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Diagnosis and Treatment of Typhoid Fever and Associated Prevailing Drug Resistance in Northern Ethiopia

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ABSTRACT

Objective: To determine diagnostic value of the Widal test, treatment pattern of febrile patients and antimicrobial drug susceptibility pattern of blood isolates.
Methods: Using cross sectional methods, blood samples were collected for culture and Widal test from 502 febrile outpatients attending Mekelle hospital and Mekelle health center with similar symptoms to typhoid. Sensitivity, specificity for anti-TH and anti-TO titers using culture confirmed typhoid fever cases, and Kappa agreement between Titer and slide Widal tests were calculated. Treatment pattern of patients and antimicrobial susceptibility pattern of the blood isolates was assessed.
Results: From the 502 febrile patients, 8(1.6%) of them had culture-proven typhoid fever. However, patients who have results indicative of recent infection by O and H antigens of the Widal slide agglutination test were 343 (68.5%), with specificity and sensitivity of 33% and 100%, respectively. Over prescription of antibiotics was seen by Widal slide test for Ciprofloxacin 268 (76.1%), Amoxicillin-Clavulanic acid 9(2.6%), Amoxicillin 8(2.4%) and Chloranphenicol 8(2.4%). Tube titer positivity was seen in 23(5.3%) patients with 75% sensitivity and 95.8% specificity. Widal slide and Tube titer tests showed poor agreement for both antigens (kappa=0.02 for O) and (Kappa=0.09 for H). A single anti-TH titer of $\geq$ 1:160 and anti-TO titer $\geq$ 1:80 higher in our study showed an indication for typhoid fever infection. Drug resistance pattern of blood isolates ranges from 0-89.7% for gram positive and 0-100% for Gram negative, with an overall multi-drug resistance rate of 61.7%.
Conclusion: Patients were wrongly diagnosed and treated for typhoid fever by Widal test. The tube titration method was relatively good but still had poor sensitivity. Blood isolates showed multi drug resistance, which may be due to the indiscriminate prescription as seen in this study. Based on our results, the slide Widal test is not helpful in the diagnosis of typhoid, hence other tests with rapid, feasible, better sensitivity and specificity are urgently needed in Ethiopia.

1. Introduction

Typhoid (enteric) fever is an important health problem.[1] Reports by the World Health Organization revealed that about 21 million cases and >600,000 annual deaths from typhoid fever occur throughout the world. Developing nations share the highest burden due to rapid population growth, increased urbanization, and limited safe water and health systems.[1,2]

Serotype Typhi isolation from blood, bone morrow, urine or stool is the most reliable way of confirming typhoid infection. Yet, this requires laboratory equipment and technical training that are not feasible for most primary health care facilities in developing world.[3–5] Thus, most typhoid infections are diagnosed on clinical grounds and treated presumptively. But as its clinical symptoms are similar with many other bacteria, it may lead patients to receive unnecessary and inappropriate antimicrobial treatment.[6]

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In many developing countries, Widal test, which was first introduced by F. Widal in 1896, is widely used in the diagnosis of typhoid fever. This is because it is relatively cheaper, easy to perform and requires minimal training and low sophisticated equipment.[7,8] This test depends on agglutination reaction between *S. typhi* somatic Lipopolysaccharides O antigen (TO) and flagellar H antigen (TH), where these antigens are shared by many other Enterobacteriaceae; for this reason, its test valueshas been debated for many years.[9,10]

In addition, interpretation of results has also been a problem as different cut-offs points have been reported from different places.[11] Moreover; patient treatment cannot wait for results obtained with convalescent phase samples. Hence, the treatment decision is made on the basis of the results obtained with a single acute-phase sample.[12]

Due to the low prevalence of typhoid, access to safe drinking water, better laboratory facilities to isolate the bacteria, and the low sensitivity and specificity of the Widal test, the test is no longer used as a diagnostic assay in developed nations[13], but it is the commonest test in developing countries. Widal test recommends a slide test to be used for screening only and positive results to be confirmed by tube titration method; however, since titer results take 18-24 hours, diagnosis is practically done on the basis of the slide agglutination results, which are available within minutes. However, this may lead to false diagnosis of typhoid, unnecessary antibiotic therapy, and emergence of drug resistant strains.[2,14]

