On the other hand, rapid IgM immunochromatographic test had better sensitivity and specificity than rapid IgG immunochromatographic test when compared to blood culture. All blood culture positive cases were also positive by rapid IgM immunochromatographic test. This agreement could be due to blood culture positive cases considered to be active infection and where IgM immunoglobulin could also predominate immune molecules in early infection. Among 30 stool culture positive cases 9 of them were negative by Widal test while among 8 blood culture positive 4 of them were negative by widal test. The false-negative Widal test results might be due to early infection of the disease before production of detectable antibody (22, 84).

In the present study, the sensitivity of Widal test against blood culture was 50% while that of against the stool culture was reported to be 70% respectively. This indicates that the ability of Widal test as compared to typhoid IgG/IgM Rapid immunochromatography test to detect true positive cases was lower. The sensitivity of widal test was comparable with study conducted in India where sensitivity was 45% (16) while the sensitivity of Widal test against stool culture was 70% as this study can be supported by the study conducted in Nekemte, western Ethiopia where the mean sensitivity Widal test is 73.5% (58). In this study the sensitivity of typhoid IgM/IgG Rapid test is higher than Widal test performed on same sample. This shows that the typhoid rapid test can perform and detect true positive result than Widal test when compared against culture result.

The PPV of Typhoid IgM/IgG Rapid test against blood and stool culture was 18% / 29%, and 82.4% / 82.1% respectively. It has paramount important as it measures the proportion of positive results in the diagnostic tests that are correctly diagnosed or true posive. The finding was comparable with the result reported 22.2% by Wasihun et al 2015 and lower than comparative study conducted by Ramyi et al. 2013 in Nigeria in which it was 83%. This difference might be explained by difference in endemicity of typhoid fever. On the other hand, the NPV value of the Typhoid IgG/IgM Rapid test against blood and stool culture was 99% /100%, and 99.2% /97.2% respectively. This shows that a negative typhoid IgG/IgM rapid test result has 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. The finding of our study is higher than the study conducted in Vietnam and it was 59% (85). On the other hand the PPV of Widal test against culture in this study was 32% which is comparable with PPV of widal test 25.32% observed in study conducted in India (86) and it can also be supported by study in Arba Minch southern Ethiopia was 24.6% (78). But the finding is lower than study conducted in Malaysia and Nekemte Ethiopia where PPV of widal test was 69% and 60% respectively(58,86,(87). On the other hand, NPV value of the Widal test was 94.7% which can be supported by study conducted in Arba Minch 90.0% (78).

The closeness Typhoid IgM/IgG Rapid test and Widal test to one another in providing precise result were assessed and showed a very good agreement 66.8% (k = 0.668) and fair agreement 37.1% (kappa = 0.371) against culture result respectively. However, these results were lower than the results observed in San Diego, USA (88) where there is excellent agreement is 99% but level of agreement for the widal test has showed comparable with study conducted in Arba Minch (kappa = 0.325) (78). Meanwhile the performances of the Typhoid IgM/IgG Rapid immunochromatographic test and the widal test were compared using receiver operator characteristics (ROC) curve keeping the culture results as a gold standard their area under the curve (AUC) and showed very good 87.9% and fair 75.5% agreement respectively and statistically significant ($p< 0.001$). This may indicate that the result of Widal test in diagnosis of typhoid fever less likely agrees with culture as oppose to the typhoid IgG/IgM rapid test. Typhoid IgG/IgM Rapid Test meets one of the criteria of an ideal diagnostic test as it has comparable results when compared to culture results and better when compared to Widal test.

Use of appropriate diagnostic method is required especially where today is the era of antibiotic resistance to prevent empirical treatment of typhoid fever. In this study all isolates of *Salmonella* species were resistant to Ampicillin (100%). This is in line with study conducted in Jimma and Harar Ethiopia (22),(62), Were as 17.1% resistance to Cotrimazole was observed which is lower than study conducted in India (89), in Bangladesh (90) and Addis Ababa (91) where resistance rate was 50% , 43% and 40.6% respectively. The difference could be due to time difference efforts made to increase awareness about consequences of drug resistance in our study area.

The success of treatment of *Salmonella is* therefore dependent on proper diagnosis of the disease that do have better sensitivity, specificity, PPV and NPV values so that correct and proper medication can be administered.

7. LIMITATION OF THE STUDY

- We have used a single blood test for serological tests due to the problem of patient recruitment for next time.
- Identification to the species level due to lack reagent.

CHAPTER VIII. CONCLUSION AND RECOMMENDATION

8.1 Conclusion

Rapid Typhoid IgG/IgM immunochromatographic has better sensitivity, specificity and predictive values than widal test. Rapid Typhoid IgG/IgM immunochromatographic has better diagnostic performance than Widal test and it should be adopted in routine clinical settings for early detection of typhoid fever.

Result of the Typhoid IgG/IgM immunochromatographic Rapid test in the diagnosis of typhoid fever better agreement with culture test than the Widal test.

8.2 Recommendation

Based on this study finding, we recommend the use of Typhoid IgG/IgM test over long known widal test for routine laboratory diagnosis of typhoid fever.

The technical simplicity, cost and ease of reading the test/interpretation, requires only a low sample volume, applicable to a wide range of settings, One-step assay, no wash steps, short time to result, possibility of multiplexing makes the rapid- Typhoid IgG/IgM test a convenient diagnostic tool including the peripheral health facilities and it would permit the clinician to institute appropriate and early treatment, thereby reducing hospital stay, morbidity and mortality in patients with typhoid fever.

Taking all these into account JUMC, health centers and private health facilities should adapt and inform the use of typhoid IgG/IgM Rapid test for the diagnosis of typhoid fever.

