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A COMPARATIVE STUDY OF WIDAL TEST WITH CLOT CULTURE IN DIAGNOSIS OF ENTERIC FEVER IN FEBRILE PATIENTS AT TERTIARY CARE HOSPITAL IN SOUTHERN RAJASTHAN

Microbiology

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ABSTRACT

AIM: To correlate the result of Widal test and clot culture in the diagnosis of enteric fever.
METHOD: Blood samples were collected from 306 febrile patients with symptoms clinically similar to enteric fever and visiting MBGH, R.N.T. Medical College, Udaipur from March to September2018 clot was seperated from blood and used to isolate S.typhi and S.paratyphi. Slide and tube agglutination test were used to determine antibody titer. An antibody titer of >1:80 for anti TO and >1:160 for anti TH were taken as a cut of value to indicate recent infection of enteric fever. The identification of isolates by phenotypic method (biochemical reactions) and confirmation was done using antisera.
RESULT: One hundred sixty three(53.2%) participants were females and one hundred forty three (46.7%) were males. 21(6.8%) cases of S.typhi and 6(1.96%) cases of S.paratyphi were identified with the total prevalence of typhoid fever 8.7%.
CONCLUSION: Widal test; unless done in paired sera can be used for screening of enteric fever. Samples yielding high titer of TO(1:80) and TH (1:160) are reliable, as maximum no. of positive clot culture (8.7%) belong to this titer one exceptional sample had TO 1:80 and TH negative, this was possibly due to sample sent in 1st week.

KEYWORDS

Widal test, Clot culture, Salmonella typhi, Salmonella paratyphi.

BACKGROUND

Enteric fever is a serious systemic infection caused by the enteric pathogen Salmonella typhi.

The infection is spread by the faecal-oral route and closely associated with poor food hygiene and inadequate sanitation. In endemic areas school children and young adults are most frequently affected during recent decades. Typhoid fever has largely disappeared from industrialized countries, but remains a serious public health problem in developing countries.[1]

The salmonellae are primarily intestinal parasites of vertebrates & which infect man, leading to enteric fever, gastroenteritis & septicaemia. The most important member is Salmonella typhi, the causative agent of enteric fever. Man is the only natural host for S.Typhi & S. paratyphiA whereas most of the other salmonellae are chiefly pathogenic in animal like poultry, pigs, cattle etc.[2]

Typhoid fever is a prolonged illness characterized by bacteremia with Salmonella typhi, a highly evolved gram-negative bacterial parasite that infects only humans. Despite the bacteriological similarities between S. typhi and other enterobacteriaceae, the clinical picture of typhoid is usually distinctive and differs in many respects from that of other septicemias caused by gram-negative organism.[3]

S. typhi is able to survive and reproduce inside monocytic phagocytes, and in typhoid fever S. typhi is reported to be confined to the monocyte-platelet fraction of the blood, fever is a prolonged illness characterized by bacteremia with Salmonella typhi.[4]

S. typhi probably invades the gut mucosa in the terminal ileum through specialized antigen-sampling cells, known as M-cells, which overlie gut-associated tissue, through enterocytes, or via a9r3paracelluar route.[5]

The bacteria adhere to the intestinal mucosa in the terminal ileum through interaction with an epithelial receptor, the cystic fibrosis transmembrane conductance regulator protein. A key, early step in the infectious process is the induction of intestinal epithelial cells to increase membrane receptor levels, with enhanced bacterial ingestion and submucosal translocation.[6]

Enteric fever continues to be a global health problem and isolation of organism from blood is the gold standard for the diagnosis. Although blood culture is the ideal method but clot bile culture has shown a higher isolation rate than blood culture.[7]

An important attribute of clot culture is that it utilises what is usually considered the left over material, giving it the potential of increasing Salmonella species isolation without requiring additional blood from patients and also the sera after removal can be used for different serological assay.[7]

The clot culture is a technique wherein serum, which may contain antibacterial activity, is removed from a blood specimen and the remaining clot is inoculated into a broth medium. The technique is highly recommended for isolation of Salmonella Typhi.

The present study was undertaken for isolation of Salmonella species in clot culture by various biochemical test & antibiotic susceptibility pattern, Mac Conkey agar media, XLD agar media.

