LABORATORY DIAGNOSIS OF TYPHOID FEVER IN ACCRA

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SUMMARY

The aim of this investigation was to evaluate the most appropriate method for the diagnosis of *Salmonella typhi* septicaemia. This involved the culture of blood samples, Widal test and antibiotic sensitivity test. Of 50 patients suspected of having typhoid fever, blood cultures of 38 (76%) yielded no bacterial growth, while 12 (24%) produced bacterial growth. Organisms encountered included *Salmonella typhi, Kblebsiella species and Staphylococcus aureus*. Antibody to the O and H antigens was detected in 20 serum samples using the Widal test. Out of this number, 7 patients diagnosed to have *S typhi* infection had antibodies titres of 1/80. Convalescent sera from all diagnosed cases of *S typhi* showed at least a two-fold rise in titre. In addition those infected with *Klebsiella sp.* and *Staphylococci* also had low antibodies titres to O and H antigens. It was also discovered that 9 patients who had no bacterial growth also had titres of <1/80 to O and H antigens. The *S typhi* isolates were all sensitive to Cefotaxime and Ofloxacin, both third line drugs so it will be prudent to keep them as such. Although one strain was resistant to Chloramphenicol, it still remains the antibiotic of choice. The widal test, like all immunological assays, cannot be positive by mere detection of antibody but based on a cut-off point. It must therefore be carefully interpreted and used together with blood cultures and clinical findings to safely diagnose *S. typhi* infections.

Keywords: Widal test, typhoid fever.

INTRODUCTION

Salmonella typhi Septicaemia (typhoid or enteric fever) is acquired through ingestion of contaminated food or water. It can also be acquired through cross-infections, as during dental extraction, bronchoscopy, needle biopsy and a variety of other medical and surgical procedures[1,2]. *Salmonella typhi* is also capable of causing bowel perforations and osteomyelitis.

The isolation and identification of *S. typhi* in blood cultures is a strong evidence of typhoid fever. In typhoid fever, *S. typhi* can be detected in the blood of about 75% of patients during the first 10 days of infection, and in about 30% of patients during the third week[1]. The organism is sometimes isolated in urine and also in stool especially after the second week of infection.

In order to reduce the work load in the laboratory and to expedite the processing of specimens, selective media are used whenever possible. Bile broth as a selective medium and Trypticase soy broth have been recommended as culture media for isolation of *S. typhi* from blood.

The purpose of Widal test is to determine the presence of *Salmonella* antibodies in the patients' sera, as a result of *Salmonella* infection, past or present. The agglutination test by itself can never afford more than a presumptive diagnosis of *Salmonella* infection, and it must always be interpreted in relation to the clinical condition of the patient an to previous experiences. There are so many limitations to this test that it should always be subordinated to direct culture[3]. In active typhoid, there is an early rise in antibody titre. Up to 70% of adult patients show a rise in antibody titres in the first week of infection. Most patients, show a two or three-fold rise in one or both agglutinin titres. About 10% of patients with active typhoid may show no rise in O and H titres as a result of severe hypoproteinaemia.

Numerous studies have produced data which have cast serious doubts on the value of widal test in the diagnosis of typhoid fever[4,5,6,7]. A large number of patients presenting with febrile illness in Accra has been diagnosed as having typhoid fever using the Widal test. Anecdotally, one hears such as statement from patients who visited our laboratory: "I had typhoid fever and was treated but after a follow-up test I am diagnosed as still having the disease". We therefore conducted this study to evalu-

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ate the methods available for the diagnosis of typhoid fever in Accra.

METHOD

Blood samples were obtained from patients attending the Departments of Child Health and Medicine as well as Emergency Wards of the Korle Bu Teaching Hospital, Accra. Fifty patients suspected of having typhoid fever, had blood samples taken before the start of antibiotic therapy. Blood samples were taken at a time when the patients were having a rise in temperature. A second batch of blood samples were taken at a time when the patients were having a rise in temperature. A second batch of blood samples from the same patients were collected 7-14 days later (during or after therapy). About 115 ml of blood was collected using aseptic conditions from each patient on each occasion. Ten millilitres was discharged into sterile thioglycollate broth for routine culture. Serum from the remaining 5ml was used for the Widal test while the clot was used for culture, in Bile and Trypticase soy broth.

The Widal test was performed as per the manufacturer's instructions (Morganville, N.J. 07751, USA).

Blood samples collected into thioglycollated broth were incubated at 37°C for 18-24 hours. The broken clot in Bile and Trypticase Soy Broths (TBS) were also incubated at 37°C for 18-24 hours. The bottles were examined macroscopically for turbidity, haemolysis, gas and pellicle formation. Subcultures of the thioglycollate broth were made onto MacConkey agar and blood agar. The bile and trypticase soy broths were, however, subcultured onto Deoxycholate Citrate Agar (DCA). A gram stain was carried out on each TSB culture. All broth cultures that showed no evidence of bacterial growth were re-incubated and examined daily for 7 days before being discarded.

All isolates were identified using standard biochemical methods and specific antisera[8]. Antibiotic sensitivity tests were also carried out using Stokes method with *Staphylococcus aureus* NCTC 6571 as the control strain. The antibiotics tested were Chloramphenicol (30mg), Ofloxacin (5 mg), Ceforaxine (30 mg).

RESULTS

Of the 50 blood samples cultured there were bacterial isolates in 12 (24%) (Table 1). The remaining 38 specimens (76%) produced no bacterial growth. Out of the 12 isolates, 7 (58%) were positive for *Salmonella typhi*. Other bacteria isolated included *Klebsiella species* 2 (16.7%) and *Staphylococcus aureus* 3 (25%).