9.0 References

1. Pokharel P, Lekhak B, Amatya R, Bhattarai S, Pokharel P. Enteric fever caused by Salmonella enterica serovar paratyphi A: An emerging health concern in Nepal. African Journal of Microbiology Research. 2016;10(42):1784-91.
2. Obaro SK, Iroh Tam P-Y, Mintz ED. The unrecognized burden of typhoid fever. Expert review of vaccines. 2017;16(3):249-60.
3. Dadia SD, Modi RR, Shirwadkar S, Potdar NA, Shinde CA, Nair AG. Salmonella Typhi Associated Endogenous Endophthalmitis: A Case Report and a Review of Literature. Ocular Immunology and Inflammation. 2017:1-6.
4. Darton TC, Zhou L, Blohmke CJ, Jones C, Waddington CS, Baker S, et al. Blood culture-PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia. Journal of Infection. 2017;74(4):358-66.
5. Barnett B, Tarpley M, Davidson M, Gbadero D. Factors Associated with Perioperative Mortality in Typhoid Fever Patients with Intestinal Perforation in Nigeria. Integr J Glob Health. 2017;1(2):12.
6. Klotz SA, Jorgensen JH, Buckwold FJ, Craven PC. Typhoid fever: an epidemic with remarkably few clinical signs and symptoms. Archives of internal Medicine. 1984;144(3):533-7.
7. Beach B, Ferrie J, Saavedra M, Troesken W. Typhoid fever, water quality, and human capital formation. The Journal of Economic History. 2016;76(1):41-75.
8. Organization WH. Background document: the diagnosis, treatment and prevention of typhoid fever. 2003.
9. Sultana S, Hossain MA, Al Maruf MA, Gani MA. Comparison of the Lytic Blood Culture Method with the Conventional Blood Culture Method in Cases of Enteric Fever in a Tertiary Care Hospital. Bangladesh Journal of Infectious Diseases. 2017;3(1):6-10.
10. Singh S. Pathogenesis and laboratory diagnosis. JIACM. 2001;2:17-20.
11. Gilman R, Terminel M, Levine M, Hernandez-Mendoza P, Hornick R. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of Salmonella typhi in typhoid fever. The Lancet. 1975;305(7918):1211-3.

12. Tarupiwa A, Tapera S, Mtapuri-Zinyowera S, Gumbo P, Ruhanya V, Gudza-Mugabe M, et al. Evaluation of TUBEX-TF and OnSite Typhoid IgG/IgM Combo rapid tests to detect Salmonella enterica serovar Typhi infection during a typhoid outbreak in Harare, Zimbabwe. BMC research notes. 2015;8(1):50.
13. Bijapur GAM, Kakkeri SR, Raysa N, Usman SM. A study to determine significant titre values of widal test in the diagnosis of enteric fever for a population of North Kerala, India. Al Ameen J Med Sci. 2014;7(1):71-7.
14. Taiwo S, Fadiora S, D. O. et al. Widal agglutination titres in the diagnosis of typhoid fever. *West African journal of medicine*. 2007;26(2):97-101.
15. Yousef S. Seroprevalence of Salmonellosis among Pigeon and its Surrounding Environment and Isolation of Salmonella Species. 2011.
16. Narayanappa D, Sripathi R, K. J. Comparative study of dot enzyme immunoassay (Typhidot-M) and Widal test in the diagnosis of typhoid fever. . *Indian pediatrics*. 2010;47(4):331-3.
17. Tarupiwa A, Tapera S, S. M-Z. Evaluation of TUBEX-TF and OnSite Typhoid IgG/IgM Combo rapid tests to detect Salmonella enterica serovar Typhi infection during a typhoid outbreak in Harare, Zimbabwe. *BMC research notes*. 2015;8(1):50.
18. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology: Elsevier Health Sciences; 2015.
19. Nüesch-Inderbinen M, Abgottspon H, Sägesser G, Cernela N, Stephan R. Antimicrobial susceptibility of travel-related Salmonella enterica serovar Typhi isolates detected in Switzerland (2002–2013) and molecular characterization of quinolone resistant isolates. BMC infectious diseases. 2015;15(1):212.
20. Balakrishna T, Sumathi S, Anuradha K, Venkatesh D, Krishna S. A comparative study of typhidot and Widal test in the diagnosis of typhoid fever. Journal of Evolution of Medical and Dental Sciences. 2013;2(21):3720-6.
21. Brown J, Shanahan P, Jesudason M, Thomson C, Amyes S. Mutations responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant Salmonella typhi in India. Journal of Antimicrobial Chemotherapy. 1996;37(5):891-900.
22. Lamboro T, Ketema T, Bacha K. Prevalence and antimicrobial resistance in Salmonella and Shigella species isolated from outpatients, Jimma University Specialized Hospital,

Southwest Ethiopia. Canadian Journal of Infectious Diseases and Medical Microbiology. 2016;2016.

23. Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. Journal of global health. 2012;2(1).
24. Storey HL, Huang Y, Crudder C, Golden A, de los Santos T, Hawkins K. A meta-analysis of typhoid diagnostic accuracy studies: a recommendation to adopt a standardized composite reference. PloS one. 2015;10(11):e0142364.
25. Gasem MH, Smits HL, Goris MG, Dolmans WM. Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia. Journal of medical microbiology. 2002;51(2):173-7.
26. Cheesbrough M. District laboratory practice in tropical countries: Cambridge university press; 2006.
27. Deng L, Song J, X. G. Host adaptation of a bacterial toxin from the human pathogen Salmonella Typhi. *Cell*. 2014;159(6):1290-9.
28. Afifi S, Earhart K, Azab MA, Youssef FG, El Sakka H, Wasfy M, et al. Hospital-based surveillance for acute febrile illness in Egypt: a focus on communityacquired bloodstram infections. *Am J Trop Med Hyg* 2005;73:392-9.
29. Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, et al. Typhoid fever in children aged less than 5 years. ; : . *Lancet* 1999;354 734-7.
30. Saha SK, Baqui AH, Hanif M, Darmstadt GL, Ruhulamin M, Nagatake T, et al. Typhoid fever in Bangladesh: implications for vaccination policy. ; : . *The Pediatric Infectious Disease Journal*. 2001;20:521-4.
31. Ferrecio C, Levine MM, Manterola A, Rodriguez G, Rivara I, Prenzel I, et al. Benign bacteremia caused by Salmonella typhi and paratyphi in children younger than 2 years. *The Journal of Pediatrics*. 1984;104(6):899-901.
32. Levine MM, Black R, Lanata C. Precise estimation of the number of chronic carriers of Salmonella typhi in Santiago, Chile, an endemic area. *The Journal of Infectious Diseases* 1982;146(6):724-6.
33. Crump JA, Lub SP, ED. M. The global burden of typhoid fever. : . *Bulletin of World Health Organization* 2004;82:346-53.

