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Comparative Study of Blood Culture and Widal Agglutination Test from the Patients Suspected of Enteric Fever

Preety Chaudhary[1*], Vijay Kumar Sharma[2], Anshu Kumari Chaudhary[1], Shashi Bhushan Chaturwedi[1] and Anima Shrestha[1]

[1]Department of Microbiology, Tri-Chandra Multiple College, Ghantaghar, Kathmandu, Nepal.
[2]Department of Microbiology, Alka Hospital, Jawalakhel, Lalitpur, Nepal.

Authors' contributions

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

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ABSTRACT

Aims: This study was performed to identify the enteric fever cases by both blood culture and Widal agglutination test and compare the results obtained from both methods.
Study Design: This research was carried out as hospital based descriptive cross-sectional study.
Methods: Blood samples collected aseptically from patients suspecting enteric fever were processed for identification of *Salmonella* species by blood culture and Widal agglutination test. The isolates were further subjected to antibiotic susceptibility testing according to CLSI guidelines. Total 1269 samples from the suspected patients were enrolled for this study and statistical analysis of the result was done by using 16.0 versions of SPSS.
Results: Among suspected patients studied, 70 (71%) and 29 (29%) cases were confirmed to be infected with *S. typhi* and *S. paratyphi* A respectively from blood culture. Out of total sera processed for Widal test, 263 samples gave agglutination with titre more than 1/80. The study showed sensitivity of 81.4% and specificity of 84.4%, positive predictive value of 31.5% and negative

**Corresponding author: E-mail: preetych44@gmail.com;*

predictive value of 98.2% and the efficiency 84.4% of Widal test in compare to blood culture. *S. typhi* isolates sensitive to the classical first line drugs- amoxycillin, chloramphenicol and cotrimoxazole were 94.3%, 97.1% and 97.1% respectively while *S. paratyphi* A isolates sensitive were 68.9%, 96.5%, and 93.1% respectively. Fifty eight (82.9%) *S. typhi* isolates were nalidixic acid resistance while 25(86.2%) *S. paratyphi* A were nalidixic acid resistant. Also, 3(3.03%) multi-drug resistant isolates were confirmed to be nalidixic acid resistant.

Conclusion: The study showed blood culture remains the gold standard for enteric fever diagnosis. Widal test alone either positive or negative should not be considered confirmatory for enteric fever However cut-off titre can be taken in the diagnosis and Widal test can be helpful in making a presumptive diagnosis of typhoid fever if interpreted with care. Azithromycin and Ceftriaxone were the most effective drugs for enteric fever cases.

Keywords: Enteric fever; blood culture; Salmonella; widal test.

1. INTRODUCTION

Enteric fever is a systemic infection caused by the human adapted pathogens *S. typhi* and *S. paratyphi* A, B, and C. These organisms are important causes of febrile illness among crowded and impoverished populations with inadequate sanitation who are exposed to unsafe water and food, and also pose a risk to travelers visiting endemic countries [1]. Globally infection by *S. typhi* is higher than *S. paratyphi* but recent researches in Asian countries including Nepal reported higher isolation of *S. paratyphi* A than *S. typhi* from enteric fever patients with growing antibiotic resistance character [2,3]. The fever is prevalent in mountains, valleys and southern belts of Nepal as an endemic disease with its peak incidence in May to August [4]. Strains which are resistant to all the three first-line recommended drugs for treatment, *i.e.*, chloramphenicol, ampicillin, and co-trimoxazole define multiple drug resistance (MDR) in *Salmonella* [5]. There are two main mechanisms of drug resistance development in *S. typhi*, first is a plasmid-mediated mechanism; the second is a chromosomal DNA-mediated mechanism [6]. Current widely used methods for the diagnosis of individuals with enteric fever include bacterial culture, microscopy and serological assays, specifically the Widal test. Blood is the most common specimen submitted for culture of *S. typhi*. The sensitivity of culture from blood is dependent on a variety of factors including the volume of blood taken (and its ratio to enrichment broth), pre-treatment with antibiotics and delay in transportation of the sample to the laboratory [7]. However, blood culture capacities are often not available in endemic areas.

