# COMPARISON OF RELATIVE EFFECTIVENESS OF CULTURE AND SEROLOGICAL METHODS IN TYPHOID FEVER DIAGNOSIS IN ABEOKUTA METROPOLIS

Akinyemi, K.O.[1], *Atayese, A.O.[2] and Oyefolu, A.O.[1]
[1]Department of Microbiology, Lagos State University, Lagos, Nigeria
[2]Department of Microbiology, University of Agriculture, PMB, 2240 Abeokuta. Nigeria.
*(Corresponding author; E-Mail: bisatfeb14@yahoo.com)

## ABSTRACT

Blood and stool samples collected from 106 typhoid-suspected patients of the Federal Medical Centre, Idi-Aba, Abeokuta, Ogun State were assessed for typhoid fever using two common methods: culturing of samples and widal agglutination test (serologic). Malaria parasite, blood and stool cultures of the patients were carried out within a period of six months. Out of the 106 patients, 35.84% were positive for widal agglutination test using 1: e"160 as the cut-off / base-line antibody titre. 33.96.0% was recorded for malaria. Blood culture revealed 1.88% bacteria pathogens in the widal positive patients out of which 0.94% isolate was of *Enterobacter sp.*, and 0.94% was of *Klebsiella sp.*, while the stool culture revealed 9.43% bacterial pathogens out of which 5.60% was *Salmonella typhi*, 1.88 % was *Salmonella paratyphi* and 1.88% was *Escherichia coli*. However 54.7% patients were on self- medicated antibiotic therapy before the tests were carried out, hence the 0% typhoidal bacillus in the blood culture. Other infectious agents such as bacterial, viral and even protozoan may mimic enteric fever. Consequently, this study further revealed that culture of stool, blood and urine samples of patients and others like bone marrow may be far superior to widal agglutination test for typhoid fever.

KEYWORDS: Antibody titre, Culture method, Diagnosis, Serological test, Typhoid fever

## INTRODUCTION

Typhoid fever has been described as acute illness in a patient previously in good health, with compatible signs and symptoms, accompanied by bacteriological recovery of the causative agent *S. typhi* (Gilman *et al,* 1975). Typhoid fever is endemic in economically disadvantaged countries in Africa, Asia and South and Central America. Typhoid fever is common in the black population of South Africa. It does not always present any distinct picture as other bacterial and even viral infections may mimic its presentation. In South Africa an epidemic of typhoid fever was reported. Out of 126 cases investigated, 33 (36%) had more than one of the six major clinical features of typhoid fever. It was concluded that the diagnosis of typhoid fever for this epidemic claim had been based virtually on the result of the widal test although; the disease is endemic in the region (Kustner *et al,* 1987).

Typhoid fever is among the major widely spread diseases affecting the population in Nigeria and has been rated eight among these common infections (Anon, 1993).Nigeria, like many other tropical and developing countries has been described as endemic zone for typhoid fever by several workers (Ikene and Anan, 1996; Mulligan, 1971, Idoko *et al,* 1988, Onuigbo, 1990; Mohammed *et al,* 1992, Talabi, 1994; Odugbemi *et al,* 1994, Oboegbulam *et al,* 1995). There was a similar study previously carried out in southern part of Nigeria in which similar conclusion was drawn (Mulligan, 1971) Onuigbo, (1990) reported 15 patients diagnosed of typhoid fever, of these malaria disease was confirmed in 70%. The diagnosis was based on Widal agglutination test. The author concluded that Widal test alone is prone to error and that any claims of a typhoid fever epidemic in Nigeria remains a mere conjecture.

In view of the purported epidemic of typhoid fever in Nigeria, Mohammed *et al,* (1992) determined the baseline titre for the diagnosis of typhoid fever using a single Widal test in Borno and Plateau states of Nigeria. Out of 172 patients with symptoms and signs of typhoid fever, 92.4% and 90.7% had reciprocal 'O' and 'H' antibody titre of 160 and above respectively. On the other hand, 95.3% and 66.3 % of the 937 healthy control subjects had reciprocal 'O' and 'H' antibody titres of 80 or less respectively. It was therefore concluded that a reciprocal 'O' antibody titre of 160 and above in persons whose symptoms are compatible with typhoid fever could be considered diagnostic in these two states, using single Widal test. Therefore, the aim of this investigation was to compare the relative effectiveness of culture to serological methods used in typhoid fever diagnosis in Abeokuta metropolis.

