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Original Research Article

Patterns of Widal test Results among Patients Suspected to Have Typhoid Fever Attending Bsuth Makurdi Nigeria

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ABSTRACT

Widal agglutination test which is the main laboratory procedure for diagnosis of typhoid fever in our rural and urban settings has received cautious acceptability and often outright rejection in other places. This study was set up to ascertain suitability or otherwise of Widal test in the diagnosis of typhoid fever among hospital attendees with clinical diagnosis of typhoid fever. Three hundred and eighty-nine patients with clinical diagnosis of typhoid fever attending Benue State University Teaching Hospital (BSUTH) Makurdi between November 2016 and April 2017 were consecutively recruited into the study. Blood and stool samples were collected where Widal test and cultures were carried out on them respectively.

Among the febrile patients 40.1% (n=156) and 59.9% (n=233) respectively had Widal agglutination tests titres of <1: 80 and ≥1: 80 while five (1.3%) of the patients grew Salmonella in stool. Analysis of the results showed the following: Sensitivity (O =73.8%, H = 25.3%); Specificity (O = 77.1%, H = 79.8%); Positive Predictive Value PPV (O = 13.7%, H = 5.0), and Negative Predictive Value NPV (O = 99.4%, H = 96.9%). Widal agglutination test alone has limited value in routine diagnosis of typhoid fever due to its poor positive predictive value and hence should not be used alone or at best with extreme caution to establish diagnosis of typhoid fever.

Key Words: Fever, Typhoid Widal Test.

INTRODUCTION

Laboratory diagnosis of typhoid fever has always been the use of Widal test in most primary and secondary healthcare centres in Benue state and indeed entire Nigeria. Many tertiary health centres across the country as well deploy this method in the establishment of typhoid fever diagnosis. The level of usefulness of Widal test in the management of typhoid fevers has received varying and divergent levels of acceptability across the globe. The test procedure however often remains the only available tool to be relied upon by health personnel in our communities to commence anti-typhoid therapy. [1-4]

In a study on 270 febrile patients in a hospital in Ethiopia, Widal test was found to have low sensitivity, specificity and positive predictive values (PPV), but was found to have good negative predictive values (NPV). On the other hand in a related study in Tanzania on 1,680 febrile children, Widal test was found to be positive in 1% (n=16)

of them. The test was found to be 75% sensitive, 98% specific, 100% NPV and only 26% PPV. Also in Benin-City in a study on 271 febrile patients showed that: 45.76% had positive Widal agglutination test, 22.10% of the blood samples cultured Salmonella species. The study found a sensitivity of 35%, specificity of 51%, PPV of 17% and NPV of 73% with an overall disqualification of Widal test procedure as a valid tool for typhoid diagnosis. [5-7]

At BSUTH Makurdi, Widal test is often the first test request clinicians often turn to commence investigation for typhoid fever. This is followed by stool and blood cultures while smaller health centres in the rural settings rely only on Widal test results to commence anti-typhoid treatment. This study is therefore set out to ascertain the usefulness or otherwise of Widal agglutination test in the management of typhoid fever among febrile patients presenting to our hospital. [7-9]

MATERIALS AND METHODS

Study Setting

The study was carried out at the Benue State University Teaching Hospital (BSUTH) over a six months period (November 2016-April 2017). Patients attending the hospital over this period who had fever (Axillary temperatures of $\geq 37.5^{O}C$) and with clinical diagnosis of typhoid fever were recruited into this study. Participation was voluntary and consents of the subjects were duly obtained. Well structured questionnaires covering relevant socio-demographic and medical information such as recent antibiotics intake were obtained. The control group which was gender and age matched was obtained from people who visited the Microbiology laboratory who had no fever such as IT students and patient's relations. Blood and Stool samples were then collected from each consented subject and analysed accordingly. Data obtained was analysed using simple descriptive methods and SPSS 16 version Statistical software. P values less or equal to 0.05 were considered significant. Ethical approval for the study was obtained from the Ethics review board of the BSUTH.

