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Blood Culture and Widal Test as Diagnostic Tools for Enteric Fever with Antibiogram in a Tertiary Care Centre at Dhaka in Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Enteric fever still exists as one of the major public health issue occurring in our country. Antimicrobials are the mainstays of treatment of typhoid fever. Due to rapidly growing antibiotic resistance, *Salmonella spp.* is required to be periodically tested for susceptibility patterns. This will also enable planning of rational use of antibiotics.
Objective: To observe the diagnostic accuracy of enteric fever by blood culture and Widal test in a tertiary care center, at Savar, Dhaka; Bangladesh. Antimicrobial sensitivity pattern of this study will guide to modify recent changes in the trends of antimicrobial use at the local level.
Methodology: Blood samples were collected from 2194 febrile patients with clinically suspected enteric fever cases at a Tertiary Care Outdoor Centre from January 2017 to March 2020. Blood

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culture was performed to isolate *S. typhi* and *S. paratyphi*. Widal tests were done for the determination of antibody titer. An antibody titer of ≥1:80 for anti TO and anti TH were taken as a cut off value to indicate recent infection of typhoid fever. Antibiotic susceptibility testing was carried out using modified disk diffusion (Kirby–Bauer) technique.

Results: Out of 111 *Salmonella* isolates, 74 (5%) were *S. typhi* and 37 (25%) were *S. paratyphi A.* Total 697 samples were positive for Widal test. A large number of isolates showed resistance to commonly used antibiotics such as nalidixic acid (94.6%), chloramphenicol (87.3%), amoxicillin (76.6%), cephradine (53.1%), azithromycin (46.8) etc. Resistance to cefixime and cefipime, ceftriaxone tended to increase than past. Imipenem, moxifloxacin and cefuroxime are escalating resistance which is alarming.

Conclusion: Blood culture is the most reliable among the diagnostic methods but it needs 5 to 7 days for delivery of final report. This delay leads to late diagnosis as well as provision of irrational usage of antibiotics. It is concluded that widal test would remain relevant as a diagnostic tool for enteric fever, which is more convenient, cheaper and faster than the other molecular tests. Our study revealed the antibiotic susceptibility of *Salmonella* isolates will be recommended for addressing the drug resistance.

Keywords: Salmonella species; susceptibility pattern; widal test; titre; blood culture.

1. INTRODUCTION

Enteric fever is still prevalent as a common public health disease and one of the major causes of in South-East Asia. Nevertheless, it is endemic in low and middle-income countries, where provision of pure water supply, sanitation and standard hygiene practices are inadequate [1]. Exposure of the individual to contaminated food or water directly correlates with the pathogenesis of enteric fever [2]. It would be a fearful disease because of its associated complications if not detected and treated promptly. Current estimates suggest those approximately 14.3 million infections and more than 135,000 deaths are caused by enteric fever worldwide each year; mostly affecting children and young adults [3]. The case-fatality ratio (the proportion of people infected with *S. Typhi* who die as a result of infection) remains poorly characterized. The study revealed case-fatality ratio of 0.3% [95% confidence interval, 0.05%–0.55%] [4]. It is worth noting that accurate diagnosis of enteric fever at an early stage is imperative for clinical management. Moreover, the detection of convalescence and chronic fecal carriage that may serve as a potential carrier and the estimation of disease burden appear warranted. Blood culture is the single most important procedure to detect systemic infection due to bacteria [5]. It provides valuable information for the management of febrile, acutely ill patients. In addition to its diagnostic significance, isolation of an infectious agent from the blood provides invaluable aid in determining antimicrobial therapy [6].

In Dhaka Shishu Hospital, Bangladesh, a study reported that the overall positivity rate of enteric fever was 3.9% (2,516/64,762; range: 1–5.7% by year) [7]. The sensitivity of blood culture is highest in the first week of the illness and gets reduced with advancing illness [8]. The average prevalence rate of *Salmonella* in the blood was 9.15% was reported in a study carried out from 2008 to 2013 [9]. Serological tests, predominantly the Widal test, are available but show very low sensitivity and specificity, and no practical value in endemic areas despite their continued use [10]. DNA test specificity over other Gram-negative organisms due to their limited genetic diversity between *S. typhi* and *S. paratyphi*. Further genomic exploration of both *S. Typhi* and *S. Paratyphi A* will identify new and better targets and then lead to novel nucleic acid based tests [11]. Advancement in genomics studies will further our understanding of molecular pathogenesis of enteric fever, which could form the basis for new molecular diagnostics. With progress in new technologies, we expect that a new generation of fast and sensitive molecular diagnostics for enteric fever will be developed in the near future.