METHODS

PATIENT INFORMATION, BACTERIAL ISOLATION, SPECIES CHARACTERIZATION.

It was a cohort prospective study were conducted in Department of Microbiology at RNT Medical College, Udaipur (Rajasthan) , the study included patients name, age, gender, and their clinical suspected enteric fever history.

The sample received in a period of 1year by OPD & IPD would be processed in the microbiology lab. Blood Samples were included as a part of study.

INCLUSION CRITERIA[8]:

Patient with clinically suspected Enteric fever (pyrexia of less than a week) from all age groups, And diagnosed for Widal test. Informed consent was obtained before collecting the blood specimen.

EXCLUSION CRITERIA[8]:

Patients with respiratory tract infections (tuberculosis ,pneumonia), Patients with urinary tract infections, Patients with malaria, Immuno-compromised patients(AIDS) ,History of fever more than a week.
By using these inclusion and exclusion criteria about 306 suspected febrile patients were recruited for this study then data and blood sample were collected from these 306 patients.

PROCESSING OF SAMPLES

Blood sample collection and inoculation: Using a sterile syringe and needle, about 8-10 ml of blood from each adult study subject was collected in a clean and dry tube under strict aseptic precaution in the clinically suspected cases of enteric fever before starting the antibiotics. allowed to clot the blood and then centrifuge in centrifugator, the serum was free from blood, and performed slide

agglutination test (by precision biomed Kit).

Qualitative slide agglutination and semi quantitative tube agglutination (titration) were performed using febrile antigen kits of Salmonella typhi (by precision biomed. kit). The slide agglutination test is used as a screening test for the presence of anti TO and anti TH antibodies in the patient's serum.

SAMPLE PROCESSED IN LAB. FOR CULTURE[9]

The clot were lysed mechanically made to semifluid with the help of sterile glass rod. The resultant produce (1-2ml) dispensed into the culture medium bottle containing 20ml bile broth containing 0.8% concentration of sodium taurocholate (bile).

QUALITY CONTROLS

Standard operational procedures were followed during processing of each sample and all the instruments used for sample processing were checked every morning for properfunctioning. E.coli ATCC 25922 was used as a reference strain as rod.[12]

Samples were subcultured on Blood agar, Mackoney agar, XLD agar media for isolation of Salmonella *enterica* serotype Typhi (*S.*Typhi) and *S. enterica* serotpe Paratyphi (S.Paratyphi) A, B, C. which would be further included in the study.

Isolates were confirmed further with the help of various biochemical test, sugar fermentation test, amino acid decarboxylase test, slide agglutination (by precision After 48 hours incubation sub culturing was performed from the bile broth on XLD agar (microgen).

After 48 hr. of incubation cultures were proceed on MACCONKEY AGAR (TM MEDIA), XLD (HiMedia, INDIA), BLOOD AGAR (HiMedia, INDIA), while negative broth culture kept forseven days and sub cultured done on the same, before reported negative.

When the growth was lactose fermented (pink or red in colour), the sample was excluded. on the other hand if the growth was non lactose fermented (colourless or pale in colour), single colonies was subcultred by streaking plate method on new XLD agar plate and Macconkey agar to simplify the colony isolation and identification by different biochemical tests including Triple Sugar Iron agar (TSI), (MERCK), simmon's citrate utilization test(MERCK), motility (Difco™), urease test (TM media ltd. India) and lysine decarboxylation (MERCK) and sugar fermentation test (MANNITOL, MALTOSE, GLUCOSE, LACTOSE AND SUCROSE 25mcg/disc HiMedia, INDIA) to determine Salmonella or other bacteria.[7]

Identification of Salmonella at genus level can be confirmed by slide agglutination using polyvalent O antisera (group A-G). Then the serotype can be identified by using type specific O antisera. (especially agglutinates with O2(group-A) antisera (DENKA SEIKEN Co. Ltd., JAPAN) in case of S.Paratyphi A[1]

Antibiotic susceptibility: The isolates were tested for their susceptibility to 08 different antimicrobial agents Amoxicillin, Azithromycin, Ceftriaxone, Ciprofloxacin, Chloramphenicol, Co-trimoxazole, Nalidixic acid and Tetracycline. (HiMedia, Lab, INDIA) on Mueller Hinton's agar by disc diffusion method. ALL the assay were performed as per the guidelines of CLSI, 2017. E. coli ATCC25922 were used as a control strain for all susceptibility assays.[12]

RESULT:

A Total 5121 serum samples tested for Widal during five month (May 2018 to Sept 2018) of duration in the central lab, in which 600 were showing positive result for Widal test, among these 600 serum samples, 306 serum samples found to be eligible according to inclusion and exclusion criteria for this study.