Widal test detects the presence of agglutinating antibodies in the serum of infected/exposed patients against lipopolysaccharide (LPS; O) and flagella (H) antigens of *S. typhi*. These antibodies present at 6 to 8 days and 10 to 12 days respectively, following infection; a 4-fold rise in either of these antibodies between acute and convalescent sera is diagnostic [8]. Widal tests are relatively inexpensive however, particularly in comparison to bacterial culture methods, and are therefore still widely used [9]. Though blood culture method has been used as gold standard method for diagnosis of enteric fever, it has limitation of time requirement, at least 3 days and positive results of only 30-70% even in well-equipped laboratory [10]. Thus a more rapid, simpler, and cheaper diagnostic method would be very useful especially in developing countries like Nepal. This study was performed to compare the sensitivity and specificity of Widal test in culture positive samples suspected for enteric fever along with antibiotic resistance trend of isolated *Salmonella enterica* and determine multi drug resistant isolates.

2. MATERIALS AND METHODS

Total 1269 samples received for blood culture were studied during the study period. All the samples were also processed for Widal test. Both male and female patients of all age groups, who were enteric fever suspected by the clinicians and requested for blood culture and Antibiotic Susceptibility Testing (AST), were included in the study. This study was conducted from March, 2013 to August, 2013 where 747 samples from male and 522 samples from female were processed.

2.1 Sample Collection

Blood samples were collected by laboratory technician at pathology department Alka Hospital, Jawalakhel, Lalitpur, using standard aseptic techniques. For culture, venous blood

sample (5 ml from adult and 2 ml from children) were collected and dispensed in culture bottle with Brain Heart infusion (BHI) broth (45 ml for adult and 18 ml for children). For Widal test, 1ml blood was collected and allowed to clot in a clean dry screw-capped test tube and centrifuged to separate serum.

2.2 Isolation and Identification

The culture bottles were incubated at 37°C. Incubation was continued for 7 days unless the visible growth was obtained. After each day of incubation blind subculture were done on Blood agar (BA), Chocolate agar (CA) and Mac Conkey agar (MA) up to seven days of incubation. The day of collection of sample was defined as the first day in this study. The culture bottles were examined daily for visual evidence of microbial growth, such as, turbidity, gas production to make presumptive diagnosis of positive culture. The identification of bacteria from isolated colonies was done by standard microbiological procedures as described in Bergey's Manual, which involve colony morphology, Gram stain and biochemical reaction. Various biochemical media were inoculated and the results were observed on following day.

2.3 Antibiotic Sensitivity Test

Antibiotic sensitivity test of the isolates to 11 antibiotics was performed by Kirby Bauer disc diffusion method with Mueller–Hinton agar using the guidelines and interpretive criteria of the CLSI (Clinical and Laboratory Standards Institute) 2012. The inoculam used for susceptibility testing was prepared in nutrient broth taking 5/6 colonies of *Salmonella enterica* that matched to 0.5 McFarland standard (1.5 X 10^8 CFU/ml). Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension and pressed inside the wall of tube above the fluid level and inoculated at 60° over the dried surface of Muller-Hilton agar (MHA) plate. After 3-5 minutes antibiotic disc were applied and gently pressed down to ensure complete contact with agar. *Salmonella* which showed resistance to all the three first-line recommended drugs for treatment, *i.e.*, chloramphenicol, ampicillin, and co-trimoxazole define multiple drug resistance (MDR) [5]. The antibiotic discs used were amoxycillin (30 µg), amikacin (10 µg), azithromycin (15 µg), cefixime (5 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), ofloxacin (5 µg) and tetracycline (10 µg). The control strains *Escherichia coli* (ATCC, 25922), *Staphylococcus aureus* (ATCC 25923) *and Pseudomonas aeruginosa* (27855) were used for the standardization of the Kirby-Bauer test by correct interpretation of the zone diameters [11].