It is important to monitor the antibiotic susceptibility patterns to provide suitable guidance for the treatment. This resistance pattern is known from the results of antimicrobial susceptibility testing. Drug resistant variants are evolving. The antibiogram of isolates obtained from blood culture as per recent studies from different regions, have shown a shifting pattern of susceptibility to conventional drugs [12,13]. In addition, the indiscriminate use and

predominantly misuse of the antimicrobials have resulted in the emergence of multidrug-resistant strains which appear as a great concern to clinicians [14].

Considering the mentioned facts and figures, the present study was aimed to evaluate the diagnostic relevance of blood culture and Widal test for enteric fever along with the prevailing data antibiotic resistance pattern of the isolates. Moreover, an attempt was made to find out the usefulness of the Widal test in the diagnosis of the disease in a feasible manner among the substantial number of blood culture negative cases.

2. METHODOLOGY

This study was a cross sectional study carried out from January '2017 to March 2020' in a Tertiary outdoor Medical Centre, Savar, Dhaka in Bangladesh. The study included patients of fever (> four days) with symptoms and signs which were suggestive of enteric fever.

2.1 Collection of Specimen

With all aseptic precautions, 5-10 ml of peripheral blood from adults and 2-5 ml from pediatric patients were collected in FAN (Fast Antibiotic Neutralizer) bottle according to WHO guidelines.

2.2 Blood Culture by BACTEC Method

After collection, the bottles were put in the BACTEC 9050 machine (Becton Dickinson and Company, Maryland, USA) where it was incubated at 37°C. All alarm positive samples were inoculated on MacConkey agar and Blood agar media (OXOID CO. UK), again incubated overnight at 37°C [15].

2.3 Microbiological Study

The isolates were identified by colony characteristics, Gram stain reaction and biochemical reaction such as catalase, oxidase, Motility Indole Urease (MIU) test, Triple sugar Iron (TSI) test, Simmons Citrate utilization test.

2.4 Serotyping

The isolates of Salmonella were further confirmed by serotyping by agglutination with polyvalent O antiserum (Mast Assure, Antiserum Mast Group Ltd. Merseyside, UK).

2.5 Antimicrobial Susceptibility Test

Susceptibility to antimicrobial agents of all isolates was done by Kirby-Bauer modified disk diffusion technique [16]. The isolated colony from the various media was inoculated on Mueller-Hinton Agar media by spreading technique. Zone of inhibition produced by each drug was considered into two susceptibility categories namely sensitive (S) and resistant (R) with the help of Clinical Laboratory Standards Institute (CLSI) 2019. Each plate was examined after 24 hours incubation at 37°C. The antimicrobial discs were used according to the standard antibiotic panel for specific sample and isolated organisms. Antibiotic discs were obtained from commercial sources (Oxoid, UK). The isolated organisms were tested against amoxycillin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), gentamycin (30 µg), Cefixime (5 µg), chloramphenicol (30 µg), moxifloxacin (5 µg), levofloxacin (5 µg), cefipime (30 µg).

2.6 Widal Test by Slide Agglutination Method

The Widal test ideally requires both an acute and a convalescent-phase serum sample taken approximately 10 days apart, and a positive result was determined by a fourfold increase in antibody titer [6]. But we performed Widal test for once; rising titre could not be identified. Qualitative slide agglutination tests were performed using febrile antigen kits of *Salmonella* typhi (Human Gesellschaft Biocemica and Diagnosticamb H, Wiesbaden, Germany). The slide agglutination test was done to detect the presence of anti TO and anti TH antibodies in the patient's serum. For the slide agglutination test, a drop of Salmonella typhi O and H antigens were added on a drop of serum on card and rotated at 100 rpm for one minute and reported as reactive or non reactive. For slide agglutinations where results showed reactive and weakly reactive titer was determined. Then the reactive and weakly reactive serum samples were serially diluted by using fresh 0.95% saline preparation from 1:80 to 1:640 for anti TO and anti TH separately. Based on the manufacturer manual, an antibody titer of 1:80 and higher for anti TO and 1:160 and higher for anti TH antibodies were taken as a cut off value to indicate recent infection of typhoid fever.

