International Journal of Biomedical and Health Sciences
Vol. 7, No. 2, June 30, 2011
Printed in Nigeria

0794-4748/2011 $5.00 + 0.00

http://www.asopah.org

IJBHS 2010205/7204

Comparative study of stool culture and Widal Test for screening of Salmonella infection in Mohammed Abdullahi Wase Specialist Hospital, Kano, Nigeria

I. Yusuf[2] and K. M. Hamid[2]

[1]Department of Biological Sciences, Bayero University, Kano, Nigeria
[2]School of Medical Laboratory Sciences, Usman Dan fodiyo University, Sokoto

(Received November 28, 2010; Accepted February 8, 2011)

ABSTRACT: A comparative study of the use of stool culture and Widal test methods for screening Salmonella infection in Mohammed Abdullahi Wase Specialist Hospital (M.A.W.S.H) Kano was carried out on out patients clinically suspected to be suffering from typhoid fever. Serum and stool samples from 150 out patients attending M.A.W.S.H were serologically (by Widal test) and culturally screened for Salmonellae. One hundred and sixteen (77.3%) patients serum were significantly reactive to Widal test, and out of these patients only 25 (16.7%) yielded positive stool cultures of salmonella on Salmonella-Shigella medium. Three (2.0%) and two (1.3%) stool samples whose serum samples were negative and non-significantly reactive to widal test respectively, yielded positive culture of Salmonella. Therefore, we cannot use Widal test alone neither stool culture alone for the correct diagnosis of typhoid fever.

Key words*: Salmonella typhi, Salmonella paratyphi*, Stool culture, Widal test.

Introduction

The genus Salmonella are Gram-Negative, motile, non-lactose fermenting, non-spore forming rods and are facultative anaerobic (Jawetz, 1984). Salmonella is one of the main causes of food borne and water borne infection worldwide. Many *Salmonella species* can infect gastrointestinal tract causing inflammation of the intestine that is gastroenteritis (Atlas, 1995).

Salmonella is widely isolated from animals, birds and man. The most prevalent species of Salmonella isolated from man are *Salmonella typhi* and *Salmonella paratyphi* which can survive in contaminated water for weeks. *Salmonella choleraesuis, S. enteritidis, S. typhimurium, Salmonella typhi* and *S. paratyphi* have been identified serologically and biochemically as important etiologic agents of clinical salmonellosis (Kelly *et al*, 1985; Lindgren *et al*, 1996).

*Author to whom correspondence should be addressed.
e-mail:- ibrahimayab@yahoo.com
Phone:- +2347037865734

S. typhi and *S. paratyphi* A, B and C are well known agents of typhoid 'enteric' fever exclusively in humans with incubation periods of 7-14 days and 1-10 weeks respectively. Their transmission and portal of entry into the body is feco-oral through ingestion of food, milk and water previously contaminated by fecal matter from patients or symptomless carriers. The case of 'Typhoid Mary' has been well documented (Thomas, 1979; Presscott *et al*, 2002). The clinical manifestation of typhoid and paratyphoid fever include dry cough, anorexia, a dull continuous headache, pyrexia, abdominal discomfort and constipation (Jawetz, 1984).

The sole dependence on laboratory Widal test for the diagnosis of typhoid fever without proper consideration of various factors such as lack of standard quality control measures, expired/fibriated antigen suspension, technical error, account for wrong diagnosis of the disease. The World Health Organization (WHO) has said that due to the various factors that can influence the results of a Widal test, it is best not to rely too much on this test. WHO instead recommends the use of cultures whenever possible. Until another simple, inexpensive, and reliable option becomes available, however, use of the Widal test will probably persist in those countries with limited resources. Research is being conducted on a different blood test called Typhidot-M that produces results in one hour, appears to have greater reliability, and may be an alternative for the Widal test in the early diagnosis of enteric fever (Anonymous, 2010).