34. Charles AM, Adam MH, El RAB MO, Morshed MG, Shakoor Z. Detection of Salmonella typhi agglutinations in sera of patients with other febrile illnesses and healthy individuals. Journal of African Medicine. 2012;10(1):41-4.
35. Omokhua AG, McGaw LJ, JF. F. A synthesis and review of its medicinal potential. *Journal of ethnopharmacology*. 2016;183:112-22.
36. Zige D, Ohimain E, MA. S. Community Based Screening of Asymptomatic Typhoid Carriers in Wilberforce Island, Bayelsa State, Nigeri. *International Journal of Health Sciences and Research (IJHSR)*. 2013;3(12):119-26.
37. Da Silva C, Wagner C, J. B. The Peyer's Patch Mononuclear Phagocyte System at Steady State and during Infection. . *Frontiers in Immunology*. 2017;8:1254.
38. Lapaque N, Walzer T, S. M. Interactions between human NK cells and macrophages in response to Salmonella infection. *Journal of Immunology*. 2009;182(7):4339-48.
39. House D, Bishop A, Parry C, Dougan G, Wain J. Typhoid fever: pathogenesis and disease. Current opinion in infectious diseases. 2001;14(5):573-8.
40. Brooks WA, Hossain A, Goswami D, Nahar K, Alam K, N. A. Bacteremic typhoid fever in children in an urban slum, Bangladesh. . *Emerg Infect Dis*. 2005;11:326-9.
41. Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, ZA. B. Typhoid fever in children: some epidemiological considerations. *Int J Infect Dis*. 2006 10:215-22.
42. Getachew Mengistu, Gebru Mulugeta, Tsehaynesh Lema, Aseffa. A. Prevalence and Antimicrobial Susceptibility Patterns of Salmonella serovars and Shigella species. J Microbial Biochem. 2014.
43. Willke A, Ergonul O, B. B. Widal test in Diagnosis of Typhoid Fever in Turkey. *Clin Diag Lab Immun*. 2002;9(4):938-41.
44. Achakzai SK, Ahmed Z, Samad A, Naeem M, Hamida H, Ali M, et al. Detection of Salmonella entericaseroverTyphi from Widal-positive blood specimens by blood culture and PCR targeting aroC and fliC genes. Rawal Medical Journal. 2017;42(4).
45. Bulbul Hasan, Sabera Gul Nahar, Shamsuzzaman AKM, Sharmina Aftab, Yusuf. A. Detection of anti-salmonella antibodies by Immunochromatographic assay at Rajshahi Medical College, Bangladesh. *J Microbiol Antimicrob*. 2013.

46. Promukh Bhattacharya, Bikram Kumar Saha, Uttam Kumar Paul, Bandyopadhyay. A. Blood Culture in Clinically Suspected Typhoid Fever. *International Journal of Scientific Study*. 2017;4 (11).
47. Pang T, S. P. Significance of widal test in diagnosis of typhoid fever in [4]endemic areas. *J Clin Pathol*. 1983;36:471-45.
48. Beig FK, Ahmad F, M E, I S. Typhidot M and Diazo test vis-àvis blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting. *Braz J Infect Dis*. 2010;14(6):589-93.
49. Siba V, Horwood PF, Vanuga K, Wapling J, Sehuko R, Siba P, et al. Evaluation of Serological Diagnostic Tests for Typhoid Fever in Papua New Guinea Using a Composite Reference Standard. *Clin Vaccine Immunol*. 2012;19(11):1833-7.
50. Choo KE, Openheimer SJ, Ismail AB, KH. O. Rapid serodiagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. *Clin Infect Dis*. 1994;19:172-6.
51. Narayanappa D, Sripathi R, K. J. Comparative study of dot enzyme immunoassay (Typhidot-M) and Widal test in the diagnosis of typhoid fever. *Indian pediatrics*. 2010;47(4):331-3.
52. Udayakumar S, Pushpalatha K, HN. S. Comparative study of Typhidot-M with Widal and blood culture in diagnosis of enteric fever. . *Indian Journal of Child Health*. 2017;4(1):64-7.
53. Enabulele O, SN. A. Typhoid fever in a Tertiary Hospital in Nigeria: Another look at the Widal agglutination test as a preferred option for diagnosis. *Nigerian medical journal*. 2016;57(3):145.
54. Neha Garg. A Comparative Study of Widal Test and Typhidot in Rapid Diagnosis of Typhoid Fever. *Int J Med Res Prof*. 2017;3(2):88-92.
55. Sanjeev H, Sweetha Nayak, Pai Asha KB, Rai Rekha, Vimal Karnaker, HR. G. A systematic evaluation of rapid dot-eia, blood culture and widal test in the diagnosis of typhoid. *nujhs* 2013;3(1).
56. Deepika verma, Sachin Kishore, ME. S. Comparative Evaluation of Various Tests for Diagnosis of Concurrent Malaria and Typhoid Fever in a Tertiary Care Hospital of Northern India. *Journal of Clinical and Diagnostic Research*. 2014 8(5):41-4.
57. Neeraj Lata, Vijay Gotwal, Tanushree Joshi YogeshGupta, DR D P Pande, BrahmPrakash. D. Cross sectional study to evaluate rapid diagnostic test (Typhidot-M) as a tool for early

diagnosis of Typhoid fever keeping blood culture as gold standard. *Int J Curr Res Med Sci*. 2017;3(3):83-93.