3. RESULTS

Table 1 showed the distribution of typhoid fever in relation to age and sex. The most common age group affected was from 1-18 years (48.65%). The least prevalence of typhoid fever was obtained among the age group above 70 years (2.7%). Males were more prone to infection than females with a male female ratio of 2.4:1.

The blood cultures were found positive in 148 (6.75%) for either salmonella species or any other bacterial isolates and rest 93.25% samples yielded no growth for any pathogenic bacterial isolates (Fig. 1).

Fig. 2 demonstrated among 148 culture positive cases 111 samples yielded the growth of salmonella spp. Among them 74 (50%) was *Salmonella typhi* , 37 (25%) *Salmonella paratyphi A* and 37 (25%) yielded the growth of other bacteria.

Table 1. Age and sex distribution of blood culture positive cases of enteric fever (n=111)

Age Group	Male	Female	Total	P value
1 to 18 Years	38 (70.40%)	16 (29.60%)	54 (48.65%)	$\chi^2 = 32.09$
19 to 36 Years	32(74.40%)	11 (25.60%)	43 (38.74%)	df=3, p=7.81
37 to 54 Years	6 (54.50%)	5(45.50%)	11 (9.91%)	and p<0.05,
55 to 72 Years	2(66.7%)	1(33.3%)	37 (2.70%)	statistically
Total	**78 (70.27%)**	**33 (29.73%)**	**111 (100%)**	significant

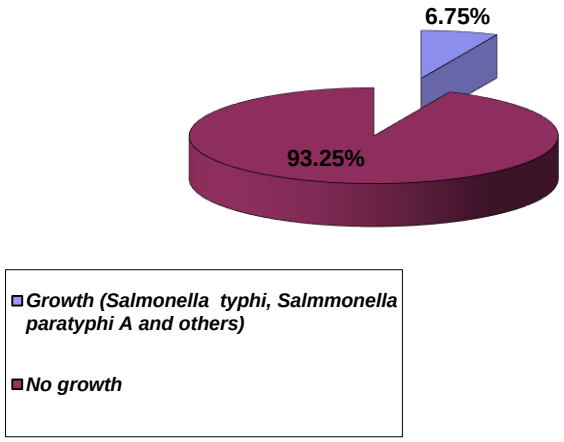

Fig. 1. Rate of isolation of *Salmonella typhi* and *Salmonella paratyphi A* and other organisms in total samples (n=2194)

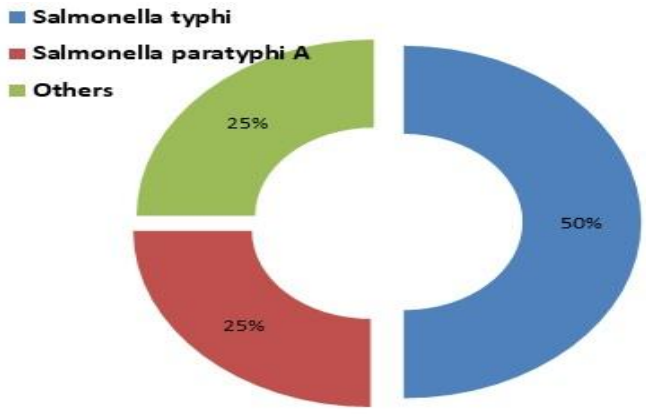

Fig. 2. Rate of isolation of Salmonella typhi and Salmonella paratyphi A in culture positive cases (n=148)

Table 2. Overall results of blood culture (n=148)

Culture result	Frequency	Percent
Salmonella typhi	74	50.0
Salmonella paratyphi A	37	25.0
Esch. coli	30	20.3
Staph. aureus	5	3.4
Streptococcs spp.	2	1.0
Total	**148**	**100.0**