In Ethiopia diagnosis and treatment of typhoid fever is by Widal test (slide agglutination); however, except for a single study done in Addis Ababa which compared the Widal test with blood culture[2], we could not find published data that evaluate test validity of the Widal test. Again the study did not address the treatment pattern of typhoid-suspected patients and the antimicrobial drug susceptibility pattern of the isolates; it was also done in a small sample size (230 patients) in a different study area in Addis Ababa, 787 km from our study area. The present study was therefore designed to address the diagnosis and treatment of Typhoid fever and the associated prevailing drug resistance pattern in Northern Ethiopia using a standard blood culture method.

2. Materials and Methods

2.1. Study design and specimen collection

Cross sectional study was conducted in Mekelle hospital (MH) and Mekelle health center (MHC) from May to December 2014 consecutively upon receiving informed consent among febrile patients suspected for typhoid fever. Study areas are located 787 km North of Addis Ababa, the capital city of Ethiopia. Five hundred and two venous blood samples, 8-10 ml from adults and 3-5 ml from children were collected aseptically using 70% alcohol and 2% tincture of iodine. Then, 5-7 ml from adults and 2-3 ml of blood from children was dispensed into a sterile bottle containing 45 ml of Tryptic soy broth culture medium (BBL^{TM} USA), mixed with the broth, and incubated at 37 °C, and the remainder was used for the Widal test. Participants already on antibiotic treatment and those who were diagnosed for other known febrile illness were excluded from the study.

2.2. Isolation and identification of bacteria

Incubated blood samples were checked for signs of bacterial growth (haemolysis, turbidity, and clot formation) daily up to 7 days. Bottles that showed signs of growth were further processed by Gram stain and subculturing onto Blood agar, MacConkey agar, and Manitol salt agar (all Oxoid, UK) and incubated at 37 °C for 24 hours. Blood culture broth with no bacterial growth after 7 days was sub-cultured before being reported as a negative result. Identification of isolates was done by colony morphology, Gram staining, Catalase test, Coagulase test, and biochemical tests using Triple Sugar Iron agar (TSI) (OXOID, UK), Citrate utilization test (BBL^{TM} USA), Urease test (BBL^{TM} USA) and Lysine motility indole test (LDC) [BBL^{TM} USA] using the standard bacteriological methods.[15]

2.3. Antimicrobial Susceptibility tests

The disk diffusion assay method was used to determine the antibiotic resistance/susceptibility pattern of blood isolates on Muller-Hinton agar (Oxoid, England) against Amoxicillin-Clavulanic acid (30 μg) (Oxoid, UK), Ceftriazone (30 μg) BBL^{TM,USA}), Vancomycin (30 μg) (BBL^{TM,USA}), Ciprofloxacin (5 μg) (BBL^{TM,USA}), Gentamicin (120 μg) (BBL^{TM,USA}), Norfloxacin (10 μg) (OXOID,UK), Doxycycline (30 μg) (OXOID UK), Erythromycin (15 μg) (BBL^{TM} ^{USA}), Nitrofurantonin and Trimethoprim-Sulphamethoxazole (25 μg) [BBL^{TM,USA}]. The criteria used to select the antimicrobial agents tested were based on the availability and frequency of prescription for the management of bacterial infections in Ethiopia. To standardize the inoculum density for a susceptibility test, a BaSO4 turbidity standard, equivalent to a 0.5 McFarland standard was used by strictly following the SOP for the preparation and standardization.[15] Multidrug resistance was defined as resistance of an isolate to three or more of the antimicrobial agents tested.

2.4. Widal test

Qualitative slide and semi quantitative tube agglutination methods were done using febrile antigen kits of *Salmonella typhi* (Chromatest Febrile Antigens kits, linear chemicals, Spain). Slide agglutination Widal tests were done by laboratory professionals who were blind to the study and were based on the manufacturers guidelines, and results were given to doctors who requested the tests for patient management. Slide test reactive serums were transported to Ayder referral and teaching hospital microbiology laboratory and further tested by standard tube agglutination test (titration) method. According to the manufacturer's manual, serum samples were serially diluted using a fresh 0.95% saline preparation from 1:20 to 1:640 for anti TO and anti TH separately in 12 test tubes. An equal amount of O and H antigens were then added to all test tubes. Based on the manufacturer's manual, an antibody titer of $\geq$ 1:80 for anti TO and $\geq$ 1:160 for anti TH antibodies were taken as a cutoff value to indicate recent typhoid infection.

