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## COMPARISON OF BLOOD CULTURE WITH SLIDE AND TUBE WIDAL AGGLUTINATION TESTS IN THE DIAGNOSIS OF TYPHOID FEVER.

Krati R. Varshney<sup>1</sup>, Shipra Singhal<sup>2</sup>, Shadma Yaqoob<sup>3</sup>, Priyanka Shukla<sup>4</sup>, Yi Singh<sup>5</sup>.

1. Junior Resident, Department of Microbiology, Era's Lucknow Medical college & hospital.
2. Junior Resident, Department of Microbiology, Era's Lucknow Medical college & hospital.
3. Assistant Professor, Department of Microbiology, Era's Lucknow Medical college & hospital.
4. Assistant Professor, Department of Microbiology, Era's Lucknow Medical college & hospital.
5. Professor & H.O.D, Department of Microbiology, Era's Lucknow Medical college & hospital.

### CORRESPONDING AUTHOR:

Krati R. Varshney,  
A-17, New Girls Hostel,  
Era's Lucknow Medical  
College & Hospital Lucknow.  
E-mail: krativarshney2001@yahoo.co.in

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**ABSTRACT: BACKGROUND:** Typhoid fever, caused by *Salmonella Typhi*, is endemic in the Indian sub-continent including Bangladesh, South-east and Far-east Asia, Africa and South Central America. The disease can occur in all age group with highest incidence among children. A simple, reliable, rapid and early diagnostic test has been a long felt need of the clinicians. Blood culture is regarded as the gold standard for diagnosis. However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing countries. To fulfill the purpose the present study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital for the period from Oct 2012 to Mar 2013. **MATERIALS AND METHODS:** 300 blood samples, collected aseptically from patients clinically diagnosed of Typhoid fever, were evaluated by blood culture, Tube and Slide Widal test **RESULTS:** This study included 300 cases having clinical suspicion of typhoid fever. Subjects were investigated by blood culture, Tube & Slide Widal agglutination tests. Among them, 42(14%) were subsequently confirmed by blood culture for *S. Typhi*, 123(41%) and 150(50%) cases were positive as having significant titre of Slide and Tube Widal agglutination tests respectively. Thus, in comparison to the gold standard test i.e blood culture, Tube and Slide Widal test were better and consumed less time. Comparing Slide and Tube Widal test, Tube is better. **CONCLUSION:** Tube& Slide Widal agglutination test is found to be better and could be applied as a good alternate in resource poor nation. Further, it is simple to perform, reliable when compared to blood culture test, and rapid, with results being available in 18 hrs when compared to 48 hours for blood culture.

**KEY WORDS:** Blood culture, *Salmonella Typhi*, Widal

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**INTRODUCTION:** Typhoid fever is a severe multisystemic illness characterized by the classic prolonged fever, sustained bacteremia without endothelial or endocardial involvement and bacterial invasion of and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes and Peyer's patches<sup>1</sup>. Typhoid fever is potentially fatal if untreated. It is caused by *Salmonella typhi* a Gram negative bacilli<sup>2</sup>. Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with other febrile infections. Incidence of typhoid fever has been estimated as approximately 22 million cases with at least 200,000 deaths occurring worldwide annually<sup>3</sup>.

Isolation of Salmonella from blood, urine or stool is the most reliable means of confirming an infection. However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world<sup>4</sup>. In addition, easy availability and widespread use of antibiotics in the community makes it frequently difficult to isolate the organism from blood culture may be difficult, which is invasive and difficult to carry out.<sup>5</sup> Blood culture is regarded as the gold standard for diagnosis and carry 70-75% diagnostic yield in the first week of illness.<sup>6</sup>

**MATERIALS AND METHODS:** This cross sectional study included 300 cases having clinical suspicion of typhoid fever. This study was conducted in Department of Microbiology, Era's Lucknow Medical College & Hospital, Uttar Pradesh, India during October 2012- March 2013. The blood samples were collected for blood culture and Widal test. The blood culture was done by using brain heart infusion (BHI) broth and it was incubated. Subsequent sub-cultures were made on MacConkey's agar and blood agar media after 24, 48 and 96 hours and the final sub-culture was made on the seventh day. The growth of *S. Typhi* was identified by the standard biochemical tests and it was confirmed by agglutination with the Salmonella polyvalent 'O', 09 and the H: d antisera. The Widal test was done by using the standard procedure<sup>7</sup>. The Widal test was done by the Slide and Tube agglutination method. It was considered as positive when a titre of equal to or more than 1:80 was observed.

## INCLUSION CRITERIA

- i) Fever for  $\geq 7$  days, with no obvious focus of infection,
- ii) Abdominal discomfort- constipation or loose motions,
- iii) Coated tongue, toxic look,
- iv) Hepatomegaly, splenomegaly,
- v) Relative bradycardia rose spot etc.