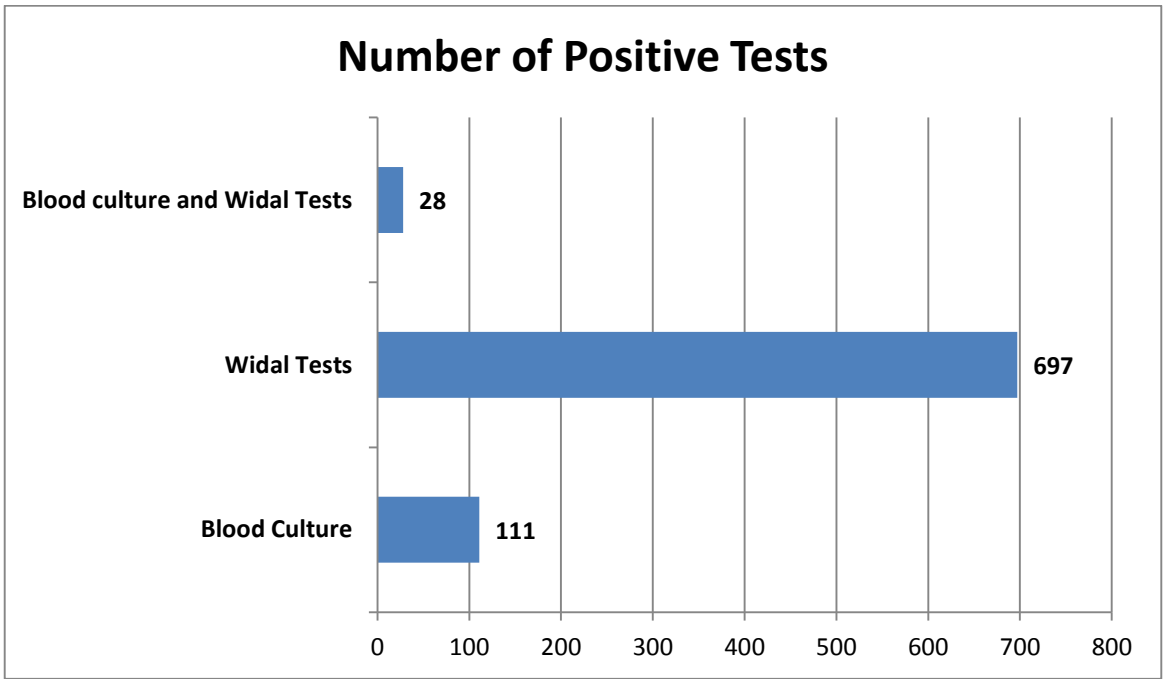

Fig. 3. Result of typhoid fever by blood culture and Widal test of febrile patients suspected of typhoid fever