2.5. Antibiotics given by doctors for positive slide agglutination results

We reviewed the treatment patterns of patients based on their Widal slide test results and clinical grounds by doctors who did not know about the ongoing study. We used patients' charts to review the treatment profiles using the chart number of the patients and date visited in both health institutions from each patient's questioner.

A senior clinician who is member of the research team handled this task in each card room of the health institutions.

2.6. Data quality control and management

A standard bacteriological procedure was followed to maintain the quality of all laboratory tests. American Type Culture Collection (ATCC) strains *S. aureus* (ATCC 25923); *S. Typhi* (ATCC 13311) and *E.coli* (ATCC 25922) were used as positive controls for culture and sensitivity testing. Negative control was performed by randomly taking prepared culture media and incubating overnight to check

for any growth. Standard operational procedures were followed during processing of each sample, and all the instruments used for sample processing were checked every morning for proper functioning. SPSS software Version 20 was used for the analysis of the data. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for Widal test using culture confirmed typhoid fever. We also calculated the Kappa to determine the agreement between the slide and tube titration method.

2.7. Ethical issues

Ethical clearance was obtained from Research Ethical committee/IRB of the College of Health Sciences. Permission was also obtained from the General Hospital and health center administration. Data and samples were collected after written informed consent was obtained from each volunteer and guardian.

3. Results

From the total 502 febrile patients involved in the study, 269 (52.3%) were female and 245 (47.7%) male. Their age ranged from 1-81 years (mean 27.97 ± 1.59 [SD]). Two hundred seventy two (54.2%) of the participants were in the age range of 15-32 years.

3.1. Qualitative Slide agglutination Widal test

Three hundred forty three (68.3%) patients were reactive for both O and H antigens, while 34 (6.8%) were reactive only for O antigen. One hundred twenty three (24.5%) patients showed no reaction result for both antigens. Overall 376 (74.9%) patients have a reactive slide agglutination Widal test by either or both O and H antigens (Table 1).

From the total 343(68.3%) patients with reactive Widal slide agglutination test, only 8 (1.6%) patients had culture proven *S. typhi* in their blood, while the remaining 66.7% were treated wrongly as typhoid fever (Fig. 1).

The Widal slide agglutination test in our study showed that both O and H antigens had 100% sensitivity and 100% negative predictive value, but showed low positive predictive value of 2.7% and 4.6% for O and H antigens respectively. Poor specificity was seen in O antigen and H antigen, 33% and 35.4% respectively.

3.2. Semi quantitative tube agglutination test (titration)

Serum samples with reactive slide agglutination test results were further analysed by standard tube Titration method. Two hundred (52. %) and 111(34.1%) of the slide reactive patients showed reaction for anti TO and anti TH antibody respectively (Table 2).

Table 1
Qualitative Slide agglutination results of Widal test among febrile patients suspected of typhoid fever in Mekelle hospital and Mekelle heath center, May-December, 2014.

Widal slide agglutination results	Frequency	Percent
Both O and H antigens reactive	343	68.3
Only O antigens reactive	34	6.8
Only H antigens reactive	8	1.6
Non reactive for O and H antigens	126	25.1
Total	502	100

Taking antibody Titer of ≥1:80 for O and ≥1:160 for H antigens as cutoff values to indicate recent typhoid infection (positive titer), 25 (4.9%) and 15 (2.9%) patients had results indicative of recent typhoid infection by O and H antigens, respectively. The total number of patients who had results indicative of recent infection by either or both O and H antigens was 23 (5.3%).

In our study we have calculated a statistical method to show agreement between slide agglutination and standard tube titer tests and we found very poor agreement for both antigens (kappa=0.02 for O) and (Kappa=0.06 for H).