58. Hylemariam M, Tilahun. K. Diagnostic Value of Widal Test in the Diagnosis of Typhoid Fever. *J Med Microb Diagn*. 2017;6(1).
59. Butler T. Treatment of typhoid fever in the 21st century: promises and shortcomings. *Clin Microbiol Infect* 2011;17:959-63.
60. Kalambhe DG, Zade NN, Chaudhari S, Shinde S, Khan W, Patil AR. Isolation, antibiogram and pathogenicity of Salmonella spp. recovered from slaughtered food animals in Nagpur region of Central India. *Veterinary World* 2016;9:2231-0916.
61. Endrias Zewdu, Cornelius. P. Antimicrobial resistance pattern of Salmonella serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Trop Anim Health Prod*. 2009;41:241-9.
62. Elsabeth Tadesse. Prevalence and drug susceptibility pattern of salmonella and shigella isolates and associated risk factors among asymptomatic food handlers in student's cafeteria at dire dawa university, eastern ethiopia. 2015.
63. Tadesse G. Prevalence of human Salmonellosis in Ethiopia: a systematic review and meta-analysis. BMC infectious diseases. 2014;14(1):88.
64. Maheshwari V, Kaore NM, Ramnani VK, Sarda S. A Comparative Evaluation of Different Diagnostic Modalities in the Diagnosis of Typhoid Fever Using a Composite Reference Standard: A Tertiary Hospital Based Study in Central India. Journal of clinical and diagnostic research: JCDR. 2016;10(10):DC01.
65. Maddocks S, Olma T, Chen S. Comparison of CHROMagar Salmonella medium and xylose-lysine-desoxycholate and Salmonella-Shigella agars for isolation of Salmonella strains from stool samples. Journal of clinical microbiology. 2002;40(8):2999-3003.
66. Testing ECoAS. Data from the EUCAST MIC distribution website. European Committee on Antimicrobial Susceptibility Testing Data from the EUCAST MIC distribution website, last accessed 09 May 2013. 2016.
67. Al-Mahdi ZKA. Validation the Best, Most Accurate and Reliable Assay for Detection of Typhoid. Kerbala Jorunal of Medicine. 2013;6(1):1561-7.

68. Islam TAB, Shamsuzzaman S, Farzana A. Prevalence and antibiogram of ESBL producing gram negative bacilli isolated from urine in Dhaka Medical College Hospital, Bangladesh. Bangladesh Journal of Medical Microbiology. 2017;9(1):17-21.
69. Mohanty S, Ramana K. Single and unpaired sera tube widal agglutination test in enteric fever. Saudi Journal of Gastroenterology. 2007;13(4):213.
70. Gardner M, Altman D. Calculating confidence intervals for proportions and their differences. Statistics with confidence London: BMJ Publishing Group. 1989:28-33.
71. Gönen M. Receiver operating characteristic (ROC) curves. SAS Users Group International (SUGI). 2006;31:210-31.
72. Zhu W, Zeng N, Wang N. Sensitivity, specificity, accuracy, associated confidence interval and ROC analysis with practical SAS implementations. NESUG proceedings: health care and life sciences, Baltimore, Maryland. 2010;19.
73. EUCAST. European Committee on Antimicrobial Susceptibility Testing. 2016.
74. Desalegn AA. Assessment of drug use pattern using WHO prescribing indicators at Hawassa University teaching and referral hospital, south Ethiopia: a cross-sectional study. BMC health services research. 2013;13(1):170.
75. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. Manual of Clinical Microbiology, Eleventh Edition: American Society of Microbiology; 2015. p. 1253-73.
76. Stemler S. An overview of content analysis. Practical assessment, research & evaluation. 2001;7(17):137-46.
77. Griner PF, Mayewski RJ, Mushlin AI, Greenland P. Selection and interpretation of diagnostic tests and procedures. Annals of internal medicine. 1981;94(4 II).
78. Gemechu A, Edemew A, Berhanu K, Bethel Y. Comparative study of Widal test against stool culture for typhoid fever suspected cases in southern Ethiopia. *Pathology and Laboratory Medicine International*. 2017;9:1-7.
79. Rasul F, Sughra K, Mushtaq A, Zeeshan N, Mehmood S, Rashid U. Surveillance report on typhoid fever epidemiology and risk factor assessment in district Gujrat, Punjab, Pakistan. Biomedical Research. 2017;28(16).
80. Nath K, Bloomfield S, Jones M. Household water storage, handling and point-of-use treatment. A review commissioned by IFH. 2006.

81. Pang T, SD. P. Significance and value of Widal test in the diagnosis of typhoid fever in an endemic area. . *J Clin Pathol*. 1983;36:471-5.
82. Hamdy MS, Abdel-Rahman S, Adbel-Mgeed M, AH. S. Evaluation of Enterocheck WB® test in Diagnosis of Typhoid Fever among Egyptian Adults. *Egyptian Journal of Medical Microbiology*. 2014 23(4).
83. www.jmitra.co.in AI. Rapid visual test for the differential and simultaneous detection of S. typhi IgM & IgG antibodies in Human Serum / Plasma. *J Mitra & Co Pvt Ltd*. 2008:26818971-73.
84. Khanna A, Khanna M, Gill KS. Comparative evaluation of tubex TF (inhibition magnetic binding immunoassay) for typhoid fever in endemic area. Journal of clinical and diagnostic research: JCDR. 2015;9(11):DC14.
85. Olsen SJ, Pruckler J, Bibb W, NTM. T, TM T. Evaluation of Rapid Diagnostic Tests for Typhoid Fever. *J Clin Microbiol*. 2005;42(5).
86. Yadav K, Parihar g, SK. Y. Diagnostic reliability of Widal slide agglutination test for enteric fever- still a query. *Int J Res Med Sci*. 2017;5(10):4407-10.
87. Mengist H, Tilahun K. Diagnostic value of Widal test in the diagnosis of typhoid fever: a systematic review. J Med Microb Diagn. 2017;6(248):2161-0703.1000248.
88. WHO. Typhoid IgG/IgM Rapid Test-Cassette evaluation. *CTK biotech, inc*. 2007.
89. DG K, Zade NN, Chaudhari SP, Shinde SV, Khan W, AR. P. Isolation, antibiogram and pathogenicity of Salmonella spp. recovered from slaughtered food animals in Nagpur region of Central India. *Veterinary World, EISSN*. 2016;9(2):176-81.
90. ANaheed A, Ram PK, Brooks WA, Hossain MA, Parsons MB, KA T. Burden of typhoid and paratyp hoid fever in a densely popula ted urban community , Dhaka, Bangl adesh. *Elsevier Ltd*. 2009.
91. Endrias Z, C. P. Antimicrobial resistance pattern of Salmonella serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Trop Anim Health Prod*. 2009;41.

10. ANNEXES

Annex I: Participant Information sheet

My name is …….... I am working as a data collector for the study being conducted in this community by Dawit Wakwoya who is studying for his Master's degree at Jimma University, Institute of Health and Medical Science. I kindly request you to lend me your attention to explain you about the study and being selected as the study participant.

The study/project Title: Evaluation for immunochromatographic test method for rapid diagnosis of typhoid fever, Jimma, Ethiopia 2017.

