CORRELATION STUDIES ON WIDAL AGGLUTINATION REACTION AND DIAGNOSIS OF TYPHOID FEVER

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Abstract. A total of 80 patients at the University of Uyo Teaching Hospital (UUTH) suspected of having enteric infections were screened for the presence of *Salmonella* species using blood, urine and stool samples along with Widal agglutination tests. Although 39 patients tested positive for the Widal agglutination test with titers ranging from 1:80 to 1:320, no *Salmonella* organism was encountered in some cultures. Statistical analysis revealed significant differences (χ^2) at the 5% probability level between the Widal test and the cultures of the clinical samples. The results suggest that serological investigations alone may not be a reliable index for the diagnosis of *Salmonella* infections.

INTRODUCTION

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). The symptoms include headache, fever, abdominal cramps, constipation and nausea. *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). Typhoid fever constitutes a serious public health problem in many parts of the world. It is unusual in Northern Europe and North America but extremely common in the tropics with a high mortality rate (Wick *et al*, 1971). According to Taylor *et al* (1985), *S. typhi* is endemic on the Indian subcontinent with a high prevalence of multiple drug-resistant strains which have been implicated as the cause of treatment failures in Ontario, Canada (Chandrashe *et al*, 1994). Prescott *et al* (2002) reported that 400 to 500 cases of typhoid fever occur annually in the United States of America.

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The diagnosis of typhoid fever may be achieved by slide or tube agglutination reactions with patients sera against known suspensions of *S. typhi* and *S. paratyphi* (Widal reaction). Although the Widal test is still extensively used in many developing countries, including Nigeria, several investigators have expressed doubts on the value of this well established test in the diagnosis of typhoid fever (Pang and Puthecheary, 1983) for several reasons. Senewirantne and Senewirantne (1997) reported the Widal test requires carefully standardized conditions and that poor or unstable agglutination suspensions can produce incorrect results. Since *S. typhi* shares the same H and O antigens with many other *Salmonella*e, a rise in titer of these antibodies is not specific for *S. typhi* (Reynolds *et al*, 1970) because immune proteins other than those induced by *S. typhi* can cross-react with the agglutinable suspensions. Such false-positive reactions have been reported in patients with active chronic hepatitis and can occur in other immunological states (Protell, 1971). The significance of the results should therefore be assessed against antibody titers found in the normal environment in endemic areas (Mohammed and Chikwem, 1992).

Salmonella agglutination may not be produced because of a poor antigenic stimulus, a defect in antibody production or as a result of the organism being out of contact with the antibody producing system. Wicks *et al* (1971) earlier reported that previous inoculations with the TAB vaccine or a previous attack by the typhoid ba-

cilli could render the Widal test valueless.

This paper is a critical appraisal of the use of Widal agglutination reaction alone in the diagnosis of enteric fever.

MATERIALS AND METHODS

Sources and sample collection

Fresh blood samples were collected from 80 typhoid patients at the University of Uyo Teaching Hospital (UUTH), St Luke's General Hospital (SLGH) and some medical diagnostic centers in Uyo, Akwa Ibom State. The samples were taken by venipuncture from patients with confirmed typhoid who were yet to undergo treatment. The fresh blood were dispensed into McCartney bottles containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The patients were also given sterile, dry, wide-necked, leak-proofed universal laboratory bottles for the collection of a mid-stream urine for culture. Stools for culture were collected in clean wide-mouth containers 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.

Screening test

Sera for the Widal Test were collected from fresh blood samples by centrifugation. Using a Pasteur's pipette, eight drops of each serum were transferred onto eight rings on a white tile. The *Salmonella* antigen reagent was also dropped into the rings. Both were thoroughly mixed using an applicator stick and the tile gently swirled for 1 minute for observable agglutination. The reacting antigens were recorded positive (+) while non-reactive antigens were classified as negative (-).

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.

Processing of samples

Fresh stools were heavily inoculated into selenite -F broth (Oxoid, in England) and incubated for 24 hours at 37ºC for the primary isolation of Salmonellae. Secondary inoculations were then made by seeding Salmonella-Shigella agar (Oxoid Ltd, England) plates with 0.5 ml of the primary culture and then incubated for 24 hours at 37ºC. Pale, non-lactose fermenting colonies were subcultured from the Salmonella-Shigella agar plates of all cultures, purified by streak plating and preserved by refrigeration at 7ºC in sterile nutrient agar (Oxoid Ltd, England) slants.

Urine culture

Urine samples were concentrated by centrifugation after which the supernatants were discarded. The fluid portion of the debris was then inoculated onto Salmonella-Shigella agar and McConkey agar (Oxoid, England) plates by spread-plating using a sterile hockey stick. The plates were then incubated for 24 hours at 37ºC. All non-lactose fermenting colonies were subcultured onto nutrient agar plates, purified and stored in slants at 7ºC.

Blood culture

0.5 ml fresh blood was dispersed into blood culture bottles containing 5 ml of tryptone soy broth and incubated at 37ºC for 48 hours. Subcultures were then made on Salmonella-Shigella agar plates by spread-plating. Inoculated plates were incubated at 37ºC for 48 hours. Culture plates that yielded no growth were further incubated for 2 weeks before being discarded.

Characterization and identification of isolates

All purified colonies were characterized and identified using the taxonomic schemes of Cowan (1985) and Holt *et al* (1994). The tests included Gram's reaction, catalase, oxidase, citrate utilization, gelatin liquefaction, ornithine decarboxylase, indole, nitrate reduction, starch hydrolysis and a sugar fermentation profile.

