

# Comparison of Blood Culture and Single Slide Agglutination Widal Test for the Diagnosis of Enteric Fever

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## Abstract

**Introduction:** Enteric fever is a leading cause of mortality and morbidity in the developing world. Unawareness of public concerning the sanitation & hygiene led to the high prevalence of the disease.

**Methods:** Blood sample of patients suspecting enteric fever attending STIDH of all age group were collected & simultaneously data on pre-disposing factors associated with systemic febrile illness were collected according to the questionnaire designated. The collected blood samples were brought immediately to the laboratory. Upon arrival, the blood samples were processed for blood culture and single slide agglutination Widal test according to the standard laboratory methods.

**Results:** Of the total 237 cases, 13 (5.48%) were confirmed as enteric fever by blood culture with 5 (38.46%) *Salmonella enterica* serovar Typhi and 8 (61.54%) *Salmonella enterica* serovar Paratyphi A. Out of the total cases, 7 (2.95%) were positive for blood culture as well as Widal test.

At the serum titre of 1:80, Widal test showed the sensitivity of 28.57% and specificity of 73.56% for O antigen whereas sensitivity of 71.42% and specificity of 61.36% for H antigen. PPVs for the test were quite low with 8% for O antigen, 12.82% for H antigen and 12.5% for AH antigen. NPVs were 92.75% for O antigen, 96.42% for H antigen and 95.08% for AH antigen. Most of the culture confirmed cases, 9 (69.23%) were positive in the 1st week of onset of fever and most of the widal positive cases, 49 (51.57%) were positive in the 2nd week of onset of fever.

**Conclusion:** Single slide Widal test of titre value  $\geq 1:160$  was found to be useful tool for diagnosis of enteric fever but the results should be correlated with clinical findings.

**Key words:** blood culture, enteric fever, *Salmonella*, Widal test

## Introduction

Enteric fever, also known as “fevers of undetermined origin” is a systemic febrile illness of prolonged duration that includes typhoid and paratyphoid fever marked by step-ladder fever, diffuse abdominal pain, frontal headache, delirium, splenomegaly, hepatomegaly and many other systemic manifestations due to bacteremia and septicemia.

Typhoid fever is prolonged febrile illness caused by a systemic infection with *Salmonella enterica* serovar Typhi (S. Typhi). Paratyphoid is caused by closely related organisms *Salmonella enterica* serovar Paratyphi A (S. Paratyphi A), *Salmonella schottmulleri* (S. Paratyphi B), or *Salmonella hirschfeldii* (S. Paratyphi C). S Paratyphi A is the only one of the paratyphi that has been isolated in Nepal<sup>4,20</sup>.

Enteric fever occurs in all parts of the world where there is substandard water supply and sanitation. It has almost been eliminated from developed countries because of sewage and water treatment facilities but remains a common disease and a major cause of morbidity and mortality in the third world countries. Exposure of the individual to contaminated food or water closely correlates with the risk for enteric fever<sup>3,19</sup>.

In Nepal, enteric fever is also known as “bisham joauro” meaning fever with poison. The fever is prevalent in mountains, valleys and southern belts of Nepal as an endemic disease with its peak incidence in May to August. Enteric fever is one of the leading diagnoses of fever in most of the hospitals in Nepal<sup>15</sup>. Many studies suggest that there is significant burden of the disease in the Kathmandu valley<sup>2,9</sup>, and other parts of the country<sup>8</sup>. Use of vegetables grown in sewage farm or washed with contaminated water, poor personal hygiene, open-air defecation, poor or unsatisfactory microbiological quality of the municipal drinking water combined with low level of public awareness and behavioral attitude concerning the sanitation and hygiene have together led to the high prevalence of the disease<sup>2</sup>.

Different techniques are used for the diagnosis of enteric fever including blood culture, bone marrow culture, rectal swab culture, urine culture, rose spot culture, duodenal string culture, Widal test, ELISA, and immunofluorescence. Widal test and blood culture remain the only universally practiced diagnostic procedure, because other methods are either invasive, have failed to prove their utility or are expensive<sup>5</sup>.

The improved living condition and the introduction of Chloramphenicol, in 1948, had resulted in a drastic reduction of enteric fever in industrialized countries<sup>13</sup>, but it still remains a major public health problem in the developing countries like Nepal<sup>8</sup>.