Collection and Processing of Blood Samples

About three to five millimetres of blood were collected from superficial veins using sterile disposable needles and syringes into plain test tubes. These were transported to the serology laboratory and Widal test carried out on the respective sera using tube agglutination test in line with standard laboratory procedures. The cut off points for somatic (O) and flagellar (H) antigens were 1:≥80 and 1: ≥160 respectively. [8-10]

Collection of stool specimens

Fresh stool specimens were collected into clean, dry, leak – proof, chemical-free and screw-crap universal containers. The samples were transported in dry containers to the Medical Microbiology Laboratory of the Benue State University Teaching Hospital, where they were processed with minimum delay.

Stool culture

Approximately 1 gram of well-mixed faeces was inoculated into deoxycholate citrate agar (DCA) and incubated overnight at $37^{o}C$.
Non-lactose fermenting colonies on DCA were sub-cultured on to nutrient agar overnight at $37^{o}C$ for purity. Pure cultures were incubated into Kligler Iron Agar (KIA) and urea agar slopes.

Test for Identification of Isolates

All isolates were identified based on their motility, morphology, biochemical and serological reactions.

Motility Test

Pure cultures from nutrient agar plates were sub-cultured in peptone waster and incubated at $37^{o}C$ for 6 hours. A ring of plasticine was made on a clean, grease-free slide. A drop of the well-mixed culture was placed on a clean cover-slip. The circular area of plasticine on the slide was inverted

and super-imposed over the drop of culture on the cover-slip. Quickly, the arrangement was inverted, with the cover-slip facing upwards. Thus, the culture hanged down in the centre of the ring of plasticine.

The preparation was examined with x10 objective lens with reduced illumination. True motility was indicated by the tumbling movement of bacteria from one spot to another. This was clearly differentiated from the Brownian movement of water molecules.

Urease Test

This test was carried out to rule out *Proteus Species* which hydrolyze urea to yield ammonia and carbon-dioxide. The presence of ammonia renders the medium alkaline and the indicator (Phonol red) changes colour to a reddish-pink.

The urease broth (OXOID) in test tubes was inoculated by shaking few colonies from pure cultures of the test organism on nutrient agar into the urea broth. All tubes were incubated at 37^{o}C for 6 hours.

Controls: Positive: *Proteus vulgaris*
Negative: *Escherichia coli*

Serological Typing

A thick saline suspension of an overnight agar culture of the isolate was made on one end of a clean, grease-free slide. Using a flame-sterilized wire-loop, a drop of the bacterial suspension was mixed with a drop of O and H antisera.

A negative control was run by mixing a drop of the bacterial suspension with a drop of sterile normal saline at the other end of the same slide. The preparations were tilted on the slide several times and the result was read within 60 seconds.

A positive result was indicated by agglutination. Isolates were confirmed as *Salmonella typhi* by their agglutination with specific antisera, in conjunction with biochemical reactions on Kligler Iron Agar (KIA). [11,12]

Test on KIA

A pure culture of the test organism on nutrient agar was inoculated on to KIA slope by using a straight wire to stab the broth and streak the slope. The tubes were plugged with sterile cotton wool and incubated at 37^{o}C overnight.

Specific Identification of Bacterial Isolates

Bacterial cultures were identified as *Salmonella typhi* if they showed the following biochemical characteristics:

Lactose ---------- Negative
Mannitol ---------- Positive
Glucose ---------- Negative
Oxidase ---------- Negative
Motility ---------- Positive
Urease ---------- Negative
Growth on KIA--- Slope: (red-pink-alkaline)
------Butt: (Yellow-acid reaction)
---------- Gas production (nil)
---------- Hydrogen Sulphide production (weak)

Antibiotic Sensitivity Testing
Preparation of inoculums

Pure cultures of *Salmonella typhi* were picked with flame-sterilized wire loop and inoculated into 10 milliliters of peptone water in McCartney bottles. The peptone water cultures were incubated at 37^{o}C for 4 hours. The turbidity of the growth was compared with that of a 0.5 Barium Sulphate turbidity standard corresponding to that of a previously prepared inoculum which yielded a dense but no-confluent growth on a sensitivity agar plate. Each inoculum was diluted to match the standard and used in flooding a sensitivity test plate.