It is worthy to note that some other related organisms share the same antibodies detected by Widal test, consequently it has been confirmed that normal healthy individuals without a history of typhoid or TAB vaccine have high titres of *Salmonella typhi* antibodies. With the following observation enumerated, it is evidently no longer wise to depend solely on Widal test for accurate diagnosis of typhoid fever. Therefore, the need to complement Widal test with other methods in the routine diagnosis of typhoid fever is worth considering. The research is aimed at comparing stool culture and Widal test for screening of salmonella infection among some patients in Kano Metropolis.

Materials and Methods

Sample Collection

One hundred and fifty outpatients attending Mohammed Abdullahi Wase specialist Hospital Kano, who were clinically suspected to be suffering from typhoid fever, and recommended for Widal test, were given sterile wide mouthed glass bottles to collect their early morning stool samples and the patients were instructed to avoid contaminating the feces with urine. Each sample container was labeled with the name of the patient, date and time of collection. Their venous blood samples were also collected using a 5-ml syringe and placed into a sterile test tube for serum extraction. Each sample container was labeled with the name of the patient, date and time of collection. The sera and stool samples were used for the study.

Isolation of Pathogens

The stool samples were inoculated into 5mls of Selenite F broth (BDH, England) in a sterile universal bottle and incubated at 37^{o}C for 24 hours before they were sub cultured onto Salmonella – Shigella agar plates (BDH, England). The plates were further incubated at 37^{0}C over night. The plates were examined for suspective organisms which appear as non-lactose fermenting (pale) colonies (Chessbrough, 2000).

Pathogen Identification

The colonial characteristic of the growth on Salmonella-Shigella agar plates were noted; and these included, the colour, shape, edge, elevation, fermentation and typical black centers due to Hydrogen Sulphide. Isolates were subjected to gram reaction and biochemical reaction which included Indole, Urease, Citrate, Triple Sugar Iron agar (TSI) and Motility test as described by (Chessbrough, 2000).

Serological (Widal) Test

Rapid (Slide) Agglutination Test

The commercial biotech stained febrile antigens were used and the method adopted by (Baker *et al.,* 1998) was used. Eight separate drops of the blood serum were placed on a clean grease free tile already labeled into 2 columns. Each column contained four separate drops of the serum. The H-antigens were added to the first column in drop wise while the 0 antigens were added to the second column. They were then mixed and rocked for 1 minute (Baker *et al*., 1998). Agglutination indicated positive widal test. Reactive titers of 1:80 and above were regarded as positive (+) while titers less than 1:80 were negative (-). All Negative slide tests were confirmed by the tube test.

Tube Agglutination Method

Ten (10) labeled test tube were placed in a rack and 4mls of saline was added to tube1 and1ml in tube 2-10. Into tube1, 1ml of serum was added and mixed, this diluted the serum 1 in 5. One (1) ml of the serum solution was transferred to tube 2 which diluted the serum 1 in 10, and these was repeated up to tube 10, which gave the serum dilution of 1:5 1:10, 1:20 1;40, 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560 respectively.

Using a fresh pipette and starting from the highest dilution, 0.5ml was transfered from each tube in to corresponding agglutination tube rack. 0.5ml of antigen was added to each tube, this gave a final serum dilution 1 in 10 in tube 1, and 1 in 20 in tube 2 and so on (Baker *et al*., 1998).

Controls were set along with test by adding together 0.5ml of saline and 0.5ml of antigen in an agglutination tube. H-antigens were incubated at room temperature for 2 hours while 0-anigens were incubated at 37^0C in water bath for 2 hours followed by refrigeration at 4^oC overnight. The result read after warming to 37^0C for 10 minutes.

Result and Discussion

The serum samples of 150 outpatients attending Mohammed Abdullahi Wase specialist Hospital Kano were qualitatively and quantitatively screened for Salmonella infection by Widal agglutination test, and their stool sample were cultured on Salmonella – Shigella agar plate for the isolation of the organism.