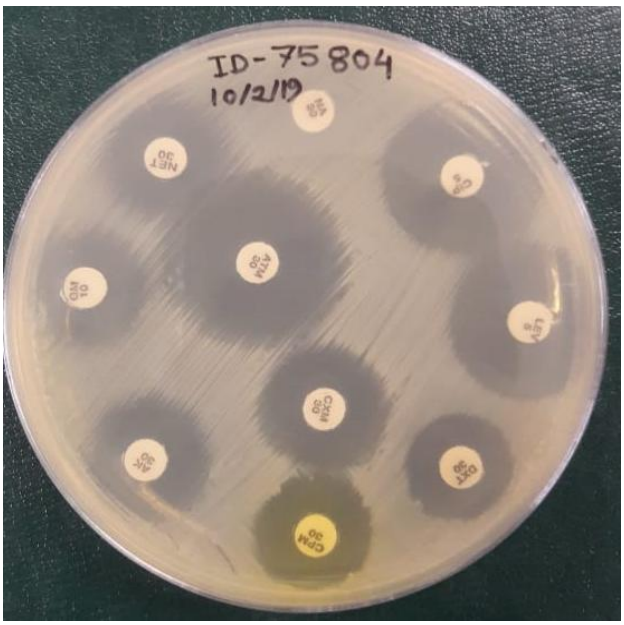

Fig. 4. Antimicrobial susceptibility pattern by Disc Diffusion Method

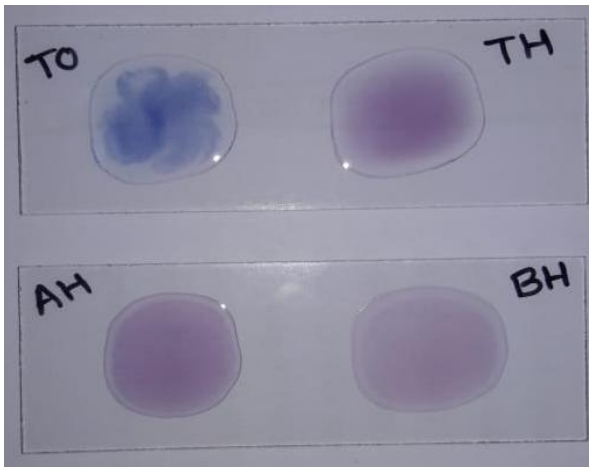

Fig. 5. Widal test by slide agglutination technique.

Table 3. Results of Single Widal tests and blood culture. (n=2194)

Test Result	Blood culture positive	Blood culture negative	Total	P value
Widal test +ve	28	669	697	$\chi^2 = 2.304$, df=1, p=3.84 and p>0.05, statistically not significant
Widal test -Ve	83	1414	1497	
Total	111	2083	2194	

Table 4. Distribution of slide agglutination titration test of Widal test in febrile patients suspected of typhoid fever

Titre	O antigen	% From Total (n=697)	H antigen	%From Total (n=697)
1:160	210(46.7%)	30.1%	240(64%)	10.9%
1:320	222(49.3%)	31.8%	132(35.2%)	6%
1:640	12(2.7%)	1.7%	3 (0.8%)	0.05%
1:1280	6(1.3%)	0.9%	0 (0%)	0%
Total	**450(100%)**		**375 (100%)**	

Table 5. Blood culture and Widal test result with the duration of onset of fever

Cases	Onset of Fever				
	1st Week	2nd Week	3rd Week	4th Week	Total
Blood culture Positive cases	**91**	**20**	**0**	**0**	**111**
Widal test Positive cases	**229**	**426**	**32**	**10**	**697**

A large number of isolates showed resistance to commonly used antibiotics such as nalidixic acid (94.6%), chloramphenicol (87.3%), amoxicillin (76.6%), cephradine (53.1%), azithromycin (46.8%), cotrimoxazole (44.1%), and ciprofloxacin (41.4%). Resistance to cefixime (41.4%) and cefipime (15.3%), ceftriaxone (11.7%) tended to increase than past. Dramatically Imipenem, moxifloxacin, cefuroxime showed increasing resistance with timer.

4. DISCUSSION

Enteric fever, though a major public health problem, but now-a-days, is mostly managed at peripheral health care facilities. Children (1-18 years) and young adult (19-36 years) groups respectively were more susceptible to *Salmonella* infection, probably because of weak immune system of underage children. Also increase fast food consumption rate further amplifies disease dilemma [17].

Out of 2194 blood samples processed during the study period, we observed a total of 697 cases of typhoid fever (31.8%) which is comparable to other study where authors [18] reported the overall positivity upto 9%. In addition, the findings of our study are in accordance with the results of a study of Bangladesh [19].

In our study, the total rate of isolation of *S. typhi* and *S. paratyphi* by blood culture were 111 (5.1%) and the widal tests were significant among 697 (32.8%) patients indicative of recent infection by either of O and H antigens. Our results correlate well with the finding of a study in Addis Ababa [20]. Authors reported that blood culture is regarded as the gold standard in the laboratory diagnosis of enteric fever [21]. They also concluded that blood culture in highly suspicious cases of enteric fever should always be done in order to avoid irrational and unnecessary usage of antibiotics so that overinflated treatment can be avoided by specific antibiogram.

In current study, the isolation rate of *S. typhi* was two times higher than *S. paratyphi A* isolates (50% vs 25%) collected from blood samples throughout the 38 months period. Similarly, in India, Choudhary et al (2013) reported 57.9% isolates of *S. typhi* and 41.6% of *S. paratyphi A* among *Salmonella spp.* in a tertiary care hospital between 2009 to 2011 [22]. Our findings also corresponds to the results of a study in Nepal [1] *S. typhi* infection is mainly due to waterborne transmission requiring smaller inoculum whereas *S. paratyphi* is due to foodborne transmission and requires a larger inoculums, [22,23] which might may be traced to the reason for this variation.