Method of Antibiotic Sensitivity Test.

The modified Kirby-Bauer's disc diffusion technique was used to determine the antibiotic sensitivity of each isolate. Commercial standardized single discs of Chloramphenicol, Cotrimoxazole,

Amoxicillin, Erythromycin Ciprofloxacin, Ofloxacin, Amoxiclav, Ceftriaxone, Gentamicin, and Tetracycline were placed on the surface of the sensitivity agar plates previously flooded with a broth culture of the test organism. All the plates were incubated overnight at 37^{o}C. [12,13]

A strain of *Escherichia coli* isolated in the laboratory was used as the control. Both test and control plates were incubated in the same atmosphere and temperature. [13-15]

Interpretation of Zones of Inhibition

An insolate was regarded as sensitive to a certain drug if the diameter of the zone of inhibition around the disc is greater than or equal to that of the control organism (18mm) and resistant if the zone diameter is less than 18mm. [13]

RESULTS

Of the 389 patients suspected patients to have typhoid fever, 61.2% (238) were males and 38.8% (51) females; the age range was four and 72 years with a median age of 56 and bi-modal ages of 41 and 52 years.

Analysis of blood samples for Salmonella antibody titres among the subjects showed that 233(59.9%) of the subjects had Salmonella antigen antibody titres ≥ 1:80. Further breakdown showed that 138 (35.5%), 16 (4.1%), and 32 (8.2%) respectively had 1:80, 1:160 and 1: 320 antibody titres against the somatic antigens. And 93 (24.0%), 63 (16.2%) and 13 (3.3%) respectively had 1: 80, 1: 160 and ≥1: 320 antibody titres against the flagellar antigens (Table 1).

In comparison, 81 (81.0) and 19 (19.0) of the control subjects had Salmonella antigen titres <1: 80 and ≥1: 80 respectively with no statistically significant difference, P > 0.05 (Table 2).

Stool culture and isolation of Salmonella species from suspected typhoid subjects serologically screened for Salmonella antibodies showed that five out of 389 (1.3%) stool samples grew Salmonella species (four *Salmonella typhi*, and one *Salmonella paratyphi*): one out of 156 (0.3%) who had titres <1 : 80, and four (4.1%) out of 233 subjects with titres ≥1 : 80. One stool sample was positive for *Salmonella paratyphi* among the control group compared to the test subjects with a strong positive correlation, P < 0.05 (Table 3).

An analysis of diagnostic accuracy in the utilization of Widal test alone, Widal test plus stool culture and stool culture alone in the diagnosis of typhoid fever showed Sensitivity (O =73.8%, H = 25.3%); Specificity (O = 77.1%, H = 79.8%); Positive Predictive Value PPV (O = 13.7%, H = 5.0), and Negative Predictive Value NPV (O = 99.4%, H = 96.9%) (Figure 1).

Table 1: Spatial Distribution of Salmonella Antigens titre among patients suspected to have typhoid fever attending Benue State University Teaching hospital Makurdi Nigeria.

	Somatic Antigen (TO, N=389)		Flagellar Antigen (TH, N=389)	
Salmonella titre Values	Number	(%)	Number	(%)
≤20	146	37.5	121	31.1
40	57	14.7	99	25.4
80	138	35.5	93	24.0
160	16	4.1	63	16.2
≥320	32	8.2	13	3.3
Total	389	(100)	389	(100)

Table 2: Comparison of Salmonella Antigens titre among suspected typhoid fever patients with that of the control

	Titres <80	Titres ≥80	Total
Test	156 (74.8)	233 (59.9%)	389 (100)
Control	81 (81.0)	19 (19.0)	100 (100)

P < 0.05

Table 3: Patterns of isolation Salmonella species from Stool samples with positive Widal test as compared to negative Widal test among suspected Typhoid patients at Benue State University Teaching Hospital Makurdi.