2.4 Widal Test

Widal test was performed on the sera collected from the patients for blood culture for the enteric fever diagnosis. Appropriate positive and negative control sera were included. Widal titres were determined by semi quantitative slide agglutination and quantitative tube agglutination Widal test. Performance testing was determined by calculating the sensitivity, specificity, positive predictive value, negative predictive value and efficiency considering blood culture as the standard method. The 95% confidence interval for sensitivity and specificity was calculated.

2.4.1 Formulae for performance testing of Widal test

Sensitivity: (True positive rate) (a/a+c) x 100%,

Specificity: (True negative rate) (d/d+b) x 100%,

Positive Predictive Value: (PPV) (a/a+b) x 100%), Negative Predictive Value**:** (NPV) (d/d+c) x 100%, and

Efficiency: (a+d/a+b+c+d) x 100% Where, a= Positive culture, and positive Widal test, b= Negative culture, but positive Widal test, c= Positive culture, negative Widal test, and d= Negative culture, and negative Widal test.

2.5 Data Management and Analysis

The data, both from the laboratory finding and from questionnaires were entered and analyzed by SPSS version 16.0. Frequency and percentages were calculated and Chi-square test was done whenever applicable with $P<0.05$ regarded as significant.

3. RESULTS

Out of 1269 samples, 99(7.8%) were culture positive while 1170 were culture negative (92.2%). Among culture positive 70(71%) *Salmonella enterica* serotype Typhi and 29(29%) *Salmonella enterica* serotype Paratyphi A. Involment of *S. paratyphi* B and C in the infection was absent. Out of 99 positive blood cultures, 69 were male and 30 were female (Table 1).

In this study, the age of the patients were ranged from patients below 10 years to above 70 years. The Highest number of patients, 41(41.4%) from Culture positive case belonged to age group 10-20 (Table 2).

Out of positive growth isolated from blood culture from March to August, there was a rise in the isolated organism. Highest isolates, 27.27% was observed in August among positive growth from culture confirmed cases (Fig. 1).

Antibiotic susceptibility test for *S. typhi* and *S. paratyphi* was performed using disc diffusion method. *S. typhi* was found to be 100% susceptible to azithromycin followed by cefixime and ceftriaxone and tetracycline (98.6%). Ceftriaxone and amikacin were found to be most effective drug against S. *paratyphi.* The resistivity pattern of nalidixic acid was highest in both *S. typhi and S. paratyphi.* Two fluoroquinolones were used, ciprofloxacin and ofloxacin. Out of 58 (82.9%) NAR (Nalidixic acid resistant) isolates in *S. typhi*, only 43(74.1%) were susceptible where as in case of *S. paratyphi* A 21(84%) were susceptible, 3(12%) (Table 3). Among 99 *Salmonella* isolates, 3(3.03%) isolates were found to be MDR (Table 4). All were resistant to nalidixic acid.

Total, 1269 sera were processed for Widal test, out of which 263 samples gave positive Widal test with titre > 1/80 (Fig. 2). Out of 99 blood culture positive samples, 83 were Widal positive and 16 were Widal negative. Out of 1170 blood culture negative sample 182 were Widal positive and 988 samples were Widal negative. Widal test was statistically significant (p=0.000) to the blood cultured cases (Table 5).Evaluation of Widal test with blood culture showed sensitivity with 81.4% and specificity of 84.4%. It was found to have low positive predictive value of 31.5% and better negative predictive value with 98.2%. The efficiency of Widal test in compare to culture was found to be 84.4%.

Out of 99 *Salmonella* isolated sample sera, only 83 gave titre to Widal agglutination test. *Salmonella* serotype Typhi gave titre toward antibody anti-O, anti-H and both anti-O and anti-H where *Salmonella* serotype Paratyphi A gave titre only to antibody anti-AH in serum samples (Table 6).