Purpose/aim of the study: The findings of this study can be of a paramount importance for Jimma University for improvement in the treatment and follow-up of the patients. Most typhoid fever is caused by infections with tiny organisms called *Salmonella.* The main purpose of this study is to find an easy way to identify some of these organisms that cause typhoid fever. If we can identify the cause of the patients earlier, we can choose the best medication to treat the infection. The results will be used to improve the management and follow-up of other cases thereby decreasing the transmission of *Salmonella* in the community general. Moreover, the aim of this study is to write a thesis as a partial requirement for the fulfillment of a Master's Program in Medical Microbiology for the principal investigator.

Procedure and duration: I will be interviewing you using a questionnaire to provide me with pertinent data that is helpful for the study. There are 20 questions to answer where I will fill the questionnaire by interviewing you. The interview will take about 20 minutes, so I kindly request you to spare me this time for the interview. After the interview you will give blood, stool, and urine specimens for laboratory examination that should be collected using a sterile and disposable syringe for blood, plastic stool and urine screw cup container. Giving these samples doesn't have any harm to your health and any other aspects, rather you will be benefited that is, if there is a positive finding in laboratory examination, we will communicate with physicians to provide treatment.

Risks and benefits: The risk of participating in this study is very minimal, but only taking few minutes from your time. There would not be any direct payment for participating in this study. But the findings from this research may reveal important information for the University.

Confidentiality: The information you will provide us will be confidential. There will be no information that will identify you in particular. The findings of the study will be general for the study community and will not reflect anything particular of individual persons. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the research.

Rights: Participation of this study is fully voluntary. You have the right to declare to participate or not in this study. If you decide to participate, you have the right to withdraw from the study at any time and this will not label you for any loss of benefits which you otherwise are entitled. You do not have to answer any question that you do not want to answer.

Contact address: If there are any questions or enquiries any time about the study or the procedures, please contact: Dawit Wakwoya, Mobile No 0911533083, Jimma University, faculty of health and medical science, department of medical laboratory technology. E-mail:-d_wakoy@yahoo.com. Jimma University Institute of Health Department of Medical Laboratory Sciences.

Office No:_____P.O.Box 235, Jimma.

Declaration of informed voluntary consent: I have read/was read to me the participant information sheet. I have clearly understood the purpose of the research, the procedures, the risks and benefits, issues of confidentiality, the rights of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to withdraw from the study at any time or not to answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my initials (signature) as indicated below.

Signature of participant: _____ Signature of data collector: _____

HIIKA AFAAN OROMOTIIN

Unka waligalte hirmaattotaa ibsu

Maqaan kiya_____-jedhama.

Yuuniversiti Jimma dame saayinsiifayyaatti barataa digirii lammattaa kan ta'e Daawit Waaqwayyaa qo'annoo gaggeessaa jirtuufodeeffanoo/data funaanaan jira. Kaayyoo qo'annoo kanaa fi haala hirmaanmaa kee siifan akkan ibsu naaf hayyami.

Mata duree qo'annichaa: Sakkatiinsa organizimoonni Salmoonnellaa jedhamu meshalee sakatiinsa adda addaaf tooftaa adda addaa fayyadamuun waldorgomsiisuu kan firii/bu'aa gaarii agarsiisu/ kennu fii kan daawaa dandamatan argamuu isaanii qorachuufi.

Kaayyoo qo'annichaa: Caalmaatti kayyoon qo'annichaa dhukkbsatoota Hospiitaala universiitii jimmaa dhibamanii dhufan irratti *Salmoonnellaa* jedhamuun dhukkaba fiduu danda'u adda baasuuf akkasumas qorichaa isaaf sirriittiadda baasuuf. Waantaheefis qo'annichi rakkoo kana beekuun tarkaanfiin furmaataa akka taasifamu nigargaara. Kanarra darbeesqo'annichi barumsa digirii lammaaffa guutachuuf godhamuufis ni oola.

Tartiibaafi turtii qo'annichaa: Gaaffii fi deebiin nuti goonu kun gaaffii 20 of keessaa kan qabu. daqiiqaa 20 ol kan hinfudhanneyeroo ta'u hundi isaa waraqaa irratti ni katabama. Kanaafun yeroo qabdanirra daqiiqaawwan kana murtanii akka nafaanaa taatanin sin gaafadha. Itti aansuunis qorannoo Laboratoriitiif kan oolu dhiiga, boolii fii fincaan xiqqoo meeshaa qulqullinnisaa eegame nuti siniif kenninuun nuuf fidda. Waan kana kennuu keessaniinis rakkoon isinirra gahu hin jiru. Qorannicha boodas yoo organizimoonni kun yoo argameef firiin isaa ogeesa siif ajajetti kennamu ni taasifama.

Faayidaa fi miidhaa qo'annichaa: Qo'annoo kanarratti hirmaachun keetiif faayidaan/kaffaltii ati argattu hin jiru. Qo'annichi erga dhumee booda organizimoonni kun irratti ergamaniif bu'aan isaanii ogeessa fayyaa isa ajajeetti deebi'ee yaalii akka argatan ni taasifina.

Icciitii hirmaattotaa: Odeeffannoon ati kennitu kun hundi icciitiidhaan kan qabamu yoota'u. qaamarrattis ta'e sammunkeerratti miidhan dhufa hin jiru. Firiin qo'annichaas dimshaashani.

Icciitii hirmaattotaa: Odeeffannoon ati kennitu kun hundi icciitiidhaan kan qabamu yoota'u, qaamarrattis ta'e sammunkeerratti miidhan dhufa hin jiru. Firiin qo'annichaas dimshaashatti, dhimmi dhunfaa kana waliin walitti dhufeenya hin qabu.Waraqaan gaaffif deebiis ta'e dhigaa, boolii fii fincaanii koodiidhan kan adda baafamu malee magaadhaan miti.

Mirga hirmattotaa: Qo'annicha kanarratti hirmachuus dhiisuufis mirga qabda. Hirmachuu erga eegaltee boodas yeroo barbaaddettis adda muruu ni dandeessa. Sababa kanaafis miidhan sirra gahu yookaan faayidaan ati dhabdus hinjiru. Gaaffii siif dhihaatu keessaas kan hin feenee deebisuu dhiisuu ni dandeessa.