Table 1 Distribution of positive blood culture and their corresponding Widal results

Case No.	Age	Bacteria isolated	Widal titre			
			Acute		Convalescent	
			O	H	O	H
1	7	*S. typhi*	1/40	1/80	1/160	1/160
3	19	*Klebsiella sp.*	1/20	1/140	1/20	1/40
7	18mths	*S. typhi*	1/80	1/80	Dead	
10	29	*Klebsiella sp.*	1/40	1/80	Discharged	
12	18	*S. typhi*	1/80	1/160	1/320	1/160
14	9mths	*S. typhi*	1/80	1/80	1/160	1/160
17	8	*S. typhi*	1/80	1/80	1/640	1/320
20	7	Staphylococcus aureus	1/20	1/20	1/20	1/40
24	11	*S. typhi*	1/80	1/160	1/160	1/320
32	23	*Staph. aureus*	1/20	1/40	1/20	1/40
40	21	*S. typhi*	1/80	1/80	1/80	1/160
49	28	*Staph. aureus*	1/20	1/20	1/40	1/20

Widal test was carried out on the 50 blood samples, and the number of serum samples having some antibody to the O and H antigens was 20. Out of this number, 7 patients diagnosed to have *S. typhi* infection had antibodies titres of ≥1/80. Convalescent sera from all diagnosed cases of *S. typhi* showed at least a two-fold rise in titre. In addition those infected with *Klebsiella sp.* and Staphylococci also had low antibodies titres to O and H antigens. It was also discovered that 9 patients who had no bacterial growth also had titres of <1/80 to O and H antigens.

Majority of *S. typhi* strains were sensitivity to Chloramphenicol but one strain was resistant (Tables 2). Two *S. typhi* isolates were resistant to Cefuroxime. *Klebsiella* and *Staphylococcu sp.* were all

resistant to Chloramphenicol but sensitive to Cefuroxime, Oflozacin and Cefotaxime.

A comparison of bile to trypticase soy broth clearly showed that more contamination occurred with trypticase soy broth than with the bile broth.

Table 2 Sensitivity pattern of all bacteria isolated

Bacteria (isolates)	CHL	CRX	DFX	CTX
Salmonella typhi (4)	S	S	S	S
Salmonella typhi (2)	S	R	S	S
Salmonella typhi (1)	R	S	S	S
Klebsiella species (2)	R	S	S	S
Staphylococcus aureus (3)	R	S	S	S

Key: a. CHL = Chloramphenicol
CRX = Cefuroxime
OFX = Ofloxacine
CTX = Cefotaxime
b. S = Sensitive
R = Resistant

DISCUSSION

This study was conducted to assess the diagnostic value of the widal test in Accra. It also evaluated the media that are used and the use of Chloramphenicol as drug of choice in the management of typhoid fever.

Antibody to O and H antigens was detected in 20 serum samples. Out of this number, 7 patients diagnosed to have *S. typhi* infection had antibodies titres of ≥1/80. Convalescent sera from all diagnosed cases of *S. typhi* showed at least a two-fold rise in titre. In addition those infected with *Klebsiella sp.* and Staphylococci also had low antibodies titres to O and H antigens. It was also discovered that 9 patients who had bacterial growth also had titres of <1/80 to O and H antigens.

These findings show that in an endemic area such as Ghana, *S. typhi* agglutinins maybe present in the general population. Care must therefore be taken in the interpretation of the Widal test as mere detection of antibody does not mean disease. Furthermore, the Widal test is an agglutination test[9] and is affected by a number of factors.

There must be a National cut-off point for interpretation of results from the Widal test. A two-fold rise in the titre of convalescent sera could also be useful when paired sera are available.

Though thioglycollate was used as the routine medium, a comparative culture of bile broth and TSB showed that contamination was more common in the TSB than in the bile broth. Furthermore, bacterial growth was enhanced in the bile broth as compared to the TSB.

Antibiotic sensitivity tests were carried out on isolates, to assist the clinician in selecting the appropriate antibiotic. It was observed that there was no single pattern of antibiotic sensitivity for a given species for the four selected antibiotics. Six (85.7%) *S. typhi* were sensitivity to Chloramphenicol, and five (71.4%) to Cefuroxime. All seven *S. typhi* strains were sensitive to Cefotaxime and Ofloxacin. Since only one isolate was resistant to Chloramphenicol, it still remains the antibiotic of choice.

From the foregoing it is recommended that typhoid fever should be diagnosed by culture of blood samples[10]. The Widal test is non-specific, poorly standardized, often confusing and difficult to interpret[11]. In our opinion, the Widal test should not be used in isolation as a diagnostic procedure for *S. typhi,* but as an additional aid. If culture facilities are not available a strong clinical suspicion, rather than Widal test, warrants therapeutic intervention. Since antimicrobial resistance is a worldwide problem, it is suggested that sensitivity testing should be made on all isolates of *S. typhi*. It is not safe to rely on previous sensitivity patterns for a given species, since the pattern of resistance may vary in different areas and with different species. These conclusions were based on only fifty samples so a larger sample size study to validate these finding is recommended.

It is, however, time for diagnostic laboratories in this country to update their techniques and adopt more modern diagnostic methods such Enzyme Linked Immunosorbent Assay (ELISA), Immunoelectrophoresis and DNA technology. Thus misuse of the Widal test and associated misuse of antibiotics could be avoided.

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