One hundred and sixteen (77.3%) of the patient's blood serum reacted significantly to Widal screening test, fifteen (10.0%) showed non significant reaction and nineteen (12.7%) were negative for the screening test (Table 1). The highest antibody titre was recorded for *Salmonella paratyphi* H- antigen with a titre of 640 while the lowest antibody titre was recorded for *Salmonella typhi* '0' – antigen with a titre of 160. However antibodies against *Salmonella typhi*. "H" – antigen and *Salmonella paratyphi* "0" – antigen had the same titre of 320 each (Table 2)

The stool samples of the same patients used for the study Yielded 30 (20.0%) positive culture of *Salmonella* organism while 120 (8%) stool samples yielded no growth of the *Salmonella* organisms on Salmonella – Shigella medium after overnight growth at 37^0C. However 10 (6.7%) stool samples yielded growth of *Shigella species* (table 3). And out of the 30 (20%) positive stool culture, 24 (16%) yielded growth of *Salmonella typhi* while the remaining 6(4%) yielded *Salmonella paratyphi* (Table 3)

A comparative results of serum Widal test and stool culture of patients samples were indicated in table 4.

In this study, out of the 116 (77.3%) patient's serum sample who were significantly reactive to Widal test, only 25 (16.7%) of them yielded positive stool culture of *Salmonella* (Table 4). The significant Widal reaction observed may probably be due to cross reaction to other infections rather than *Salmonella* infection. According to Parker (1990) some positive widal reaction had been observed in the cross reaction between malaria sera and known isolate of *Salmonella typhi* antigens. Ramachandran *et al.,* (1974) suggested that *Plasmodium falciparum* malaria may predispose to non-typhoidal *Salmonella* septicaemia in children under 5 years of age. Grange (1994) also noted that some other related organisms share the same antibodies detected by Widal test. Consequently, He also confirmed that normal healthy individual without history of typhoid or TAB Vaccine has high titre of *Salmonella typhi.* Also a study conducted in Sudan by Weil (1953) observed that *Salmonella typhi* O –agglutinin had titres of 1:320 in 10.5% of healthy individual.

However, stool samples of 3(2.0%) and 2(1.3%) patients whose serum samples were negative and non significantly reactive respectively to Widal test, yielded positives culture result of *Salmonella*. (Table 4). This probably may be due to the fact that the Widal test might have been carried out at the time when the antibodies

against the *Salmonella* were yet to produce. Bowry (1977) indicated that antibodies are not detectable in the blood serum from 1 – 5 days of the infection. Thus is the diagnosis of typhoid fever was carried out only on Widal test, these patients would have been wrongly diagnosed and may eventually not treated with antibiotics and this may lead to typhoid complication (intestinal perforation).

Table 1: Widal slide screening reaction of the patients' serum samples

Widal Reaction	**Number of Samples**	**%**
Significant reaction	116	77.3
Non significant reaction	15	10.0
Negative reaction	19	12.7
Total	**150**	**100**

Table 2: Quantitative titre distribution of significantly reactive serum samples

Specific antigen	**Antibody reacted to**	**Titre**	**Number of samples**	**%**
Salmonella typhi	O	160	60	40
Salmonella typhi	H	320	30	20
Salmonella paratyphi	O	320	13	8.7
Salmonella paratyphi	H	640	13	8.7
Total			**116**	**77.3**

Table 3: Distribution of isolates from positive stool culture

Isolate	**Identity**	**Number**	**%**
Non-lactosefermenter 1	*Salmonella typhi*	24	16
Non-lactosefermenter 2	*Salmonella paratyphi*	6	4
Non-lactosefermenter 3	*Shigella species*	10	6.7
Total		**40**	**26.7**

Table 4: Result of serum Widal test and stool culture of patient's samples

Widal reaction	Number of serum sample	%	Number of positive stool culture	%
Significant reaction	116	77.3	25	16.7
Non significant reaction	15	10.0	2	1.3
Negative reaction	19	12.7	3	2.0
Total	**150**	**100**	**30**	**20**

Conclusion

The proper and an accurate method of typhoid fever diagnosis depend on the isolation of the causative agents from clinical specimens of which stool sample is inclusive. This is because the salmonella are present in an appreciable numbers; therefore, stool samples should be collected for culture. Finally over dependence on Widal agglutination test for the diagnosis of typhoid fever could be misleading, because the method is not specific and accurate.

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