3.3. Blood culture results

Out of 502 blood cultures, there were 8(1.6%) *S.typhi* isolates, and 107(21.3%) other non-Salmonella pathogenic bacteria. No bacteria growth was seen from blood cultures of 387 (77.1%). Non-Salmonella species included: *Staphylococcus aureus* (n=41), Coagulase negative *Staphylococcus* (n=39), *Escherichia coli* (n=12), Citrobacter species (n=9), *S.pyogen* (n=6), Pseudomonas spp (n=2) and Klebsiella spp (n=3).

Anti TO agglutination titer of 1:80 and higher were detected among 7 (87.5%) of the culture confirmed typhoid cases by *S.typhi* as compared with 12 (9%) by other non salmonella bacterial species. But positive titer for TH was seen in 6 (75%) and other non salmonella bacteraemia 8 (6%). The specificity and NPV of both antigens was high but the PPV which is important measurement in the diagnosis of the disease was very low (28% and 33.3% for TO and TH, respectively) (Table 3).

In this current study, the overall Widal Titer positive among culture confirmed patients of *S.typhi* was 6(1.2%), which all have positive titer of anti TH and one has also a positive titer for anti TO. Thus the sensitivity, specificity, PPV and NPV of the overall positive titer was 75%, 95.9%, 22.2% and 99.6%, respectively (Fig. 2).

Patients' charts of Widal slide test positive results showed that the following antibiotics were over prescribed: Ciprofloxacin 268 (76.1%), Amoxicillin- Clavulanic acid 9(2.6%), Amoxicillin 8(2.4%), Chloranphenicol 8(2.4%) and 24(4.7%) other antibiotics. Twenty four patients had no chart available during collection time; hence no information was obtained about their treatment.

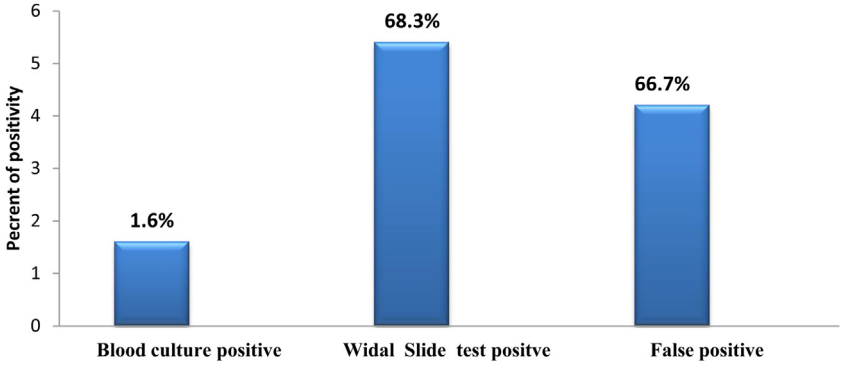

Fig. 1. Coparsion between blood culture and Widal slide test for typhoide diagnosis.

Table 2
Frequency of distribution of semi quantitative tube agglutination test in febrile patients suspected of typhoid fever, May-December, 2014.

Titer	O –Antigen			H-Antigen		
No agglutination	Frequency	% (n=377)	% (n=502)	Frequency	% (n=351)	% (n=502)
	137	36.3	27.3	99	23.7	19.3
1:20	144	38.2	28.7	129	36.8	25.7
1:40	63	16.7	12.6	91	25.9	18.2
1:80	12	3.2	2.4	17	3.5	3.4
1:160	15	4	3	8	2.3	1.7
1:320	0	0	0	5	1.4	1.2
1:640	0	0	0	2	0.7	0.4
Total	377	100	74	351	100	69.9

Table 3
The sensitivity, specificity, PPV, and NPV of titers of anti TO (≥1:80) and anti TH (≥1:160) Widal tests for diagnosis of typhoid among febrile patients in Mekelle hospital and Mekelle heath center, 2014

Measurement	O-Antigen (%)	H -Antigen (%)	Both antigens (%)
Sensitivity	87.5	62.5	75
Specificity	96.4	98	95.9
PPV	28	33.3	22.2
NPV	97.8	99.4	99.6

3.4. Antimicrobial susceptibility pattern of blood culture isolates

The In vitro antibiotic susceptibility pattern (Table 4) showed that gram positive bacteria resistance ranges from 0 to 89.7%. Thirty six (87.7%) *S. aureus* isolates were resistant to Trimethoprim-sulphamethoxazole, 34(82.9%) to Ceftriazone and 31(75.6%) to Doxycycline. In Thirty five (89.7%) and 25(64%) resistance was seen by CoNS to Trimethoprim-sulphamethoxazole and Doxycycline, respectively. Vancomycin resistant was seen in 22% *S.aureus* and 23% CoNS.