Thus this study will focus on the changing etiology of enteric fever and antibiotic resistance trend of the isolates and will try to find the usefulness of the Widal test in the diagnosis of the disease. It will also try to find the way to manage the false results of the test and can play an important role in determining the agglutination titre of the general population.

## Methods

### Isolation of Bacterial pathogen

From 10ml of collected sample, 5ml was transferred directly to the sterile 1% BHI broth so as to make the blood to broth ratio of 1:10. The remaining blood was allowed to

clot & centrifuged at 3000 rpm for 2 minutes in a dry, screw-capped test tube to separate serum samples for Widal test. The culture bottles were incubated at 37°C. incubation was continued for 7 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 sub-culture was done on Mac-Conkey agar and Blood agar. Repeated sub-cultures of the culture bottles were made at different times during their incubation from 24 hours to 7 days. The sub-culture plates were examined after overnight incubation. Mac-Conkey agar plates were examined for growth of non-lactose fermenters.

### Identification of Bacterial pathogen

Identification of bacteria from positive culture plates were done with the use of standard Microbiology technique which included colony morphology, Gram stain, Biochemical reaction and Serotyping.

### Widal Screening Slide test (Qualitative)

All the reagents were brought to room temperature before use. The glass slide supplied in the kit was cleaned well and was wiped free of water. One drop of undiluted test sera was put on each of the first four circles (1-4) and drop of positive control sera in each of the last two circles (5 and 6). One drop of antigen O, H, AH and BH was placed in circle 1, 2, 3 and 4 respectively. And O antigen in circle 5 and anyone of the H antigen (H), (AH), or (BH) in circle 6. The content of each circle was mixed with separate wooden applicator stick and was spread to fill the whole area of the individual circle. The slide was centrifuged or rotated at 150 rpm for minute and observed for agglutination. If agglutination was visible within one minute, quantitative slide test was proceeded for the quantitative estimation of titer of the appropriate antibody.

To obtain reliable result, quality control was applied in specimen collection, blood culture and during Widal test.

## Results

Single Widal Slide Agglutination Tests & bacteriological culture

Out of 237 included samples, 5(38.46%) were found to be positive for Salmonella Typhi whereas 8 (61.54%) were found to be positive for Salmonella Paratyphi A (Fig 1).

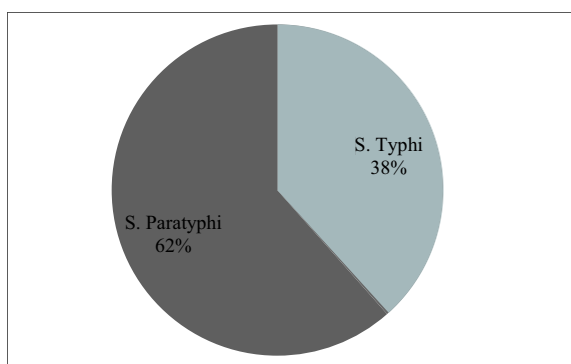
The widal test was found to be significant with blood culture ( $p=0.58$ ) (Table 1)

**Table 1: Results of Single Widal Agglutination tests for Salmonella Typhi & Paratyphi 'A' and bacteriological culture.**

Culture	+ve	-ve	Total
<b>Widal test</b>			
+ve	7	88	95
-ve	6	136	142
Total	13	224	237

#### Microbial Pattern of the result.

Out of 13 isolates, 5 (38.46%) were *S. Typhi* & 8 (61.54%) were *S. Paratyphi* A



**Figure 1: Percentage Distribution of *Salmonella* spp Isolated from Blood Culture**

Distribution of Single Widal Slide Agglutination Titre according to bacteriological culture.

Out of 237 enteric fever suspected patients sera processed for the Widal test, 30 (31.58%) had the agglutinin titre  $\leq 1:80$  and 65 (68.42%) had the agglutinin titre of  $>1:80$ . Out of the total 13 culture confirmed cases, 7 (53.84%) were positive by the single widal slide agglutination test (Table 2).