DEMOGRAPHIC CHARACTERISTIC:

Out of 306 serum samples there were 143 (46.7%) males and 163(53.2%) female were included as a part of the study.

AGEWISE DISTRIBUTION:

Out of 306 serum samples, 5 (1.63%) were belong to age group 11-20 years, 83 (27.12%) belong to age group 21-30 years, 97 (31.69%) belong to age group 31-40 Years , 52 (16.99%) belong to age group41-50 Years, 34 (11.11%) belong to age group 51-60 years and 35(11.43%) belong to 61-70 years. Most affected age group belong to 31-40 (31.69%) followed by age group 21-30 years (27.12%).

TABLE NO.1

Sex-wise distribution of total sample

TOTAL NO. OF SAMPLE 306		
Age group (Yr.)	No. of samples	Percentage (%)
11-20	05	1.63
21-30	83	27.12
31-40	97	31.69
41-50	52	16.99
51-60	34	11.11
61-70	35	11.43

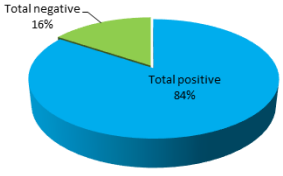
Qualitative slide agglutination reaction result of Widal test of febrile patients suspected for typhoid fever.

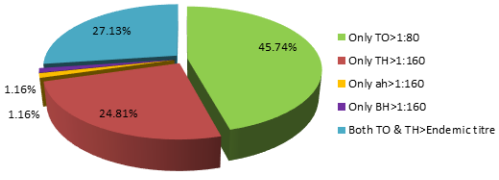
Table no. 2

Total sample	Only TO>1:80	Only TH>1:160	Only ah>1:160	Only BH> 1:160	Both TO & TH>Endemic titre	Total positive	Total negative
306	118	64	03	03	70	258	48

70 (22.9%) patients had reactive reaction for both O and H antigens while 118 (38.5%) have reactive only for O antigen. Only 64 (20.9%) of patients have reactive reaction for H antigen only. Overall, 258 (84.3%) patients had reactive slide agglutination test by either or both of O and H antigens. Among 258 positive for Widal Test there were 26 samples clot culture positive and 48 negative for Widal there was only 01 sample positive for clot culture.

GRAPH





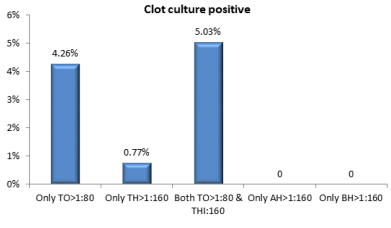
Percentage of clot culture positivity among positive (having significant titer) Widal 258 samples.

Table no.3: Percentage of clot culture positivity among positive (having significant titer) Widal 258 samples.

Significant Widal Titer	No. (258)	Clot culture positive
Only TO>1:80	118	11 (4.26%)
Only TH>1:160	64	02 (0.77%)
Both TO>1:80 & THI:160	70	13 (5.03%)
Only AH>1:160	03	0
Only BH>1:160	03	0

Among the 306 samples 258 samples have significant Widal titer,26 samples were clot culture positive, there were only 2 samples among positive culture having titre TO & TH 1:320 AH 1:160 and Salmonella paratyphi isolated in it.48 samples have insignificant titer for Widal test there was only 01 sample positive for clot culture at titre TO1:80.

GRAPH



The distribution of clot culture results of febrile patients suspected of typhoid fever at MBGH Hospital.