In our study, 111 (5.1%) were positive by blood culture, and 697 (31.8%) were positive by Widal titre (1:160 and more). Among the culture positive cases, 28 (1.3%) were also found positive by both blood culture and significant Widal agglutination titre. The sensitivity in terms of ratio of Widal test to blood culture is found to be 6.3:1.Although, the noticeable drawback of this test is cross-reactivity with different bacteria of same genus [24]. This is similar with the study conducted in St. Paul's general Specialized Hospitals in Ethiopia, in Addis Ababa where total prevalence of typhoid fever by blood culture was 4.1% and total number of patients who have recent infection by either of O and H antigens of Widal test was 32.6% [20].

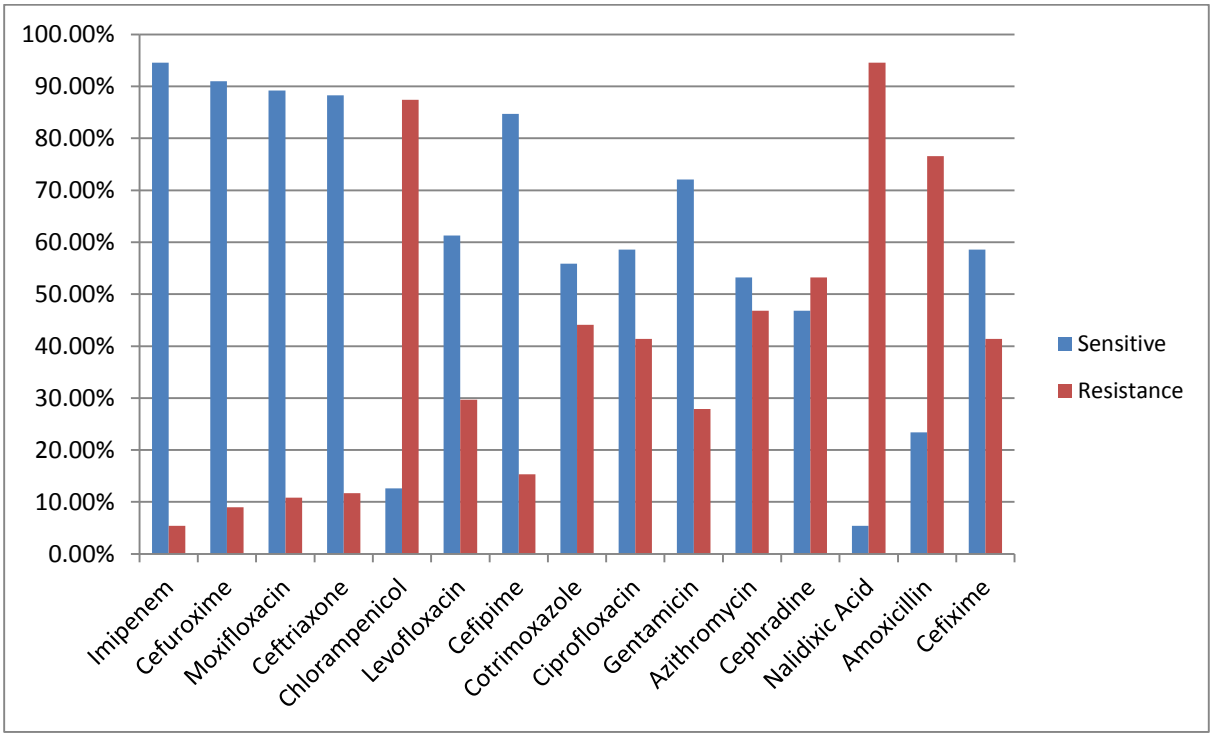

Fig. 6. Antimicrobial susceptibility pattern of identified *Salmonella typhi* and Salmonella paratyphi A (n=111)

In our study, the total number of patients who were observed as indicative of recent infection by either O and H antigens of Widal test were 697 (31.8%). Our study reflects similarity with the finding of another study in India [24]. Some studies manifests with various findings of *Salmonella* spp. were isolated ranges from 40% to 60%. This variation may be due to prior antibiotic use, duration of fever and amount of blood taken for culture. We could not confirm typhoid fever by Widal test serologically by observing rising titre which is a limitation of our work.