Over all, high resistance was seen by gram positive bacteria to Trimethoprim-sulphamethoxazole 89.7%, Doxycycline 75.6% and Ceftriazone 82.9%. Relatively, Amoxicillin-clavunilic acid and Vancomycin were effective against Gram positive isolates in our study.

Resistance levels of gram-negative organisms ranged from 0 to100%. *E. coli* showed high resistance to Ceftriazone 75% and Nitrofurantonin 66.7%. Isolated *S. typhi* were resistant to Nitrofurantonin 75%, Doxycycline 62.5% and Trimethoprim-sulphamethoxazole 50%. Overall gram genitive isolates showed high resistance to Nitrofurantonin75.9%, Trimethoprim-sulphamethoxazole 69%, Ceftriazone 62% and Doxycycline 55.6%. On the other hand, low level of resistance was seen by gram negative bacteria to Ciprofloxacin 14%, Gentamicin 28% and Amoxicillin-clavunilic acid 31% in this study.

Antibiogram drug resistance pattern showed that 65.9%, 68.9% and 50% of *S.aureus*, CoNS and *S.pyogen* showed multi drug resistance, respectively with an overall gram positive MDR rate of 66.3%. On the other hand, 50% MDR was seen for *E.coli*, Citrobacter spp and *S.typhi* with overall gram negative MRD rate of 44.8%. In general the multi drug resistance rate of in this study was seen in 71(61.7%) of the isolates (Table 5).

4. Discussion

Though definitive diagnosis of typhoid is by isolation of the bacteria from blood, bone morrow or other body fluids, most developing nations like Ethiopia due to limited access to laboratory facilities, use the old Widal test[7,8,13]. In our current study 343 (68.3%) of the febrile patients showed positive slide Widal test. This test was found good as a screening test [p=0.002] with 100% sensitivity and negative predictive values. It was however, very low specificity for both antigens (33% for O and 35.4% for H. This result was similar with the study report from India.[14]

Since positive predictive value (PPV) represents the proportion of patients with positive test results that are correctively diagnosed, it is considered as the most important clinical diagnosis method.[2,15] In our current result the PPV was very low for both antigens [2.7% for O and 3.02% for H]. Similar results were reported from the study finding of febrile patients from India.[14], which proves that slide test is good in screening out negative samples but not helpful in the diagnosis of the disease. This is the reason why previous studies have found it the test performing worst in the diagnosis and recommended that it should not be used for the diagnosis of the disease.[14,16]

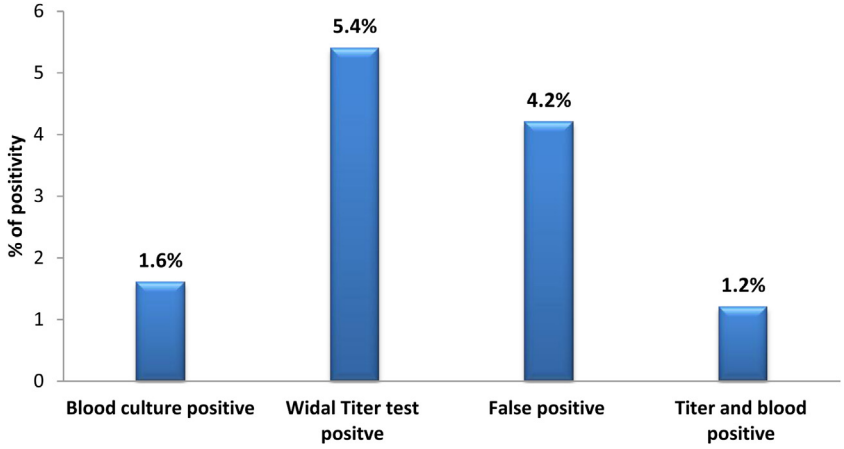

Fig. 2. Comparsion of blood culture and Widal titer tests.