Table 1. Distribution of positive blood culture among sex

Sex	Frequency	Growth positive	Positive %
Male	747(58.9%)	69	69.69%
Female	522(48.9%)	30	30.30%
Total	1269(100%)	99	7.80%

Table 2. Age distribution of culture positive patients

Age (years)	Male	Female	Total	
	N	N	N	%
<10	10	6	16	16.16
10-20	30	11	41	41.4
21-30	22	10	32	32.3
31-40	2	2	4	4.04
41-50	2	1	3	3.03
51-60	2	-	2	2.02
61-70	-	-	-	-
>70	1	-	1	1.01

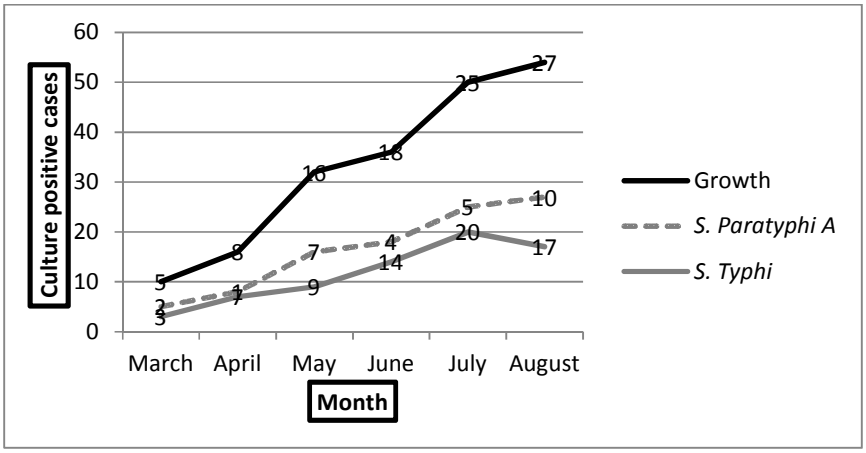

Fig. 1. Distribution of culture positive cases by month

Table 3. Antibiotic susceptibility pattern of *Salmonella typhi* and *S. paratyphi*

Antibiotic used	Antibiotic susceptibility pattern					
	Sensitive (%)		Intermediate (%)		Resitant (%)	
	S. typhi	*S. paratyphi* A	*S. typhi*	*S. paratyphi* A	*S. typhi*	*S. paratyphi* A
Amoxycillin	94.3	68.9	-	-	5.7	31.1
Azithromycin	100	96.5	-	-	-	3.5
Amikacin	97.1	100	-	-	2.9	-
Cefixime	98.6	96.5	-	3.5	1.4	-
Ceftriaxone	98.6	100	-	-	1.4	-
Chloramphenicol	97.1	96.5	-	-	2.9	3.5
Ciprofloxacin	77.1	86.2	4.3	10.3	18.6	3.5
Cotrimoxazole	97.1	93.1	-	3.5	2.9	3.5
Nalidixic acid	17.1	13.8	-	-	82.9	86.2
Ofloxacin	77.1	86.2	4.3	10.3	18.6	3.5
Tetracycline	98.6	93.1	-	-	1.4	3.5