Widal test was performed in clinically suspected for typhoid fever 2194 patients. Antibody titer of 1:160 for O antigen and 1:160 for H antigens were taken as cut off values to indicate recent typhoid infection. Among positive cases for Widal agglutination in Table 4, 450 (64.5%) had titre of 1:160 or higher for O antigen and 375 (53.8%) had titre of 1:160 or higher for H antigen which is supported by another study [25]. Hence, the 'TO' titre may be considered to be of greater diagnostic significance. Willke et al observed similar finding [26]. There was no titre of 1:1280 for H antigen. We had no control group, so risk factors for negative blood culture or insignificant Widal test could not be evaluated and sensitivity and specificity of the test could not be done.

In this study, the culture positive cases mostly occurred in 1st week of fever and rest was positive in 2nd week no cases were positive after 2nd week of fever. Similar study showed the highest positive culture cases of 96.9% in the 1st week of the onset of fever [27]. In this study, the highest number of 426 cases were Widal test positive in the 2nd week of the onset of fever followed by 229 cases were positive in 1st week. This observation is similar to the finding of a study conducted inTropical and Infectious Disease Hospital, Teku, Nepal [1]. The Widal test positive results in the early week in our study can be attributed to the regular sub-clinical sensitization of the patients with the Salmonella strains. High positivity of Widal test in the 1st week may be associated with hyper-immune state of the patients.

The emergence of antimicrobial resistance enteric fever, especially the multidrug resistance (MDR) to amoxicillin, chloramphenicol and cotrimoxazole, is a major public health problem, particularly in developing countries [28]. A more useful definition of Multi drug resistant *Salmonella* typhi (MDRST) is reserved for strains resistant to all three first-line antityphoidal antimicrobial agents, namely amoxycillin, chloramphenicol, and trimethoprim-sulphamethoxazole [29]. The present study showed a high resistance level to nalidixic acid (94.6%) which is comparable to a study [30]. We observed that other quinolones such as levofloxain and ciprofloxacin resistance was 41.4% and 29.7%. Many researchers around the world reported increased resistance of *Salmonella* spp. against nalidixic acid [29, 31] and in the same manner, increase in nalidixic acid-resistant isolates was observed in this study. Nalidixic acid resistance may be an indicator of treatment failure to ciprofloxacin; hence both these drugs should be simultaneously tested for sensitivity. Fluoroquinolone resistance has increased, which has established the use of the third generation cephalosporin (ceftriaxone). After fluoroquinolone resistance has increased, requiring the use of the third generation cephalosporin (ceftriaxone) [32]. This study reflects the similar pattern [33]. For last few years, scattered cases of ceftriaxone or azithromycin resistant strains have also been reported [32] which resemble with the present study.

We found 5.4% isolated *salmonella spp*. were resistant to Imipenem, whereas Singh CL et al found no Imipenem resistant *Salmonella spp* [34]. With time the development of Imipenem resistant *Salmonella* strains already is creating a panic to the clinicians. Appropriate antimicrobial treatment depends on an understanding of local patterns of antimicrobial resistance and is maintained by the results of antimicrobial susceptibility testing of the *Salmonella* isolates.

5. CONCLUSION

Enteric fever continues as a great problem to the local community. Appropriate diagnosis of this infection depends on blood culture. Because of emergence of multi-drug resistance strains of Salmonella, culture should be preferred along with Widal test to select the appropriately directed antibiotics. Although blood culture is more acceptable, our results showed that Widal test may also be a reliable adjunct to reach the proper diagnosis of enteric fever with some limitations. It can be inferred that blood culture would be helpful in the diagnosis and thereby preventing the spread of antibiotic–resistant *salmonella spp*. which might lead to the development of MDR strains. Our findings emphasize the need for continuous evaluation as

well as judicious use of antimicrobial drugs. We recommend that further multicentre case-control research works with a large series of study population comprising all the available diagnostic modalities to generate hard data while formulating strategies in the early diagnosis and proper management of enteric fever.

CONSENT

An informed consent was taken from the patients and guardian before the sample collection.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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