Table 4
Antimicrobial resistance pattern of bacterial isolates of febrile patients attending Mekelle hospital and Mekelle heath center, May-Dec, 2014, No (%).

Antibiotics Tested	Bacteria isolated							
	S.aureus (n=41)	*CoNS* (n=39)	*S.pyogen* (n=6)	*E.coli* (n=12)	*Citrobacter* spp (n=6)	*S. typhi* (n=8)	*Klebsiella* spp (n=3)	*Total* (n=115)
AMC	7(17.1)	9(23)	2(33.3)	3(25)	2(33.3)	2(25)	2(75)	27(23.5)
CRO	34(82.9)	21(53.8)	1(16.7)	9(75)	4(66.6)	2(25)	3(100)	73(63.5)
CN	16(39)	7(18)	0	3(25)	2(33.3)	3 (37.5)	0	31(27)
Do	31(75.6)	25(64)	4(66.7)	6(50)	4(50)	5(62.5)	3(100)	78(67.8)
CIP	18(43)	10(25.6)	1(16.7)	1(8.3)	3(50)	1(12.5)	1(33.3)	35(30.4)
SXT	36(87.7)	35(89.7)	5(89.3)	10(83)	1(16.7)	4(50)	3(100)	94(81.7)
F	NA	NA	NA	8(66.7)	5(83.3)	6(75)	3(100)	22(19.1)
E	21(51)	19(48.7)	1(16.7)	NA	NA	NA	NA	41(35.7)
NOR	18(43)	14(36)	1(16.7)	5(41.7)	1(16.7)	3(37.5)	3(100)	45(39.1)
V	14(34)	9(23)	0	NA	NA	NA	NA	23(20)

CoNS=Coagulase negative *Staphylococci*, AMC=Amoxicillin-clavunilic acid, CRO=Ceftriazone, CN=Gentamicin, E=Erythromycin DO=Doxycycline, CIP=Ciprofloxacin, SXT=Trimethoprim-sulphamethoxazole, NOR=Norfloxacin, F=Nitrofurantonin, V=vancomycin, NA=Not Applicable.

Table 5
Multiple drug resistance patterns of bacterial isolates from blood of febrile patients in Mekelle hospital and Mekelle heath center, May-December, 2014.

Bacterial isolates and their Antibiogram (Resistance pattern) No (%)								
	S.aureus (n=41)	*CoNS* (n=39)	*S. pyogen* (n=6)	*E.coli* (n=12)	*Citrobacter* spp (n=6)	*S.typhi* (n=8)	*Klebsiella* spp (n=3)	Total (115)
R_0	3(7.3)	5(9.1)	___	___	___	3(37.8)	1(33.3)	12(10.3)
R1	7(17.1)	4(9.1)	1(16.7)	4(.3)	1(16.7)	1(12.5)	___	18(15.7)
R2	4(9.8)	8(20.5)	2(33.3)	2(13.3)	1(16.7)	2(25)	2(75)	21(18.3)
R3	5 (12.2)	8(20.5)	1(16.7)	2(20)	1(16.7)	1(12.5)	___	18(15.7)
R4	5(12.2)	5(13.6)	1(16.7)	3(26.7)	2(33.3)	___	2(75)	18(15.7)
R5	4(9.8)	3(6.8)	1(16.7)	___	___	1(12.5)	1(33.3)	10(8.7)
R6	6(14.6)	5(9.1)	___	1(6.7)	___	___	___	12(10.3)
R7	4(9.8)	5(9.1)	___	___	___	___	___	9(7.8)
R8	3(7.3)	1(2.3)	___	___	___	___	___	4(3.5)
R9	___	___	___	___	___	___	___	___

CoNS - coagulase negative Staphylococci; R0 - sensitive to all antibiotics tested; R1, R2, R3, R4, R5, R6, R7, R8, R9, resistant to one, two, three, four, five, six, seven, eight, nine antibiotics, respectively.