**Table 2: Widal test Versus Culture result.**

Salmonella agglutinin	Widal positive and culture positive (a)	Widal positive and culture negative (b)	Widal negative and culture positive (c)	Widal negative and culture negative (d)
O antigen	2	23	5	64
H antigen	5	34	2	54
Both O and H antigen	1	7	6	81
AH antigen	4	31	3	58

H antigen was found to have the highest sensitivity of 71.42% while both O and H antigen was found to have highest specificity of 92.04%. The lowest sensitivity was observed with that of both O and H antigen i.e. 14.28% whereas the lowest specificity was that of H antigen i.e. 61.36%. H antigen had the highest PPV of 12.82% and highest NPV of 96.42%. O antigen had the lowest PPV and NPV of 8% and 92.75% respectively. Highest efficiency was observed with both O and H antigen i.e. 86.31% (Table 3).

**Table 3: Evaluation of the Widal test with culture**

Salmonella agglutinin	Sensitivity (%)	Specificity (%)	Predictive values (%)		Efficiency (%)
			Positive	Negative	
O antigen	28.57	73.56	8	92.75	70.21
H antigen	71.42	61.36	12.82	96.42	62.10
Both O and H antigen	14.28	92.04	12.5	93.10	86.31
AH antigen	57.14	65.16	12.5	95.08	64.58

#### Cases Versus Onset of fever

Most of the culture confirmed cases i.e., 9 (69.23%) were positive in the 1<sup>st</sup> week of the onset of fever. Most of the widal positive cases i.e., 49 (51.57%) were positive in the 2<sup>nd</sup> week. There was no culture confirmed cases obtained 3<sup>rd</sup> week onwards. Very few of the widal positive tests were obtained in the 3<sup>rd</sup> week onwards (Table 4).

**Table 4: Blood culture and Widal test result Vs duration of onset of fever**

Cases	Onset of fever				Total
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	
Blood culture positive cases	9	4	0	0	13
Widal test positive cases	32	49	13	1	95

#### Discussion

A total of 237 samples were included in the analysis. The statistical analysis of result revealed no significant differences ( $p=0.58$ ) between the Widal agglutination reaction and culture diagnosis of clinical samples. Though blood culture is taken as gold standard but the results shows

that even Widal test may be reliable alone for diagnosis of enteric fever infections. Similar findings revealed that Widal test can be of diagnostic value when blood cultures are not available or practical<sup>1</sup>.

In this study, out of the total 237 enteric fever suspected patients sera processed for the Widal test, 65(68.42%) had the agglutinin titre >1:80. Among the total 13 culture confirmed cases, 7(5.38%) were positive by Widal test. Similar findings studied at Dhulikhel Hospital has reported the Widal test found to be positive in 59% of the enteric fever suspected cases<sup>18</sup>.

In present study, the Widal test was performed from the enteric fever suspected cases, at the cut-off of 1:80. Sensitivities of O, H, both O and H, and AH antigens were found to be 28.57%, 71.42%, 14.28% and 57.14% respectively; specificities were found to be 73.58%, 61.36%, 92.04% and 65.16% respectively. PPVs for the test were quite low; 8%, 12.82%, 12.5% and 12.5% respectively; and NPVs were 92.75%, 96.42%, 93.10% and 95.08% respectively. Efficiency of the test was highest with both O and H antigen (86.31%), lowest with H antigen (62.10%) followed by 64.58% for AH antigen and 70.21% for O antigen. Similar findings showed that the sensitivity of 46.67% and 60%; specificity of 75.68% and 72.4%; PPVs of 13.59% and 15.12%, and NPVs of 94.53% and 95.66% were reported for the O and the H agglutinin respectively<sup>7</sup>. In our country, the standard diagnostic titre of Widal test has not been set yet. So, the diagnostic titre suggested in the manufacturer's reference was followed throughout the study. In a similar cross sectional study at NPHL, the sensitivity of 10.35%; specificity of more than 90%; PPV of 50% and 75%, and NPV of 62.19% and 62.63% were reported for the O and the H agglutinin respectively<sup>9</sup>.

