Diagnostic methods for typhoid fever in Nigeria

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ABSTRACT

In this study, 65 patients are screened for *Salmonella typhi* by conventional culture and the Widal test. In addition, the patients undergo full blood count are screened for malaria parasites. Of the 65 patients, 50 report febrile conditions, while the remaining 15 are used as a control population. In the febrile group, 13 (26%) were positive for *S. typhi*, while in the control group only one (7%) was positive for *S. typhi*. Overall, 36 (64.3%) patients had malaria parasites. Patients with a higher O antibody titre (≥ 1 in 80) by Widal test were found to have consumed both tap water and pure water. More females (10/14; mean age: 33) had typhoid fever as a result of *S. typhi* infection, the majority of which were isolated from stool samples (57%). Nine of the isolates were also positive for malaria parasites, seven of which were in the trophozoite stage. *Plasmodium falciparum* was the predominant parasite (78%), the remainder being *P. malariae*. The majority of patients (12/14) with typhoid fever had normal PCV values. In conclusion, it is recommended that tests for the diagnosis of typhoid fever in Nigeria should include malaria parasites, *S. typhi* culture from faecal samples, and the Widal test.

KEY WORDS: Bacterial culture. Malaria. Parasites. Salmonella typhi. Widal test.

Introduction

Typhoid is an important public health problem throughout the world. Globally, there are more than 21 million cases of typhoid each year, with more than 700,000 deaths. It remains an important cause of morbidity and mortality in many developing countries. In Africa, about 4.36 million cases occur out of an estimated population of 427 million.[1] This febrile disease is among the major widespread diseases that affect both young children and young adults.

In many regions where typhoid fever is endemic, the clinic diagnosis of typhoid may be complicated by the presence of other febrile conditions such as malaria, diarrhoeal diseases and respiratory tract infections, all of which may present with signs and symptoms resembling typhoid fever. As a consequence, confirmation of a clinical diagnosis is of critical importance in ensuring prompt identification and appropriate treatment of patients with typhoid fever.

There are several laboratory methods available for the diagnosis of typhoid fever (e.g., Widal test, isolation and culture of *Salmonella. typhi* from clinical specimens [blood, stool, urine, bone marrow aspirate and intestinal secretions]). Culture is often regarded as the gold standard; however, other methods used include antigen detection using enzyme-linked immunosorbent assay (ELISA), dipstick assays and molecular methods.[2–4]

In Nigeria, the demonstration of a four-fold or greater rise in antibody on a single Widal test result on paired sera during the acute phase of illness is regarded as diagnostic. However, there is still disagreement about whether an increased O titre or H titre is of greater diagnostic value in endemic countries.

Previous reports abound on the non-specific nature of the Widal test in some Asian countries[3,5] and in Nigeria.[6,7] In the latter, malaria and typhoid are endemic and often occur together. So, when a patient presents with a febrile illness, a Widal test or malaria parasite test (or both) are performed and treatment is based on the results. Very few laboratories culture for *S. typhi*.

In recent years in Nigeria, the prevalence of *S. typhi* has increased from 13% in 1995[8] to approximately 34% in 2003.[9]

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Antibiotic susceptibility patterns from different studies show that *S. typhi* is generally resistant to the drug of choice, chloramphenicol.[10–12] No previous report from Nigeria has compared the results obtained with the Widal test, culture and malaria parasite test for the definitive diagnosis of typhoid fever. The poor management of febrile conditions in Nigeria may have been the cause of significant loss of life. Therefore, there is a need to resolve the diagnostic and management problems associated with the most common causes of febrile conditions in Nigeria, of which typhoid fever is one.

This study aims to discover the incidence of *S. typhi* infection and malaria in Nigeria, in order to achieve the proper diagnosis of typhoid fever.

Materials and methods

Blood, stool and urine samples obtained from 50 patients presenting with febrile illness and from 15 afebrile controls were cultured for *S. typhi*. In addition, serum samples were screened by Widal test and conventional techniques were employed for full blood count and malaria parasite tests. A widal antibody O titre of 1 in 80 was regarded as indicative of typhoid fever.

Blood (5 mL) was added aseptically to 45 mL brain–heart infusion (BHI) broth and transferred to a BHI agar slant in a

Table 1. Clinical symptoms reported by patients (%) infected with *S. typhi*.

Symptom	Patients (%)
Headache	100
Fever	93
Weakness	93
Dizziness	57
Abdominal cramp	43
Diarrhoea	21
Vomiting	21

medical flat container to form a biphasic medium. This was incubated at 37°C. Blood cultures were subcultured daily for seven days on a salmonella–shigella (SS) agar plate and incubated at 37°C for 24 h.

Stool samples were inoculated into selenite F broth and incubated overnight. Stool cultures were then subcultured onto SS agar and incubated at 37°C for 24 h. Early morning midstream urine was collected aseptically in a universal container and 0.02 mL was inoculated onto MacConkey, blood and SS agars. These were incubated at 37°C for 24 h.

Strains were confirmed as *S. typhi* by biochemical characteristics[13] and serotype tests.