Teessoo: Maqaa nama qorannoo gageessuu: Daawit Waaqwayyaa, Mobayila: 0911533083

Dhaabbata irraa Dhufte: Yunivarsitii jimmaa, Lakk. Bilb: _____
Lakk.S.Posta:396, JIMMAA

Unka waligalte hirmattootaa mirkanneessu: Ani waraqaa walii galtee kana dubbisee (naaf dubbifamee) sirriitti hubadheera. Kaayyooqo'annichaa, tartiibaa fi tartiisaa, faayidaa fi miidhaasaa, icciitii hirmaataa mirga Hirmattotaa,yoon gaaffii qabadheef teessoo nama qo'annichaa gageessuuakkasumas qo'annicha keessa yeroon fedhetti bahuu akkan danda'u hubadheera. Kana hunda beekees Mallattoo kiyyaan nan mirkaneessa.

Mallattoo Hirmaataa ----------

Mallattoo nama oddeffanoo/data funaanuu----------

Annex 2: Consent Form

JUMC, SCHOOL OF MEDICAL LABORATORY SCIENCES 2017

Consent Form for Febrile Patients who suspected for Typhoid Fever

Participant code Number _____

I am informed fully in the language I understand about the aim of this research. I understood the purpose of the study entitled with "A comparative study of rapid test strips and blood and stool culture in the diagnosis of typhoid fever in febrile patients attending Jimma University Medical Center (JUMC), Jimma Ethiopia 2017. I have informed this study which involves collecting blood, and stool specimen. During collection of specimen I have been told that there is no harm and I have also read the information sheet or it has read to me. In addition, I have informed that all information is kept confidential. I understood that I have a full wright not to participate in the study and no punishment for that. I_____, after being fully informed about the detail of this study, hereby give my consent to participate in this study and approve my agreement with my signature.

Patient Name _____sig _____Date _____

Investigator Name _____sig_____ Date_____

Annex 3: Questionnaire for Sociodemographic Data

Collage of Health Science

Master of Medical Microbiology Program

This questionnaire is to be administered to those febrile patients attending Jimma University Medical Center where the study was conducted.

Eligibility questioner

1	In the last 2 weeks, did you have any of the following sign and symptoms? 1. Fever 2. Diarrhea 3. Nausea 4. Vomiting 5. Abdominal pain	
2	Are you taking antibiotic drug?	1. Yes 2. No
3	If yes for the above question, when?	_____ _____

Patient's name_____

Patient ID_____

Card No_____

Date _____

S.No		Response

1	Sex	1. Male 2. Female
2	Age	_____
3	Occupation	1. Employee 4. Merchant 2. Farmer 5. Daily laborers 3. Student 6. Other
4	Educational background	1. Illiterate 3. High school 2. Elementary 4. College & above
5	Residence	1. Urban 2. Rural
6	Is there domestic animal in the house	1. Yes 2. No
7	If yes, which domestic animals?	1. Poultry 3. Cattle 2. Goat 4. Sheep
8	Annual Income (ETB)	
9	Presence of fever	1. Yes 2. No
10	Did the patient take medication in the past 7 days	1. Yes 2. No
11	Presence of abdominal cramps	1. Yes 2. No
12	Presence of vomiting	1. Yes 2. No
13	Sources of water used for drinking	1. Tap water 3 Pond water 2. Stream water 4. Well water
14	Latrine usage	1. Yes 2. No

15	Do you wash your hands before meal?	1. Yes 2. No
16	Do you cook vegetables before eating?	1. Yes 2. No
17	Do you cook/boil animal product before eating?	1. Yes 2. No
18	Do you often use or buy drug or antibiotic without prescription of medical doctor?	1. Yes 2. No
19	Sources of drug	1. From Drug vender 2. One left from other 3. Other sources
20	Who did/do you consult to buy and use them?	1. Advice from drug seller 2. Decided by yourself 3. Physician

ጅማዩንቬርስት

<u>የጤናሳይንስእንስቲትዩት</u>

ለድህራምራቀማሚያጤናለይየሚሠራመረጃመሰባሰቢያ

የተከሚውስም_____

መለያቁጥር_____

1	በሁለትሰምንትውስጥየትኞቹምልክቶችተይ ቶቦውተል? 1.ትኩሰት 2.ተቅማጥ 3.መቅለሽለሽ 4.ማስመላስ	

	5.የሆድቁርጣት	
2	መድሀንትወስዳውያውቃሉ?	1.አዎን 2.አይ
3	ከሆመቼ?	_____ _____

የተከሚዉስም_____

የበሽተኛውመለያቁጥር_____

የከርድቁጥር_____

Ken_____

ቁጥር		መልስ
1	ጾታ?	1.ወንድ 2.ሴት
2	እድሜ?	_____
3	ሥራ?	1.ተቀጣሪ 2.ጋበሬ 3.ተማር 4.ናጋዴ 5.ቀንሰራተኛ 6.ሌለምካለ
4	የት/ትዳረጃ?	1. ያልተማረ 2. 1ኛ/ዳረጃ 3. 2ኛ/ዳረጃ 4. ዩኒቨርሲት5. ሌለምካለ

5	የማኖርያአከበቢ?	1.ከተማ 2.ገጠር
6	የቤትእንስሳትበቤትውስጥስለማኖራቸው?	1.አሉ 2.ያሉም
7	ቤትውስጥያሉየእንስሳትዓይነቶች?	1.ዶሮ 2.ፍየል 3.ከብቶች 4.በጎች
8	የወርገቢ?	------
9	ትኩሰትአለቦዊት?	1.አዎ 2.አይ
10	ባዚህሳባትቀንውስጥተከመውያውቀሉ?	1.አዎ 2.አይ
11	ያስመልስዋታል?	1.አዎ 2.አይ
12	የሆድቁርጠትአለብቸ?	1.አዎ 2.አይ
13	የምጣጡትውሃምንድንነው?	1.የቧንቧውሃ2.የምንጭውሃ 3.የኩሬውሃ 4.የጉርጓድውሃ
14	ሽንትቤትይጠቀማሉ?	1.አዎ 2.አይ
15	ከመመገቦትበፍትእጅዎንይታጠበሉ?	1.አዎ 2.አይ
16	አትክልትከመመገቦበፍትያበስላሉ?	1.አዎ 2.አይ
17	የእንስሰትተዋዕጾአብስለውይጠቀማሉ?	1.አዎ 2.አይ
18	መድህኒትየምገዙትበህክምትዕዘዝወይስ?	1.አዎ 2.አይ

19	መድሀኒትየሚገዙትኬየትነው?	1.ከፋርማሴ 2.ከሌለየተረፋ 3.ከሌለም
20	የመደህንትአወሳሰደንማንነየመከራሉ?	1.ድረግስት 2.በራሴ 3.በነሮቤት

UNKA GAAFFII FI DEEBII (Afaan Oromootiin)

Unki kun dhukkubsatoota Hospiitaala Unversiitii Jimmaati walla'anamuuf dhufaiif qofa.