## MATERIALS AND METHODS

Study population and sample collection

A total of 106 patients referred to the hospital laboratory with suspected cases of typhoid at the Federal Medical Centre, Idi-Aba, Abeokuta, Ogun State were used for this investigation. Widal agglutination reaction test, Malaria parasite, stool and blood cultures were performed on the patients. Blood and stool samples were collected from patients using standard procedures as used by Christie (1974) and Talabi (1994). The blood specimens of five healthy individuals were obtained and used as negative control. All blood specimens collected in tubes containing EDTA were stored at 4°C until processing. Questionnaires were administered among the patients.

Microbiological analysis

A clean spatula was used to take small quantities of stool sample into tubes of selenite 'f' broth. Ratio 1:20 dilutions of the blood samples were made. Five millilitres of diluents were inoculated into 50mls sterile broth medium of selenite 'f.' broth. Incubations were at 37°C for the first 3 days before sub-culturing into the following media for isolation of bacteria: Blood agar, chocolate agar and MacConkey agar (Oxoid, U.K.). Incubations were at 37°C for up to seven days after which plates with no growth were recorded as negative. Colonies growing on the incubated plates were randomly picked and purified by repeated sub-culturing. Distinct representative colonies from over-night culture plates were then tested for Gram reaction (Claus, 1992) morphology and motility. The isolates were subjected to further biochemical characterization such as pattern of sugar fermentation, utilization of various proteinous substrates and production of some enzymes (Olutiola, 1991). The discriminatory scheme of Sneath *et al* (1986) was used to identify representative isolates.

Serological analysis: typhoid diagnosis

Using widal bacteria suspension kits (Linear chemicals, Montgat-Barcelona Spain) the Widal agglutination test commonly used in typhoid fever diagnosis was carried in this work. The assays were performed by testing the stained antigen against unknown samples. The bacterial suspensions were stained to facilitate reading and interpretation of the results. Using normal saline as diluents, serial dilution of each serum sample was prepared. One drop of the suspension was added to each tube and incubated at 50°C for two hours, and then examined macroscopically for agglutination.

## RESULTS

Out of the total 106 patients with clinically suspected typhoid fever, 35.84% were positive for widal agglutination test using 1:e"160 as the cut-off/base- line antibody titre while 33.96% were recorded for malaria. Five organisms were isolated from blood and stool cultures (Table 1). Blood culture revealed that only 1.88% bacterial pathogens were isolated from the widal positive patients out of which 0.94% isolate is of *Enterobacter* sp., and 0.94% is of *Klebsiella* sp. Stool culture revealed that 9.43% bacterial pathogens were isolated out of which 5.6% were *S. typhi*, 1.88% were *S. paratyphi* and 1.88% were *E. coli*. Through questioning, it was established that before these tests were carried out 54.71% patients by self- medication had been on antimicrobial therapy (Fig. 1). From the above only 7.80 % was actually confirmed as typhoid patient from cultural methods.

## DISCUSSION

In this study out of the 106 patients tested for widal agglutination, 35.84% were positive for typhoid fever, using 1:e"160 at the cut off antibody titre based on the healthy individuals in Abeokuta Ogun state. This result is in line with the recent report of Akinyemi *et al* (2002) who used the base line antibody level of typhoid fever patients in Lagos State to be 1:e"160. The importance of using blood culture in the isolation of the aetiological agents of typhoid and paratyphoid fever as definitive diagnosis in areas where this febrile disease is endemic cannot be over emphasized. This is because although, Widal test had been used as a screening test to increase the suspicion index for the infection, most suspected cases of typhoid fever have been erroneously confirmed using the antibody titre based on single serological test. Other infectious agents such as bacterial, viral and even protozoans may mimic enteric fever (Christie, 1974; Akinyemi *et al,* 2002).