Full blood count was performed on a Coulter AT8 series analyser. A questionnaire was used to obtain information on symptoms and to identify the sources of drinking water and food.

Statistical analysis was performed using SPSS (version 11) and Fischer's exact test (using EPI-INFO).

Results

Of the 65 patients screened, 14 (22%) were positive for *S. typhi* by culture. Table 1 shows the clinical symptoms reported by the patients with *S. typhi*. Four of the 14 patients were also positive for *S. paratyphi* A, B and C. With a Widal titre of at least 1 in 80, 31 (48%) had a significant titre for O antigens and 36 (55%) had malaria parasites. Nine of 14 (64%) patients were positive for both *S. typhi* and malaria parasites (seven *P. falciparum*, two *P. malariae*; mainly in the trophozoite stage). More women (10) than men (four) had typhoid fever, and the mean age was 33 years. Of the 14 *S. typhi*-positive cases, six were isolated from blood and eight were isolated from stool samples. *S. typhi* was not isolated from urine culture (Table 2).

From the questionnaire, 51/65 (78%) of the patients ate in canteens or at the kerbside. Of those included in the study, 32% had taken antibiotics prior to attending the clinic. The most common source of drinking water among the *S. typhi*-positive patients was tap water (90%) and 'pure water' (90%). The majority (12/14) of the *S. typhi*-positive patients had full blood count results within normal limits.

Discussion

Clearly, the possibility that a patient can be positive for *S. typhi* and malaria parasites simultaneously is apparent. Many hospitals/clinics do not actually culture for *S. typhi* and reliance on malaria tests and the Widal test may lead to an incorrect diagnosis.[14]

Although blood culture has proved more specific for the diagnosis of *S. typhi*, its sensitivity is low. One reason for this could be that only small numbers of bacteria are needed to cause severe infection and a positive blood culture is directly related to the volume of blood collected.[15] Hence, positive culture yields are low and definitive diagnosis may be difficult to achieve. Other limiting factors include prior antibiotic treatment. In Nigeria, across-the-counter purchase of drugs is commonplace and 32% of the patients in the present study had received prior antibiotic therapy.

Bone marrow aspirate culture is claimed to be most sensitive,[4] and one report from Nigeria corroborated this view, showing a sensitivity of 38.1% compared to 33% and 28.6% for stool and blood samples, respectively. However,

Table 2. Parameters used for the diagnosis of *S. typhi*.

Strain No*	Sex	Age	Widal OD	Widal HD	MP	Sample
1	F	29	1:160	1:160	+	Stool
2	F	25	1:20	1: 20	+	Stool
3	F	20	1:80	1:160	+	Stool
4.	F	36	1:320	1: 80	–	Blood
5	F	42	1:320	1:80	–	Blood
6	F	24	1:80	1:80	+	Blood
7	F	59	1:160	1:20	+	Stool
8	F	23	1:80	1:20	–	Stool
9	M	39	1:80	1:20	–	Stool
10	F	35	1:20	1:20	+	Blood
11	M	33	1:160	1:80	+	Blood
12	F	21	1:20	1:20	–	Blood
13	M	36	1:20	1:20	+	Stool
14	M	40	1:20	1:20	+	Stool

* Culture-confirmed strains

bone marrow aspiration is only used as a last resort in difficult cases.[16]

Typhoid fever can also be associated with leucopenia (with a relative lymphocytosis) and anaemia. This was corroborated in the present study by those *S. typhi*-positive patients with a high Widal titre (Table 2). However, some patients with high Widal titres (≥ 1 in 160) proved to be negative for *S. typhi* by culture. In the face of such high titres, Nigerian physicians simply prescribe antibiotics to treat what they believe to be an *S. typhi* infection. The results of the present study suggest that a high Widal titre should be confirmed with an *S. typhi*-positive culture to avoid the possibility of misdiagnosis.

In cases where patients present with febrile illness and are negative by Widal test and for malaria, other febrile conditions should be considered and investigated. In the present study, however, the patients generally presented with symptoms that were typical of typhoid fever, and similar symptoms were reported in a review article by Crum.[4] In general, the present study showed that *S. typhi* and malaria coexist and that it is advisable to always culture for *S. typhi* as a confirmatory test before prescribing antibiotic therapy to patients with febrile illness.

A high proportion (78%) of the patients in the present study ate in canteens or at the kerbside, and food obtained at the latter is often cheaper than in canteens but is prepared and sold in considerably less hygienic conditions. Furthermore, most of the patients (90%) infected with *S. typhi* had used tap water and/or so-called pure water as their source of drinking water. It would appear, therefore, that the level of chlorination of the public water supply might be inadequate. On the other hand, pure water is sold in sachets in Nigeria and is presumed to be free of contamination; however, this may not always be the case and more vigorous work should be undertaken by the Federal Food and Drug Agency to check on those who sell impure water and ban them.

In conclusion, the proper diagnosis of typhoid fever in Nigeria requires a combination of Widal test, faecal culture of patient samples for the presence of *S. typhi*, and examination for malaria parasites.

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