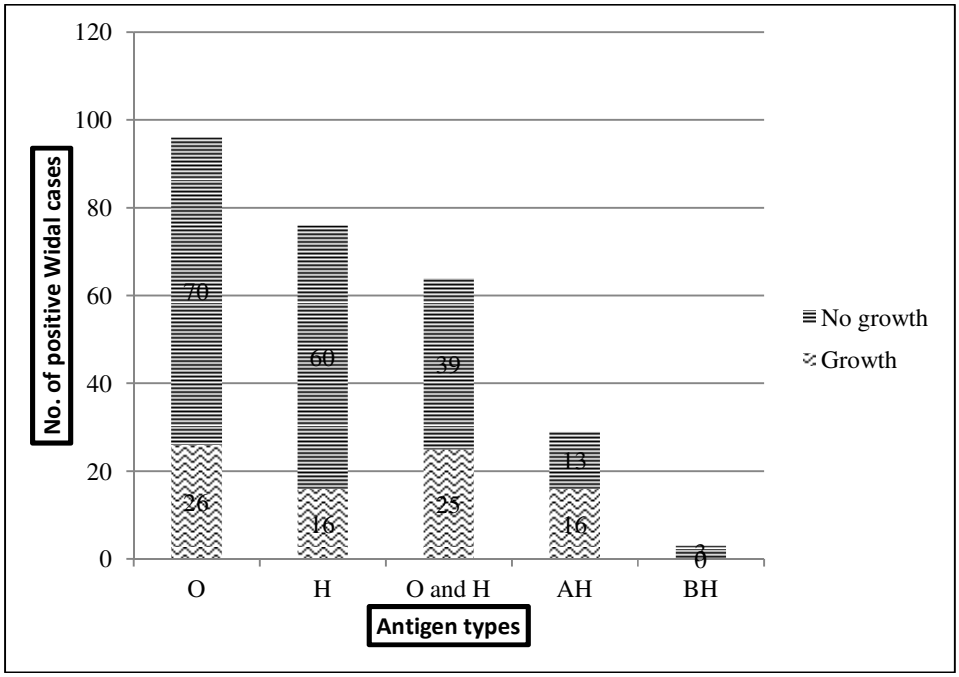

Fig. 2. Distribution of positive samples for agglutination in Widal test

Table 4. MDR among *Salmonella* isolates

Organism	Number	MDR isolates	Total (%)
S. typhi	70	2	2.86
S. paratyphi A	29	1	3.45
Total	99	3	3.03

4. DISCUSSION

Out of total samples 99(7.8%) were culture positive. Low positive rate might be due to the use of antibiotics prior to sample collection, due to insufficient blood withdrawn for culture, quality of media and time of collection of blood during fever. Another reason might be that most of the enteric fever suspected patient might be patients with pyrexia of unknown origin and similar other. Similar incidence of positive culture was reported in some studies [12,13]. The study showed higher percentage of male in suspected as well positive cases. Out of 1269 sample 747(58.9%) were male and 522(48.9%) were female. Among 99 growth positive cases, 69(69.69%) were

Table 5. Comparison of blood culture with Widal test

		Blood culture			*P* value
Widal		**Positive**	**Negative**	**Total**	
	Positive	83	182	263	
	Negative	16	988	1006	0.000
	Total	99	1170	1269	

Table 6. Antibody titre verses *Salmonella* isolated

Antigen	**Antibody titre value**				**Total**	***Salmonella* serotype**
	1/80	**1/160**	**1/320**	**1/640**		
O		19	6	1	26	*Salmonella typhi*
H	-	-	13	3	16	*Salmonella typhi*
O and H	-	3	18	4	25	*Salmonella typhi*
AH	1	10	4	1	16	*Salmonella paratyphi* A

male and 30(30.30%) were female. The ratio of male: female was found to be 2.3:1. Similar finding has been reported in a study conducted in eastern Nepal where the ratio of male: female was 2:1 [13]. The difference in ratio may be due to more outdoor exposure of males. The study also showed the highest number of patients, 41(41.4%) from culture positive cases belonged to age group 10-20. This age group belongs to studying population including school children thus due to eating and drinking outside, having street food, poor hand washing and other hygiene habits etc. may be the reason of their high prevalence [14]. Where typhoid is endemic, most cases of infected persons are aged 3-19 years (WHO, 2003). In a recent study conducted in five Asian nations, 5% of the growths were from the age below 15 years [15]. In both males and females, *S. typhi* was the predominant etiological agent across all age groups, which is consistent with observation that *S. typhi* is more prevalent than *S. paratyphi* A in this location [13].

In this study highest isolates, 27.27% was observed in the August among positive growth from culture confirmed cases from March to August. *S. typhi*, 28.6% was observed in the month of July whereas *S. paratyphi* A with a fluctuation in the growth rate was observed maximum, 34.5% in August. Transmission through the water supply is supported by the seasonal variation in disease incidence [4]. There have been reports of seasonal typhoid outbreaks with recent one in 2002 in Bharatpur, a central town of Nepal. The Multi-drug resistant typhoid epidemic affected more than 6,000 patents in a 4 to 5 weeks period and was from a single source of the municipality water supply [16].