We found 335(66.7%) febrile patients with Widal false positive and treated wrongly as typhoid fever while only 8(1.6%) patients were culture-proved to have typhoid fever. This high false positive rate may be due to cross reacting antibodies of other bacterial and non bacterial infections.[11] We also reviewed the treatments given for those slide positive patients by the clinicians from each patient chart and found that patients were give: Ciprofloxacin 268 (76.1%), Amoxicillin- Clavulanic acid 9(2.6%), Amoxicillin 8(2.4%), Chloranphenicol 8(2.4%) and 24(4.7%) other antibiotics. This incorrect treatment based on Widal slide test results and clinical round of patients may lead to unnecessary treatment costs and pressure the normal gut flora to develop drug resistance, and most importantly, highly fatal diseases of febrile patients such as malaria, non typhoidal salmonelasis, endocarditis and urinary tract infection may be missed, ultimately leading to bad patient outcomes.[5] Again we found that one of the reasons that may lead clinicians to the misdiagnosis of patients is the way Widal results are reported i.e. results should be reported as 0(no agglutination), +1 [25% agglutination], +2[for 50% agglutination], +3[for 75% agglutination] and +4[for 100% agglutinations)[11,13] rather than reactive or non reactive. This problem in reporting was also seen in our study areas and needs to be corrected.

The tube titration method was done for those patients whose serum was positive for slide Widal test, and from the 343 a positive titer ($\geq$1:80 for TO and $\geq$1:160 for TH) was seen among 23 (6.7%) with sensitivity and specificity of 75% and 95.8% respectively. Similar findings were reported by other researchers.[2,7,14] A study from Kenya has shown much lower sensitivity (26%) of Titer result.[17] This low sensitivity of Titer test could be due to variation in the blood collection time. Titer test showed very low PPV (22.2%) and high NPV (99.6%). Similar reports were seen from Egypt (5.7% of PPV and 98% of NPV)[18] and Ethiopia (98.9% NPV and 5.7% NPV).[2]

Though not as high as the slide Widal test, a significant number of patients were still reported falsely as positive (PPV=22.2%) by Tube titration methods, which could be due to cross reacting antibodies by infections other than typhoid fever.

In our study positive titer was found in 12 (9%) and 8 (6%) patients for TO and TH, respectively by other non salmonella species. This was clearly seen in a study conducted in Cameroon where out of the total in febrile patients clinically similar to typhoid, 45% were malaria cases and only 2.5% were true typhoid cases[4], proving that there are febrile infections that induces cross reacting antibodies with the somatic and flagellar antigens. In this study there were two culture confirmed cases of typhoid but had a negative titer. Possible reasons for this are early blood collection time before disease or inadequate bacterial inoculation to induce antibody production[11], and more importantly previous antibiotic treatment of patients even if no patients told us of taking any antibiotics during our study.

Slide agglutination and standard tube titration results were compared and results revealed that there was statistically poor agreement between both antigens (kappa=0.02 for O) and (Kappa=0.09 for H), similar to the study conducted in India with poor agreements between the tests[14], but in contrast to this, fair agreement results were reported from other areas[2,19].

The antimicrobial susceptibility pattern of blood isolates was determined for the commonly available and prescribed antibiotics. Overall the range of drug resistance pattern for gram positives was from 0% - 89.7% and from 0% -100% for gram negative bacteria, which is similar to the result from other part of Ethiopia, which was

0- 85.7% and 0% - 100% for gram negative and positive respectively.[20] This increased resistance in this study may be an indication of indiscriminate and continuous use of antibiotics as clearly seen in our study, where more patients (66.7%) were put on the wrong treatments.