Statistical analysis

This was carried out using the chi-square as earlier described (Sokal and Rohif, 1981).

RESULTS

Correlation of the Widal agglutination test with urine, stool and blood cultures

The results obtained revealed that out of the 80 patients (not shown for convenience) at the University of Uyo Teaching Hospital suspected to have enteric infections, 39 (48.75%) tested positive for the Widal agglutination reaction with titers ranging from 1:80 to 1:320 while 41

(51.25%) tested negative (Tables 1 and 2). Urine and stool cultures from 25 (31.25%) patients whose sera tested positive for the Widal agglutination reaction yielded growth of either *Salmonella typhi* or *Salmonella paratyphi* or both; other *Salmonella*e were also encountered. Urine and stool cultures from 7 (17.95%) patients whose sera produced a negative Widal agglutination also yielded a positive growth of either *Salmonella typhi* or *Salmonella paratyphi,* but never both, while similar cultures from 3 (7.69%) patients were ignored because they yielded growth in one sample only, either urine or stool. Only patients with any two samples that yielded growth on culture were regarded as positive in this investigation. All the blood cultures investigated yielded no growth of *Salmonella* species, although bacteremia was established in two cases. Statistical analysis revealed significant differences (χ^2) at the 5% (p = 0.05) probability level, between the cultures and the Widal test of the clinical samples.

Frequency distribution of *Salmonella* according to agglutinins by the sexes and ages of the patients

Of the 80 patients investigated 43 (53.75%) were males and 37 (46.25%) were females of various age groups. Twenty (51.28%) out of the 39 cases that tested positive for the Widal agglutination reaction were from male patients while 19 (48.72%) were obtained from females. The age group 26-30 years had the highest combined incidence of 7 (17.95%), with 4 (10.26%) positive cases for males and 3 (7.69%) for females, followed by the age groups 21-25 and 31-35 years with a combined total of 6 (15.38%) cases each (Table 3). No positive *Salmonella* agglutinins were encountered in age groups 6-10 and 56-60 years.

Table 1
Correlation of positive Widal agglutination test with positive culture in diagnosis of typhoid infection.

Sex	Positive Widal test No. (%)	Positive culture No. (%)
Male	20 (25)	13 (16.25)
Female	19 (23.75)	12 (15.0)

Table 2
Correlation of negative Widal agglutination test with positive culture in diagnosis of typhoid infection.

Sex	Negative Widal test No. (%)	Positive culture No. (%)
Male	23 (28.75)	30 (37.50)
Female	18 (22.50)	25 (31.25)

Table 3
Frequency distribution of Salmonella agglutinin according to sex and age group.

Age (year)	No. of positive male	No. of positive female	Total
6-10	0	0	0
11-15	1	0	1
16-20	4	1	5
21-25	3	3	6
26-30	4	3	7
31-35	2	4	6
36-40	1	3	4
41-45	3	2	5
46-50	0	3	3
51-55	2	0	2
56-60	0	0	0
Total	20	19	39

DISCUSSION

The isolation of *Salmonella* from the urine and stools of Widal negative patients could be a reflection of a carrier state and not of active infections, and underscores the need for a clinical diagnosis along with the Widal test. Widal positivity is more of an epidemiological test rather than clinical because a rising titer repeated after two weeks duration should be demonstrated before it is of clinical significance, although this has also been under serious criticism in recent years. This is, according to Reynolds *et al* (1970) and Protell (1971), because immune proteins other than those induced by *S. typhi* can cross react with the agglutinable suspensions and produce false positive results. Stokes and Ridgeay (1980) re-

ported that symptomless carriers harboring *S. typhi* and *S. paratyphi* in their gall bladders and excreting them in their stools are the main source of outbreaks of this disease in many countries. The confirmation of infection by isolation of *Salmonella* species from Widal positive cases is also important because according to Prescott *et al* (2002), laboratory diagnosis of typhoid fever is by demonstration of typhoid bacilli in the blood, urine or stools and serology (the Widal test). Results obtained from this investigation are also in congruence with an earlier report by Bellow *et al* (2001) that blood, bone marrow aspirates, stool and urine cultures should be carried out alongside looking for the malarial parasite. Apparently some suspected cases of enteric fever may have been chronic malaria infections, hepatitis or other immunological disorders (Pang and Puthucheary, 1983).

The results also revealed that 20 (51.28%) out of the 39 cases that tested positive for the Widal agglutination test were from male patients and 19 (48.72%) from females. This marginally higher percentage incidence in males is not significant. The absence of *Salmonella* species on some culture plates from the Widal positive cases and their presence in similar cultures from some Widal negative patients may be attributed to the stages of development of the pathogen and whether the patient had taken self medication before visiting the hospital. The results are in agreement with an earlier report (Prescott *et al*, 2002) that as the pathogen colonises the small intestine, penetrates the epithelium and spreads to the lymphoid tissues, blood, liver and gallbladder, some individuals stop shedding the pathogen in their feces after three months of infection. Individuals that continue to shed the typhoid bacilli from their feces for extended periods and show no symptoms become carriers as the pathogen reaches the intestine through the bile duct. Symptomless carriers are the main source of outbreaks of enteric fever in many countries. The results of statistical analysis revealed significant differences ($p<0.05$) between the Widal agglutination reaction and cultural diagnosis of clinical samples and strongly suggests that serological investigation alone may not be a reliable diagnosis for enteric fever infections.

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