**Table 1**: Characteristics of microorganisms isolated from blood and stool cultures

| PROPERTY | *E. coli* | *S. typhi* | *S. paratyphi* | *Enterobacter* | *Klebsiella* |
|---|---|---|---|---|---|
| Motility | - | + | + | + | - |
| VP test | - | - | - | + | - |
| Indole test | + | - | - | - | - |
| $H_2S$ production | - | + | + | - | + |
| Gas from glucose | + | + | + | + | + |
| Acid from lactose | + | - | - | + | - |
| Urease | - | - | - | + | + |

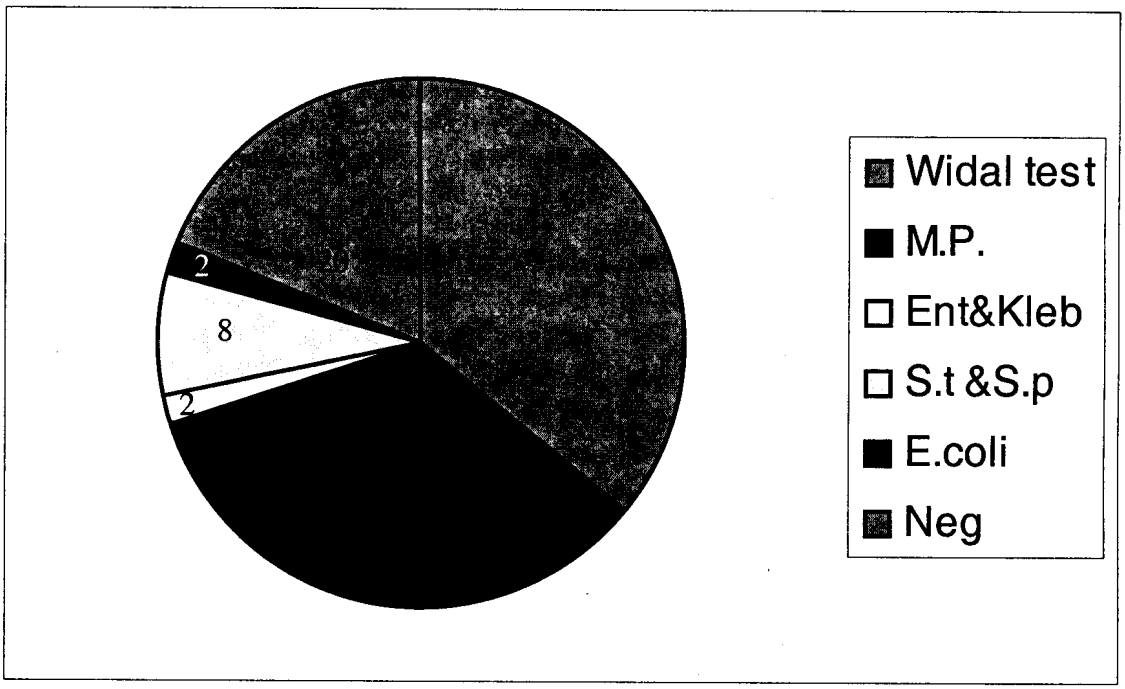


Fig. 1 Profile of patients with suspected cases of typhoid fever in Abeokuta metropolis

Blood culture revealed that only1.88% bacterial pathogens were isolated from the widal positive patients. It is important to note that in this study no typhoidal bacillus was isolated from blood culture. The reason for non-isolation of *S. typhi* and *S. paratyphi* from the blood samples may be attributed to the observations made by direct questionnaires completed by the patients during the course of the study, as 54.71% of patients had been by self-medication on antimicrobial therapy. Gross abuse of antibiotics among Nigerians had been well documented by several workers (Grossan, 2003; Wallinga, 2002). Other bacteria isolated from the study were *Enterobacter sp*, *Klebsella sp*, and *E coli*. The result of the blood culture revealed that the isolation of bacterial pathogens other than *S. typhi* and *S. paratyphi* from a widal positive blood suggests that a clinically diagnosed patient does not necessarily confirm a true *S. typhi* and *S. paratyphi* infections since some other isolated enteric bacterial probably interfere and mimic both the 'O' and 'H' antigens that form the core of widal test. Data showed statistical significance difference between positive Widal test, which is 34.84% and positive blood culture in which the (1.88%) organisms isolated were not even *S. typhi* and *S. paratyphi*.