Thirty six (87.7%) of the *S. aureus* isolates were resistant to Trimethoprim-sulphamethoxazole, 34(82.9%) to ceftriazone 31(75.6%) to doxycycline, 51% to erythromycin, 39% to Gentamicin. Resistance of Trimethoprim-sulphamethoxazole was comparable with reports from other parts of Ethiopia.[21,22] Our present study reveals lower resistance to vancomycin by *S.aureus* (22%) and CoNS (23%) than the study done on surgical wound infection from South Ethiopia, which was 100% and 65.2%, respectively.[22] The consequences of using ineffective drugs in rigorous bacterial infections could be devastating as this can complicate the management and increase morbidity and mortality.[21,23,24]. CoNS were mainly recognized as a contaminant until the 1970's, nevertheless, several studies have reported an increasing incidence of infections due to these bacteria[25,26]. This was similar to our current study. *E. coli* was 75% and 66.7% resistant to Ceftriazone and Nitrofurantonin respectively. Resistance to ceftriaxone by *E. coli* may be due to production of Amp C enzymes and BAL TEM genes (Beta-lactamse enzymes) which inhibit the Beta-lactam rings of cephalosporin.

S. typhi isolates were resistant to Doxycycline (62.5%), Trimethoprim-sulphamethoxazole (50%) and Nitrofurantonin (75%). Conversely, among the antibiotics used for susceptibility testing Amoxicillin-clavunilic acid and Ciprofloxacin were relatively effective for gram negative bacteria isolates. Even if Ciprofloxacin was prescribed right and left for almost all febrile slide-test-positive patients in this study area, it was effective for most gram negative bacteria isolates, which could be because of its broad spectrum, because it is new-generation and has not been used for long, because the patients visiting the hospitals during the study period were taking the antibiotic for the first time, and it could also be the isolates did not develop resistance. However, if this indiscriminate type of antibiotic prescribing continues and no rational use of antibiotics is implemented, it would not be long to miss these antibiotics as well.

A general overview of the anti biogram of all the bacterial isolates indicates that multi drug resistance was observed in *S.aureus* 65.9%, CoNS 68.9%, *S.pyogen* 50% Citrobacter spp 50% and *E .coli* 50% and *S.typhi* 50%. The overall multi drug resistance rate in our study was 71(61.7%). This suggests a high-resistance gene pool perhaps due to gross misuse and inappropriate usage of the antibacterial agents[27] which we came across in this study for the diagnosis of typhoid fever.

Amoxicillin clavulanic acid was found to be effective against both gram positive and gram negative isolates in this study. Unlike our current findings, other studies reported Ciprofloxacin is effective[28,29] for both gram positive and negative, but Ciprofloxacin was found to be effective against gram negative isolates here in our study, which is in line with findings that others reported.[21,23]

5. Conclusions

Prevalence of typhoid fever in the study area was low; however, due to the poor diagnostic value of the Widal test, patients were wrongly diagnosed and treated for typhoid fever. The tube titration method was relatively good but still had poor sensitivity. The test has scarce PPV value, specificity and correlation with serological testing tube. Therefore, the culture must be the reference test for diagnosis of typhoid fever. There is no doubt about the value of the presented study and its potential local impact. However, these data have been previously reported and the impact on other countries is very limited because these techniques are no longer used.

The antimicrobial drug susceptibility pattern of blood isolates showed high multi drug resistance to commonly used antibiotics which may be due to the indiscriminate prescription of the antibiotics as seen in this study. Hence it should be a call to health authorities for the establishment of programs for the appropriate use of antimicrobials to control the emergence of drug resistant bacterial strains. Based on our results, the Widal test is no longer important in the diagnosis of typhoid fever. Hence, other tests that are rapid, feasible, and have good sensitivity and specificity are urgently needed in Ethiopia.

Limitation of the study: We have used a single blood test due to the problem of patient recruitment for next time.

Competing interest: All authors declare that they have no competing interest.

Authors' contributions: Araya Gebreyesus was the principal researcher, conceived the study, designed and collect data, laboratory works, conducted data analyzed and drafted the manuscript for publication. Letemichale Negash involved in data collection, Laboratory works and data analysis. Senay Aregawi in collecting the antibiotics prescribed by clinicians to patients by their slide Widal test results from the two health institutions and reviewed the initial draft manuscript. Tsehaye Asmelash, Tadesse Dejenie, Abadi Luel conceived the study and contributed designing the study, Saravanan Muthupandian contributed designing the study, analysis and interpretation of data and reviewed the initial draft manuscript. All authors read and approved the final manuscript.

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