Among the test criteria for the performance testing of Widal test, PPVs were lowest i.e. the probability that enteric fever is present when Widal test is positive when is very low. This is due to the higher number of the false-positive results of the Widal test, considering the blood culture as the gold standard method. The false positive results in Widal test may be due to cross-reacting antigens or an anamnestic response. Salmonellae are divided into serological groups on the basis of O or somatic antigens. Other salmonellae share the H (flagellar), and antigen D with *S. enterica* serovar Typhi. Cross-reactions, producing a false-positive O-antigen titre in the Widal test, can therefore occur with any of these serovars. There are more than 40 cross-reacting antigens between *S. enterica* serovar Typhi and other Enterobacteriaceae<sup>12</sup>.

The other reason for both false-positive and false-negative results in Widal test is the lack of antigen standardization. This in turn is due to many factors like lack of knowledge of baseline titre of population and unavailability of the

antigens of the local isolates<sup>10</sup>. The false negative Widal test results lead to a low sensitivity and negative predictive value for the test<sup>11</sup>.

Out of the 13 culture confirmed cases, 6 (46.15%) were Widal negative at the titre of 1:80. Similarly, among the 13 culture confirmed cases, 5 (38.46%) were negative for anti-O agglutinin and 2 (15.38%) were negative for anti-H agglutinin at cut-off of 1:80. Although the agglutinin may be present in lower titre, it is well documented that patients with confirmed typhoid fever may have a negative Widal test throughout the course of their illness. Lack of antibody response among patients with blood culture-positive typhoid fever is observed because of collection of blood sample in the acute-phase, high level of background antibodies in an endemic region and, though not clear yet, the early administration of antimicrobial which in some individuals blunts the antibody response or because of the undefined host or bacterial factors or prior antibiotic treatment<sup>14</sup>.

In present study, among the culture confirmed cases, the highest positive number i.e. 9 (69.33%) were found in the 1<sup>st</sup> week of the onset of fever. Similar study showed the highest positive culture cases of 96.9% in the 1<sup>st</sup> week of the onset of fever<sup>7</sup>. The highest number of 49(51.57%) positive Widal cases were found in the 2<sup>nd</sup> week of the onset of fever. In contrast, the highest positive Widal cases of 85.16% were found in the 1<sup>st</sup> week of the onset of fever. Some of the Widal positive results in the early week as in this study can be attributed to the regular sub-clinical sensitization of the patients with the *Salmonella* strains. High positivity of Widal test in the 1<sup>st</sup> can attribute to the hyper-immune state of the patients.

The most widely used serological test in enteric fever is to detect antibody against O, H, AH and BH antigen of *Salmonella* Typhi and *Salmonella* Paratyphi A & B by widal test. The cut off value of Widal agglutination test was considered as 1:80 for all O, H, AH and BH<sup>16</sup>. Although Widal test usually become positive from 2<sup>nd</sup> week, in this study, out of 5 culture positive typhoid patients, 1(33.33%) had an initial O and H titre  $\geq 160$  in the 1<sup>st</sup> week of illness. Closely similar findings found that 33(47.8%) out of 69 culture positive cases in the 1<sup>st</sup> week of illness<sup>6</sup>.

This findings were most probably attributable to a hyper immune or immunologically sensitized population which is continuously exposed to *Salmonella* Typhi and other Salmonellae<sup>17</sup>. This observation is also of practical importance as second specimens are often not sent to the laboratory. The results obtained are also of relevance to the concept that specimens taken in the 1<sup>st</sup> week are of little use in the serodiagnosis of typhoid.

## Conclusion

Our finding revealed that, *S. enteric* serovar Paratyphi A was found to be more prevalent than *S. enterica* serovar Typhi. Statistical analysis revealed no significant differences ( $p=0.58$ ) between the Widal agglutination reaction and cultural diagnosis of clinical samples. Though blood culture is taken as gold standard but the results showed that even Widal test may be reliable alone for diagnosis of enteric fever infections. Culture positivity 5.4% and widal positivity 40% irrespective of demographic status single slide agglutination test of antibody titres  $\geq 1:160$  is considered to be reliable for diagnosis of enteric fever.

All the isolates were 100% susceptible to Chloramphenicol and 100% resistance to Nalidixic acid. Out of 13 isolates, 8 were found to be multi drug resistant. Because of emergence of multi-drug resistance, culture should be preferred along with Widal to determine the antibiotic of choice and minimize spread of antibiotic resistance.

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**Conflict of interest:** None declared

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