However the result of malaria parasite revealed that 33.96% out of 106 (100%) were positive, indicating that malaria parasitaemia also mimic and interfere with the 'O' and 'H' antigens of commercially available widal agglutination kits. Similar observation had been made in Lagos by Akinyemi (2002). It is important to note that exclusive reliance on serology for the diagnosis of typhoid fever can be misleading, as individuals with pyrexia are assumed and erroneously treated as having typhoid fever based on single widal agglutination test, whereas potential fatal illness such as malaria, non-typhoid salmonellosis, endocarditis, parasitaemia and other gastrointestinal infections may have been responsible for the high antibody titre.

In conclusion, isolation of the causative organisms from blood and stool samples of patients and by extension other materials like bone marrow, urine and rose spot may be far superior to serological test in typhoid fever diagnosis.

## REFERENCES

Akinyemi, K.O, Oyefolu, A.O, Omonigbehin E.O, Akinside K.A and Coker (2000) Evaluation of Blood Collected From Clinically Diagnosed Typhoid Fever Patients In The Metropolis Of Lagos, Nigeria *Journal of the Nigerian Association of Infection Control* Volume 3 (2) 25-30

Anon (1993) Nigeria Bulletin of Epidemiology. Routine Disease Reports for 1992. 3: (2) pp. 19

Christie, A.B. (1974). Infectious Diseases: Epidemiology and Clinical Practice, 2nd ed. Chapter 3. Churchill Livingstone Ltd., Edinburgh.

Claus D.C 1992) A Standardized Gram-staining procedure Wor. *J. Microbiol. Biotechnol.* 8:451-452.

Gilman, R.H. Terminel, M. Levine, M.M. Hermandez-Mendoza, P., Hormick, R.E. (1975). Relative efficacy of blood, urine, rectal, swab, bone marrow and rose sport cultures for recovery of Salmonella typhoid fever, Lancet, 1 (7818): 1211-1213.

Grossan M. (2003) Excessive use and misuse of antibiotics *http://www.ent-consult.com/*

Idoko, J.A., Anjonrih, F.I. and Lawande, R.V. (1988). Typhoid fever in Zaria, Northern Nigeria. *Nig. Med. Pract.* 15: 21-23.

Ikene, A.C. and Anan, C.O. (1996). A clinical review of typhoid fever in Ibadan, *Nigeria. J. Trop. Med. Hyg.* 69: 15-21.

Kustner, H.G.V., Van Wyk, I.E. and Steinberg, W.J. and Collie, A. (1987). Widal Testing for Typhoid (Letter). *S. Afr. Med. J.* 72:511.

Mohammed, I., Chikwem, J.O. and Gushau, W. (1992). Determination by widal agglutination of the base line titre for the diagnosis of typhoid fever in two Nigerian states. *Scan. J. Immu.* 36: 153-156.

Mulligan, T.O. (1971). Typhoid Fever in Ilesha, Western Nigeria. West. Afr. Med. J. 20:353-268.

Obuegbulam, S.I.. Oguike, J.U. and Gugnanai, H.C. (1995) Micro Biological Studies on cases diagnosed as typhoid/enteric fever in South-East, Nigeria. J. Commun. Dis. 27:29-100.

Odugbemi, T.O., Oduboye, O. and Animashaun, T. (1994). Typhoid fever- microbiological aspects. Nig. Post Graduate Med. J. 1: 39-43.

Olutiola, P.O. Famurewa, O. and Sonntag, H.G. (1991) An introduction to general microbiology. A practical approach. Hygienic- Institut der Universitat Heidelberg, Germany. 267pp.

Onuigbo, A.C. (1990). Diagnosis of typhoid fever in Nigeria: Misuse of the Widal test, Trans. Royal Soc. Trop. Med. Hyg. 84: 129-123.

Sneath, P.H.A. Mair, N.S. Sharpe, M.E. and Holt, J.G. (Ed.) (1986) Bergey's Manual of Systematic Bacteriol. Vol. 2. Williams and Wilkins Co. Baltimore.

Talabi, H.A, I. (1994). Medical aspect of typhoid. The Nig. Post. Grad.Med. J. 1: 51-56.

Wallinga D. (2002) Misuse of Antibiotics *http://www.keepantibioticsworking.org/pages/*