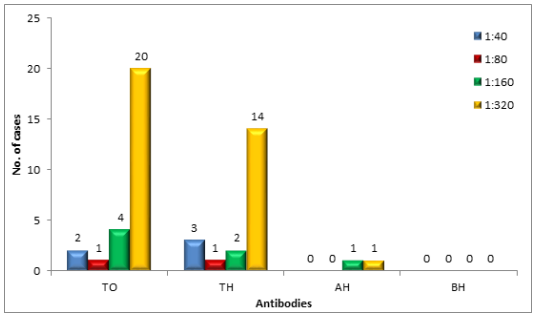
Table No. 4 : The distribution of clot culture results of febrile patients suspected of typhoid fever at MBGH Hospital

Bacteria	No. of isolates (%)
Salmonella typhi	21 (6.8%)
Salmonella paratyphi A	06 (1.9%)
Other bacteria	150 (49%)
Negative clot culture	129 (42%)
Total	306 (100%)

Among 306 samples 21 (6.8%) Salmonella typhi, 06 (1.9%) Salmonella paratyphi A were isolated, 129 samples show no growth while 150 samples were positive bacteria other than salmonella species.

Table no. 5: Distribution of different antibody titer among 27 clot culture positive patients

Different Antibody Titer Among Clot Culture Positive Patients				
Antibody	1:40	1:80	1:160	1:320
TO	02	01	04	20
TH	03	01	02	14
AH	00	00	01	01
BH	00	00	00	00



DISCUSSION

A Total 5121 serum samples tested for Widal during five month (May 2018 to Sept. 2018) of duration in the central lab, in which 600 were showing positive result for Widal test, among these 600 serum samples, 306 serum samples found to be eligible according to inclusion and exclusion criteria for this study.

Typhoid fever remains as an important cause of morbidity even at present. In the present study, 306 blood samples were taken from patients with clinically suspected enteric fever and clot culture and Widal test were done for the diagnosis.

In our study we found 163 (53.2%) were female and 143 (46.7%) were male patients., a similar study done by Gizachew Andualem[13] et al in yr. 2014, among 270 febrile patients One hundred and eighty six (68.9%) participants were females and eighty four (31.1%) were males.

The age group 31-40 years (31.69%) was mostly affected in this study, these findings were much similar to the study carried out by Renu Mathew et al[10]. Among the 306 cases studied, clot culture were positive in 27 patients of which 21 (77.77%) isolates were *Salmonella typhi* and only 06 (22.22%) were *Salmonella paratyphi* A. Culture positivity was 8.8%. Significant Widal titers were seen in 258 (84.3%) samples.

In a similar study done by Krishnan P et al[14] in Chennai, 70% of isolates were *Salmonella typhi* and 30% were *Salmonella paratyphi* A, which was in accordance with our study. Among 306 sera 84 blood samples shown <1:80 antibody titer for both antigens, 15 were culture positive for *Salmonella typhi* in both blood and clot culture method. Out of 194 samples that had shown <1:80 antibody titer for both O and AH antigens, 6 blood samples were positive for *Salmonella paratyphia*. Another similar study done by Renu et al[10] in 2013. Among the 290 cases studied, 117 patients were positive for enteric fever with either positive Widal test and or with culture positivity. Culture was positive in 40 patients (34%). Out of 117 positive cases of enteric fever, 89 (76%) had typhoid fever and 28 (24%) patients had paratyphoid fever. This was on the basis of significant Widal titers and isolation of Salmonella from blood or clot, Significant Widal titers were seen in 96 patients[10].

In our study among 27 isolates of Salmonella (21 S.typhi and 6 S.paratyphi A) showed that 100% susceptibility for ampicillin, ceftriaxone, cotrimoxazole, azithromycin, chloramphenicol, nalidixic acid and pefloxacin while as for ciprofloxacin and nalidixic acid 14.28% and 4.76%. Salmonella typhi were showing resistant pattern respectively.

Our study is in accordance with Smitha et al[11] found that Strains (both *S. typhi* and *S. paratyphi A*) were sensitive to drugs like Ampicillin, Chloramphenicol, Co-trimoxazole, Gentamicin, Ceftriaxone and 17% of *S. Typhi* and 40% of *S. paratyphi A* were intermediately sensitive to ciprofloxacin. The strains were 100% resistant to nalidixic acid. Nalidixic acid resistance is considered a marker of low level resistance to ciprofloxacin among Salmonella and also an indicator of treatment failure with ciprofloxacin[11].

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