Re-appraising Widal test as a diagnostic tool for typhoid fever in Nigeria

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Abstract

The standard for the diagnosis of typhoid fever depends on the isolation of the organism from the patient's bone marrow, blood, urine or stool through culture. However, the Widal test can be used as a serological test for the same purpose if a fourfold antibody titer can be obtained from the patient. The problem is that many laboratories do not receive a second serum sample and so, reliance is placed on one sample for diagnosis. This is not the correct methodology for Widal test and therefore, the result should not be authentic. Clinicians are advised to be more careful while examining their suspected patients with typhoid fever especially knowing that there may also be false positive results with Widal tests.

Key words: *Widal test, typhoid fever, diagnosis, Nigeria*

Typhoid fever is an enteric infection caused by *Salmonella typhi* (*S.typhi*) and *Paratyphi* (*S. paratyphi*), which are members of the *Enterobactericeae* family of organisms that are commonly transmitted through contaminated water and food.[1]It is commonly found in poultry products and sea foods. The usual incubation period for typhoid fever is 10-14days. The infective oral dose of *Samonella typhi* required to cause disease in human volunteers is between 10^5 and 10^8 organisms.[2] The invasion of the small intestinal mucosa by *S. typhi* is followed by multiplication in the mesenteric lymph glands. During the later stages of the incubation period, the organism localizes in the reticuloendothelial system (RES) and gall bladder. Septicaemia later results from the release of organisms from the RES into the blood. The start of septicaemia coincides with the onset of fever.[3] Common symptoms during the first week of the fever include malaise, headache, non-productive cough, constipation, abdominal pain and mental confusion, which are usually non- specific.

By the second week, the causative organism *S.typhi*, has started to cause localized lesions in the Peyer's patches of the small intestine and diarrhoea frequently commences. Physical signs of relative bradycardia, abdominal rose spots or splenomegaly occur in a minority of patients[4] and many patients show leucopenia at this point. The organism often persists intracellularly in macrophages and this may help to protect it from the humoral antibody defense mechanisms and incidentally, also against some antibiotics.

Complications of typhoid fever may occur between 2 and 5 weeks after the onset of the illness and include intestinal perforation, intestinal haemorrhage, myocarditis, osteomyelitis and meningitis. Death occurs in about 10% of patients not receiving antibiotics.[5] Relapses of typhoid fever occur after an initial recovery in about 10% of patients. The severity of the illness is usually much less during the relapse than in the original illness. Permanent faecal carriage, or less often, urinary excretion of *Salmonella typhi* following the illness occurs in up to 5% of patients.[6] Prolonged faecal carriage may be associated with persistence of infection in the biliary tract.[7]

Salmonella typhi is isolated from the blood cultures of over 90% of patients with typhoid fever during the first week of the illness.[8] Three sets of blood cultures should be collected and these are preferably taken just as the temperature starts to rise. Blood cultures are also frequently positive during the second to third week of the illness. This is the gold standard for laboratory diagnosis. Due to the production of hydrogen sulphide from thiosulfite, diagnosis can be done on differential media such as Bismuth sulfite agar.[9]

Bone marrow culture is rarely worthwhile, but may be carried out if bone marrow biopsy is performed in a patient with PUO to investigate the possibility of other diagnoses such as leishmaniasis.[10] The chances of isolating *S.typhi* from faeces and urine increase during the second and third weeks of the illness. One urine sample for salmonella culture is sent together with the other initial samples for culture and further urine samples are only collected when the early cultures are negative.[11]

Due to the fact that culturing for salmonella species is time consuming and requires a lot of manpower input, the Widal test was developed by Georges-Fernand Widal in 1896. It was meant to be a presumptive serological test for enteric fever and undulant fever.[12] This is a serological test

that tries to detect the presence of antibodies in the serum of the affected patient for *Salmonella typhi* infection.[13] In an infected patient, it usually takes 7-14 days before antibody rises significantly and this usually limits its applicability in early diagnosis.[14] Usually, the Widal test is positive if the "O" antigen titer is more than 1: 160 in an active infection, or if "H" antigen titer is more than 1:160 in past infection or in immunized persons. A single Widal test is of little clinical relevance due to the high number of cross-reacting infections, including malaria.[15] To make a diagnosis with Widal test, a fourfold increase in the titer (e.g. from 1: 40 to 1: 640) in the course of the infection, or a conversion from an IgM reaction to the IgG reaction of at least the same titer would be consistent with a typhoid infection[12.] The procedure can be a tube test, slide test or both. The methods are semi-quantitative methods. However, the limitations of Widal test are more than the advantages. These are:

1. It is time consuming because of the need for serum to be collected twice and often times when the diagnosis is reached; it is too late to start an antibiotic regimen.
2. The Widal test should be interpreted in the light of base line titer in a healthy local population and this is not usually possible because patients only present at the time of illness.
3. The Widal test may be falsely positive in patients who have had previous vaccination or have been previously infected with S. *typhi.*[7]
4. Besides cross reactivity with other Salmonella species, the test cannot distinguish between a current infection and a previous infection or vaccination against typhoid.[7]
5. Widal titers have also been reported in association with the dysgammaglobulinaemia of chronic active hepatitis and other autoimmune diseases.[5]
6. False positive Widal results are also known to occur in typhus, acute falciparum malaria (particularly in children), chronic liver disease associated with raised globulin levels and disorders such as rheumatoid arthritis, myelomatosis and nephrotic syndrome.[5]
7. False negative results may be associated with early treatment, with hidden organisms in bone and joints, and with relapses of typhoid fever. Sometimes, the infecting strains are poorly immunogenic.[11]
8. Severe hypoproteinaemia may also prevent a rise in 'O' and 'H' antibody titers.[16]

9. The antibody levels found in a healthy population may vary from time to time and in different areas, making it difficult to effectively establish a cut off level of baseline antibody in a defined area and community.[16]
10. In low typhoid endemic areas, weak and delayed 'O' and 'H' antibody responses limit the usefulness of the Widal test. Variations also exist between laboratories in the performance and reading of Widal tests which compromise further the reliability of the tests.[16]

The World Health Organization has said that due to the various factors that can influence the results of a Widal test, it is best not to rely so much on this test. The misuse of Widal test for diagnosis has been acknowledged in Libya by Zorgani et al.[16] This was due to paucity of training of the Laboratorians. Even Ley et al, while evaluating the Widal tube agglutination test for the diagnosis of typhoid fever among children admitted to rural hospital in Tanzania, noted that despite Widal test being a rapid test, the positivity was 26%.[9] Kariuki and colleagues reported in 2004 that typhoid fever was over reported in Embu and Nairobi, Kenya[17,18], buttressing the point that Widal test might be over rated.

Some researchers have suggested that instead of the habit of relying on a single serum sample, which is dangerous and not very reliable, a sequential Widal test should be done with different Widal brands.[19] This is because none of the four brands in the market has a real comparative advantage on the others.[19] A few others have tried evaluating the typhoid/paratyphoid diagnostic assay (TPTest) to detect anti salmonella IgA in secretions of peripheral blood lymphocytes and they had encouraging results.[20] Yet, we are still saddled with single serum sampling serological Widal test for typhoid diagnosis in Nigeria in general and Rivers state in particular.

Recently in Rivers state, Nigeria, there has been a pseudo epidemic of typhoid following results obtained from privately owned laboratories using mostly single serum Widal test reactions. This is in sharp contrast to what obtains in the University of Port Harcourt Teaching Hospital. Following a one year review of results of Widal tests in the Department of Medical Microbiology and Parasitology (1st January 2015 – 31st December 2015), 590 single serum samples were submitted for Widal tests and using our standard antibody titer of 1:160 for positivity, none was positive (unpublished data). This was not surprising because of the unreliability of single serum Widal tests

results that has been stated above and the proficiency of the laboratorians. The patients were predominantly out-patients with no means of contact tracing and so the second serum samples were not collected to get a probable fourfold increase for the positive cases.

This is in contrast to Ley et al and Omuse that had 26% in Tanzania and Kenya, respectively.[9,17] The case of Tanzania may be due to the rural situation of the hospital where water borne diseases may be prevalent, unlike the University of Port Harcourt Teaching Hospital which is an urbanely situated hospital in a cosmopolitan city. This is also in sharp contrast to the findings of Saha et al in Bangladeshi[10], where Widal test was found to have 80% sensitivity and specificity, and even Taiwo et al had 92.5% and 95% sensitivity and specificity, respectively.[12]

The way forward is that clinicians should take detailed histories from patients and meticulously/properly examine their patients before sending them to the laboratory for Widal test and remember that their clinical acumen is also very important in making diagnoses; but most importantly, when in doubt of any laboratory results, they should seek a second opinion. It is most profitable for the patient if the result is interpreted by a clinical Microbiologist.

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