S. typhi was susceptible to azithromycin (100%), cefixime, ceftriaxone and tetracycline (98.6%). Chloramphenicol, cotrimoxazole and amikacin (97.1%) showed better susceptibility and amoxycillin (94.3%) weak susceptibility pattern toward *S. typhi*. A re-emergence of chloramphenicol sensitivity was also reported by Prajapati et al. [17] from Nepal. Resistance (1.4%) toward cephalosporin was also found but in low rate. Isolates showed high resistance to nalidixic acid followed by Fluoroquinolones, ciprofloxacin and ofloxacin Another studies reported that fluoroquinolones particularly ciprofloxacin was the most frequently used antibiotics in *S. typhi* and *S. paratyphi* case but none of the isolates were resistance to this antibiotic [18].

In *S. paratyphi* A, ceftriaxone and amikacin was found to be 100% susceptible which was supported by a study done in Teaching Hospital of Kathmandu [19]. With respect to prescribing azithromycin, most of the antimicrobial susceptibility standards do not mention the MIC breakpoints of azithromycin for *Salmonella*. CLSI also have no guidelines to interpret it. However, it is still being prescribed worldwide with many clinical trials suggesting its superior clinical efficacy [20]. In *S. paratyphi* A; NAR isolates were higher in comparision to *S. typhi*. The nalidixic acid resistivity was statistically significant to growth of *Salmonella.* A study carried out in Nepal in 2005, 73.3% and 94% of *S. typhi* and *S. paratyphi* A strains showed the resistance to nalidixic acid [21]. Some of the

researcher say that moreover, the clinical effectiveness of fluoroquinolones for *S. typhi* isolates, for which MICs of ciprofloxacin were high, but which were positive for nalidixic acid susceptibility is unknown [22]. The prevalence of MDR in this study was of very low percentage similar to previous studies [19,20]. A study conducted in Nepal concluded the antibiotics against MDR *S. typh*i and *S. paratyphi* A, carbapenems (ertapenem and imipenem) and cephalosporin were highly active against MDR isolates [23].

It was found in Widal agglutination positive agglutinins to *S. typhi* were the most prevalent among the sera of various dilutions which were tested. The levels of the agglutinins for *S. paratyphi,* AH and BH were found to be low, comparable to findings reported by a study conducted in hilly region of India [24]. Widal test sensitivity was 81.4% and specificity of 84.4%. It was found to have positive predictive value of 31.5% and better negative predictive value with 98.2%. The efficiency of Widal test in compare to culture was found to be 84.4%. Although the Widal test at cut-off titer (≥1:80) was performed relatively well in terms of sensitivity, specificity and NPV, its PPV was low. A study conducted for evaluation of Widal test in children in a hospital of Tanzania also found low PPV, indicating that testing a single serum sample is inadequate for the confirmation of typhoid fever [25]. In a similar study sensitivity was 77% and specificity 89%, positive predictive value 32% and negative predictive value was 98% [26]. The sensitivity of Widal test increased to 77.6% when the cut-off was taken as 1/160 for “O”antigen and 1/320 for “H” antigen of *S. typhi*. A Seroprevalence rate measured in the Widal test was generally much higher than isolation rates. From many patients with a Widal test positive result an organism other than *Salmonella* was isolated, that showed the Widal test is highly non-specific and likely overestimates the prevalence of *Salmonella* infection [27]. However, Widal test is rapid, with results when compared to 48 hours for blood culture. Ideally a fourfold rise in antibody titre in a paired serum (collected within 2 week) is more diagnostic [28].

5. CONCLUSION

Blood culture remains the gold standard for enteric fever diagnosis. Azithromycin and ceftriaxone are the principle alternatives antibiotics for the treatment of enteric fever caused by MDR and fluoroquinolone-resistant *Salmonella* isolates. In case of Widal test the cut off value of ≥1:80 were found to be valid in this study more ever cutoff value for H agglutinin should be increased to >1:160 for more effective result. Widal test can be taken into consideration in case of early antimicrobial administration or lack of culture facility on the basis of clinical background. However, Widal test alone either positive or negative should not be considered confirmatory for enteric fever. Widal test can be used as a complimentary serological diagnostic tool as and when it is required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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