## EXCLUSION CRITERIA

- i) Persons who were immunized with typhoid vaccines.
- ii) Patient suffering from fever other than typhoid.
- iii) Patients on antibiotics

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## RESULTS

**Table 1: Positive cases by blood culture**

Age in years	Blood culture Positive
1-5 = 102	15(14.70)
6 - 10 = 81	12(14.81)
11-15 = 72	09(12.50)
>15 = 45	06(13.33)
Total = 300	42(14.00)

Among 300, 42(14%) were subsequently confirmed on the basis of positive blood culture for S. Typhi

**Table 2: Positive cases by Slide Widal agglutination test (SWAT)**

Age in years	SWAT Positive
1-5 = 102	30(32.35)
6 - 10 = 81	18(22.22)
11-15 = 72	36(50)
>15 = 45	39(86.66)
Total = 300	123(41)

123(41%) cases were positive as having significant titre of Slide Widal agglutination test in first sample.

**Table 3: Positive cases by Tube Widal agglutination test (TWAT)**

Age in years	TWAT Positive
1-5 = 102	33(32.35)
6 - 10 = 81	21(25.92)
11-15 = 72	48(66.66)
>15 = 45	48(66.66)
Total = 300	150(50)

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150(50%) cases were having significant titre of Tube Widal agglutination test. 150 cases neither had blood culture positive result nor significant titre on Widal test, but still were included due to clinically in favour of typhoid fever.

**Table 4: Comparison of Blood culture, Slide Widal Agglutination tests (SWAT) and Tube Widal Agglutination tests (TWAT) of first sample among clinically suspected typhoid cases.**

Test	Positive	Negative
Blood culture ( n = 300 )	42 ( 14.00 )	258 (86.00)
SWAT ( n = 300 )	123 ( 41.00 )	177 (59.00)
TWAT ( n = 300 )	150 ( 50.00 )	150(50.00)

Figures in parenthesis indicate percentage.

Thus, in comparison to the gold standard test i:e blood culture, Tube and Slide Widal test were better and were less time consuming. Comparing Slide and Tube Widal agglutination test tube accounted to be better.

**DISCUSSION:** Isolation of the causative agent by culture has remained the gold standard for diagnosis of enteric fever. Blood culture has got its limited diagnostic utility due to low sensitivity. Although the Widal test has been used for more than a century in many developing countries but it is non-specific, poorly standardized, often confusing and difficult to interpret<sup>8</sup>. Moreover, sharing of O and H antigens by other Salmonella serotypes and other members of *Enterobacteriaceae* makes the role of Widal test even more controversial in diagnosing typhoid fever<sup>9</sup>.

Most of the children presented in the second week of their illness and we used a cut-off titer of 1:80. The use of a higher cut-off titre would have further reduced the sensitivity of the test. Although this has been questioned,<sup>10</sup> antibiotic therapy has been shown to alter the antibody response to *S. Typhi* infection,<sup>11</sup> and given that a large number of the patients had previously received antibiotics, this factor may have altered the antibody titers against O antigens. The Tube Widal Agglutination was significantly more sensitive than the Slide Widal Agglutination test, although the sensitivity lowers than those reported from Malaysia and The Philippines.<sup>12-14</sup> Our findings of sensitivity were also lower than values 90% reported recently by Karamat and others<sup>15</sup> from northern Pakistan.

These differences may be due to several factors including the genomic diversity among *S. Typhi* isolates in the region<sup>16</sup> and differences in antigenic epitopes. Other factors responsible for reported differences in areas of high endemicity are various stages of the illness and the rate of increase of IgG antibodies to the outer membrane proteins (OMPs), which may interfere with identification of concomitant IgM antibodies. Most of our patients presented in the second week of their illness, whereas information on duration of illness is lacking in other studies.<sup>12,13,16</sup> The relative low sensitivity of the blood culture in diagnosing typhoid fever is understandable in the wake of widespread antibiotic use<sup>17</sup> and the difficulties of obtaining large enough blood volumes

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for cultures. Although bone marrow cultures significantly increase the yield from cultures,<sup>18-19</sup> the comparative study with bone marrow culture is not done in our study. It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among *S. Typhi*, they remain an essential investigation. In many circumstances, especially among partially treated cases presenting to health facilities, combining cultures with a serological test may reduce the diagnostic difficulty in typhoid fever. Our data indicate that combining the blood cultures with a Widal Slide & Tube agglutination tests will significantly improve the diagnostic yield.

**CONCLUSIONS:** After analyzing the findings of the present study it was concluded that although blood culture is gold standard for diagnosis of typhoid fever, the rising titre of Widal test is also helpful for diagnosis of typhoid fever. Our data indicate that combining the blood cultures with a Widal Slide & Tube agglutination tests will significantly improve the diagnostic yield. Tube & Slide widal agglutination test is found to be better, less time consuming and could be applied as a good alternate in resource poor nation. Further, it is simple to perform, reliable when compared to blood culture test, and rapid, with results being available in 18hrs when compared to 48 hours for blood culture.

There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for typhoid fever.

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