GAAFFII ULAGAA QO'ANNICHAAF GAHAA TAHUU MIRKANEESSU

Lakkofsa	Gaaffileewangaafatama	Deebii
1	Torbanlamaandabran kana keessattidhukkubbin? 1. QaamaHo'isaa 2. garaa-hammachu 3. garaakaasuu 4. ooldeebisuu	
2	Dawaafudhachuuttijirtaa?	1. Eyyee 2. Lakkii
3	Eyyeeyoota;eyoom?	

GAAFFII WARRA ULAGAA GUUTANIIF DHIHAATU.

Maqaa_____

Lakkoobsadhukkubsataa._____ Lakkoobsakaardii_____ Guyyaa _____//_____//_____		
Lakkoofsa	Gaaffileewangaafataman	Deebii
1	Saala	1. Dhira 2. Dhalaa
2	Umuriikee/keesan?	
3	GaheeHojii?	1. Mindeffamaa 2. Qonnanbulaa 3. Barataa 4. Daldalaa 5. Kanbiraa
4	Sadarkaabarnoota?	1. Kanhibaratin 2. Sadarkaagadii 3. Giddugaleesaa 4. Unversiitiifisaaol 5. Kanaaalatti
5	Teessuma ?	1. Magaalaa 2. Baadiyyaa
6	Beeladin mana keesajiruu?	1. Eyyee 2. Lakkii
7	Yoojiraatanmeemaalfaa?	1. Hadaaqoofaa 2. Saawa 3. Re'ee 4. Hoolaa
8	Galiinji'aa ?	
9	Qaamaisinho'isaajiraa ?	1. Eyyee 2. lakkii
10	Turban darban kana keessatti mana	1. Eyyee

	yaalaadeemtanii?	2. Lakkii
11	Garaakeesanisinhammeesaa?	1. Eyyee 2. Lakkii
12	Olisindeebsaa?	1. Eyyee 2. Lakkii
13	BishaanDhugaatiiisakamiinfayyadamtu?	1. Bishaanboombaa 2. Burqitubishaanii 3. Kuufamabishaanii 4. Bishaanboollaa
14	Fayyadama mana fincaanii?	1. Eyyee 2. Lakkii
15	Nyachuu dura harkanidhiqattaa /tuu?	1. Eyyee 2. Lakkii
16	Nyachuu dura kuduraafiimuduraaniaffeeltuu/ bisheesituu?	1. Eyyee 2. Lakkii
17	Nyatamaddiisaaniibeeladairraata'enyachuun dura bisheestaniinyaatuu?	1. Eyyee 2. Lakki
18	Ajajaogeessaamaleedawaanibittuu/liqmsituu?	1. Eyyee 2. Lakki
19	Dawaaeessaabitattuu?	1. Mana dukkanaaqorchaa 2. Hafteekanbiraairraa 3. Bakkabiraa
20	Gorsaenyutiinbittaniifayyadamtuu?	1. Ogeessafayyaa 2. Ofumakootii 3. Ollaairraa

Annex 4: Laboratory Procedures

Annex 4.1 Culture and Identification

Specimen Collection

A) Procedure of Blood Sample for culture

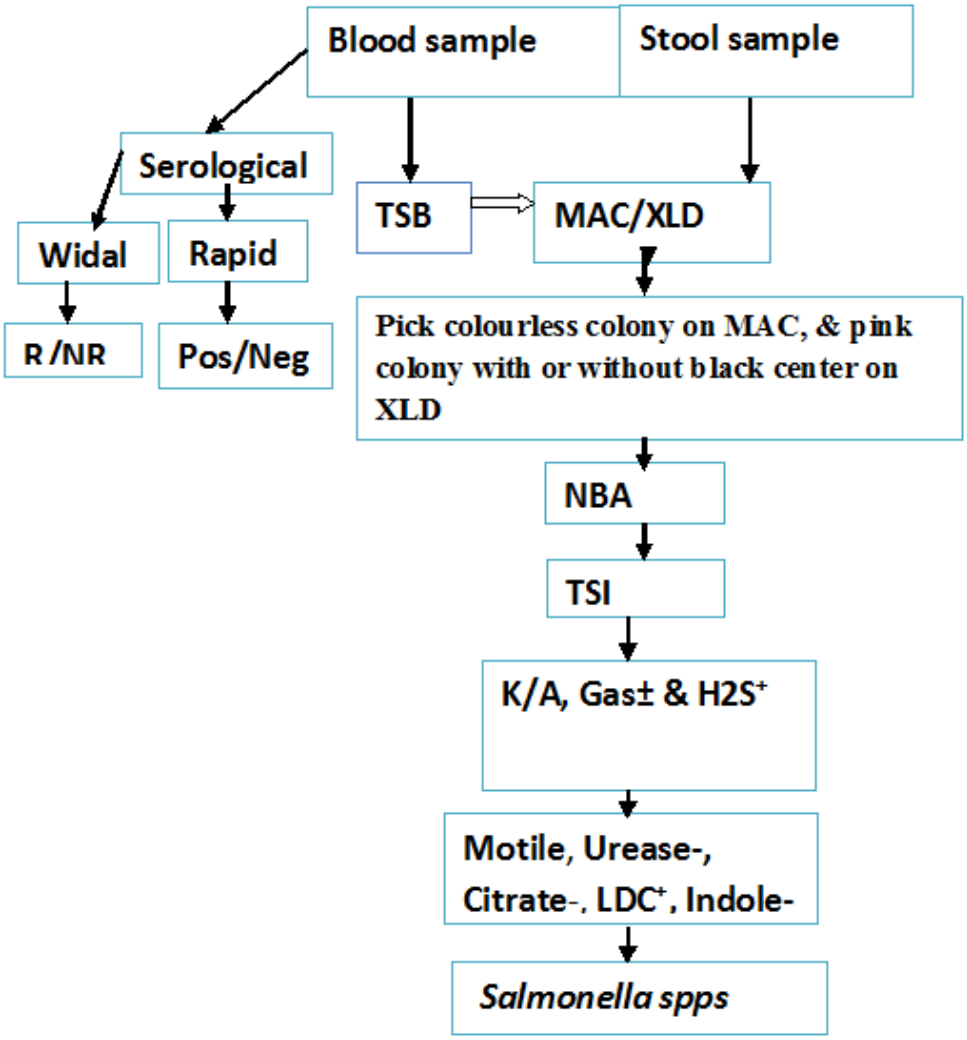
1. Collect under aseptic precautions, 5ml from children and 10ml from adults respectively transfer and inoculated into Tryptic Soy Broth (TSB).
2. Blood culture bottles were incubated aerobically at 37^{o}C at least for 24 hours.
3. If growth observed, Sub-cultures were made on XLD and MacConkey agar and incubated at 37^{0}C for 18 – 24 hours on the same selective.
4. Observe the Medias for the growth of specific colony characteristics.