Salmonella Antigen Titres	Positive Stool Culture (%)	Negative Stool Culture (%)	Number of Subjects (%)
< 80	1 (0.6)	155 (99.4)	156(100)
≥ 80	4 (1.7)	229 (98.3)	233 (100)

P < 0.05

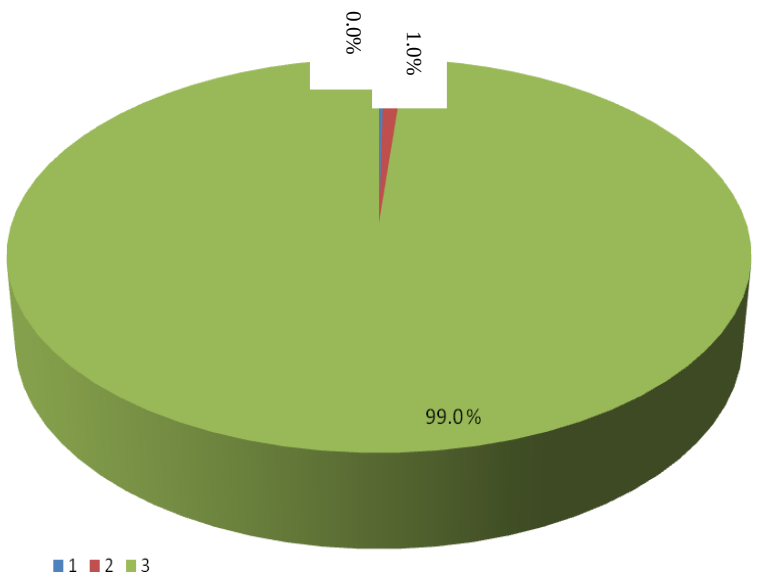

Figure 1. Comparison of diagnosis of typhoid fever using Widal test alone, stool culture alone, and Widal test plus stool culture combined (N=389).

Key: 1 Stool Culture only (n= 1)
2. Widal test plus stool culture combined (n= 4)
3. Widal test only (n= 384)

Sensitivity (O =73.8%, H = 25.3%); Specificity (O = 77.1%, H = 79.8%); Positive Predictive Value PPV (O = 13.7%, H = 5.0), and Negative Predictive Value NPV (O = 99.4%, H = 96.9%).

DISCUSSION

Of the 389 patients with clinical diagnosis of typhoid fever, five (1.3%) of them Salmonella species from stool samples; one (0.0%) from the 156 (40.1%) patients with Widal test titres <1: 80, and four (1.0%) from the 233 (59.9%) patients who had Widal agglutination test titres ≥1: 80. This is compared to the one subject in the control group with Widal agglutination titres of ≥ 1: 80 with positive Salmonella stool culture. The findings from this study show that Widal test can to a large extent detect the absence typhoid fever in a patient but fail to sufficiently diagnose an active infection.

The isolation of Salmonella from a non-febrile subject with supposedly high Widal agglutination test without any febrile symptoms could have been as a result typhoid carriage state or patient recovering from the disease who failed to disclose. A general screening of the populace in this community for typhoid carriage would further offer a better explanation. The implication of the above findings is that if Widal agglutination test alone is relied upon to establish laboratory diagnosis of typhoid fever many cases would be missed. This is the major challenge affecting effective management of typhoid fevers in our both rural and urban settings. [16-19]

In comparison to the present study findings from Ethiopia similarly showed low sensitivity and positive predictive values similar to the findings in Tanzania where the PPV was found to be 26% while in Nigeria a PPV of 17% was obtained. This compares favourably with the PPV of 13.7% and sensitivity of 73.8% obtained in the present study. The comparatively lower PPV obtained in the present study could be attributed to the different nature of sample used for culture. While blood samples were used for culture in the other studies stool samples were used for culture and isolation

of Salmonella species. A combination of blood, stool, urine, bone marrow and illeo-caecal aspirates' culture is bound to give a more accurate and precise result. [5-7,20]

This study was however limited by the fact that Selenite F was not used as a culture medium for the stool cultures so some isolates of Salmonella species may have been missed, and the findings should be interpreted in this regard.

In conclusion, this study has shown that Widal agglutination test alone is unreliable in establishing diagnosis of typhoid fever and should be used with extreme caution especially where there are no facilities for culture.

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Declaration

We declare no conflict of interest.

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