    - ✓ On MacConkey agar, *Salmonella* produces lactose non-fermenting smooth colonies, while On XLD *Salmonella* appears as red colony with black center)

B) Procedure for Stool Sample

1) Instructing the patient or parents or the guardian to collect stool sample the specimen should contain at least 5g of feces.
2) Once the specimen has been placed in the specimen container, the lid should be sealed.
3) Stool was transported to Jimma University microbiology research lab for analysis.
4) Prepare a faecal suspension by suspending approximately 1g of the stool sample in a tube containing 1 ml of sterile saline. If the stool sample is liquid, saline does not need to be added.
5) Inoculate on MAC and XLD and add three or more loops full of faecal suspension to the enrichment broth (selenite F broth for 18hrs).
6) Subculture selenite F broth on selective plating media (MAC, XLD).
7) Incubate the plates for the isolation of *Salmonella* at 37 ^{o}C for 24 hrs.

8) Pick *Salmonella* suspected colony and subculture on non-selective media (Nutrient agar)
9) Identified and characterized by standard biochemical testing flow chart



Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.668	.064	11.221	.000
N of Valid Cases		275			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.371	.067	6.609	.000
N of Valid Cases		275			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Annex 4.2 Biochemical Test

ID	TSI	H2S	Gas	Motility	Urease	Citrate	Indole	Oxidase	lactose	LDC	strain
1	k/A	**(+)**	-	+	-	-	-	-	-	+	*S. typhi*
2	k/A	-	+	+	-	-	-	-	-	-	*S.paratyphi A*
3	k/A	+	+	+	-	+	-	-	-	+	*Other Salm.sp.*

For KIA: K = alkaline (red); A = acid (yellow); G = gas production; + = black H2S produced (weak); – = no H2S

For LIA: K= alkaline (purple); A= acid (yellow); G = gas production; + = black H2S produced (weak) – = no H2S

Annex 4.3 Typhoid IgG/IgM Rapid Test-Cassette (Serum / Plasma)

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into lavender, blue or green top collection tube (containing EDTA) by vein puncture.

2. Separate the plasma by centrifugation.

3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube without anticoagulants by vein puncture.

2. Allow the blood to clot.

3. Separate the serum by centrifugation.

4. Carefully withdraw the serum into a new pre-labeled tube.

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix\the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device

on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into the

Sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

NB: Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

Annex 4.4 Tube Agglutination for Widal Test

1. Take 2 sets of 8 Kahn tubes/test tubes and label them 1 to 8 for O and H antibody detection.

2. Pipette into the tube No.1 of all sets 1.9 ml of isotonic saline.

3. To each of the remaining tubes (2 to 8) add 1.0 ml of isotonic saline.

4. To the tube No.1 tube in each row add 0.1 ml of the serum sample to be tested and mix well.

5. Transfer 1.0 ml of the diluted serum from tube no.1 to tube no.2 and mix well.

6. Transfer 1.0 ml of the diluted sample from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7 in each set.

7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.

8. Tube No.8 in all the sets, serves as a saline control. Now the dilution of the serum sample achieved in each set is as follows: Tube No.: 1 2 3 4 5 6 7 8 (control) Dilutions 1:20 1:40 1:80 1:160 1:320 1:640 1:1280.

9. To all the tubes (1 to 8) of each set add one drop of the respective WIDALTEST antigen suspension (O & H) from the reagent vials and mix well.

10. Cover the tubes and incubate at 37° C overnight (approximately 18 hours).

Dislodge the sedimented button gently and observe for agglutination

Annex 4.5 Antibiotic Susceptibility Test

1. Pick Colony on TSI and make suspension in nutrient broth or normal saline

2. Check turbidity against 0.5 McFarland standard

3. Streak on MHA by sterile swab to get confluent growth

4. After drying it place antibiotic disk carefully

5. Incubate at 37oc for 24 hrs.

6. Look for inhibition zone around the disk after overnight incubation period

7. Measure the diameter using straight ruler

8. Report as resistant or sensitive according to EUCAST/CLSI guideline.

Drug Susceptibility

Salmonella Isolate	CHLOR	AMPI	CIPRO	CEF	CEFOT	NAL	COTRI	NORF

NB: CHLOR = Chloramphenicol, AMPI = Ampicillin, CIPRO = Ciprofloxacin,

CEF = Ceftriaxone, CEFOT = Cefotaxime, NAL = Nalidixic Acid

COTRI = Cotrimoxazole, NORF = Norfloxacin

Annex 5 Evaluation of clinical value of serologic tests against culture

Test		**Culture**				
		Positive	Negative	Total	PPV	NPV
Test to be evaluated	Positive	TP	FP	TP + FP	TP/TP+FP	
	Negative	FN	TN	TN +FN		TN/TN+FN
Total		TP +FN	TN +FP	TP +FN+TN+FP		

Declaration

I, the undersigned, declare and affirm that Evaluation of Alternative Rapid Test for Diagnosis of Typhoid Fever in Resource Limited area, Jimma, south west Ethiopia is my work. I have followed all ethical principles in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced necessary sources I have used in this document.

Name: Dawit Wakwoya Fufa

Signature: _____

Date: July 24, 2018

School/Department: _____

Examiner:

Dr.Getnet Beyene

Signature: _____Date_____