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MICROBIOLOGICAL STUDIES OF BLOOD SPECIMEN FROM PRESUMPTIVELY DIAGNOSED TYPHOID FEVER PATIENTS IN ZARIA, NORTHERN NIGERIA.

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ABSTRACT

Three hundred and fifteen blood samples were obtained from presumptively diagnosed typhoid patients who were referred for Widal Serological test at four diagnostic centres. The blood samples were subjected to bacteriological investigations. Salmonella and non-Salmonella organisms isolated were identified according to standard identification schemes. The Salmonella serological O - and H - antigen titre values of the patients whose blood samples were cultured, were also collated and compared with the bacteriological findings.

There was generally low correlation between the antigenic (O and H) titre value and cultural isolation of the causative organisms of typhoid fever. However, as the O-antigen titre value increased from 1:20 to 1:160, the percentage of samples in which Salmonella spp. Were isolated, rose from 5.6% to 50%. There was also significant variation in the percentage values among the four diagnostic centers (varying from 46% to 83% at O-tire value of 1:160). Beside Salmonella spp. Other organisms comprising mostly members of the Enterobacteriaceae Family, Psuedmonas spp. and Streptococcus were isolated from the blood of patients presenting high O-antigen titre values.

KEY WORDS: Typhoid Fever Diagnosis

INTRODUCTION

Typhoid fever is a debilitating systemic infection caused by *Salmonella typhi* and *paratyphi* with a contagious incidence of as much as50% (1). It is ogften fatal if allowed to progress for long, undetected and untreated. It has continued to pose serious epidemiological problems due to its high mortality and morbidity rates as well as its adverse economic effects in countries where it is endemic. Prognosis is good once the infection is diagnosed early and prompt treatment is commenced. Proper management of the infection largely depends on its early and prompt diagnosis. Many of the clinical symptoms presented in typhoid such as continuous fever, headache, malaise, bradycardia and early constipation, at the early state of the infection, closely resembles those presented by other similar feverish infections like malaria, hence other identification methods are usually employed to aid its diagnosis in patients.

Proper diagnosis and confirmation of clinical symptoms presented by typhoid suspected patients is only achieved by isolating and identifying the causative organisms coupled with serological examination of antigenic properties. Diagnostic methods currently in use are broadly classified into two: conventional methods and rapid methods. The conventional methods involve the isolation and identification of the causative organisms by culturing in non-selective, selective enrichment and differential media (cultural method), followed by serolohical confirmation. Cultural method is highly time-consuming and may constitute a problem where prompt and early institution of therapy of the

infection is urgently desired. Generally, presumptive result takes about 3-4 days while definite positive result is obtained only after 5-6 days. The serological test, which examines the patient' serum for salmonella antibodies is a rapid tool in the diagnosis of enteric fever, but can afford an indirect evidence of infection and can not differentiate between recent infection from past exposure or previous treatment with TAB (typhi, paratyphi A and B) vaccine.

The rapid methods which provide reliable and precise results within 24-27 hours, still lay emphasis on the detection of the causative organisms of typhoid. They involve the amplification of the target organisms as the case with immunomagnetic separation (IMS), bacteriophage amplification, enzyme-linked immunosorbent assay (2,3,4,), or amplification of the DNA of the causative organism, for example polymerase chain reaction, pulsed field field gel electrophoresis and hybridization (5,6,7). Most of the rapid methods especially the molecular ones require expensive materials, specialized facilities and trained personnel to be carried out, which are not readily available in developing countries like Nigeria.

In Nigeria and many other developing countries, bacteriological culturing and serological tests are the only available diagnostic methods employed in typhoid fever confirmation. In the recent times, morbidity and mortality from typhoid has been on the increase in (8). Most of the deaths that have resulted from typhoid in Nigeria have largely been attributed to incorrect diagnosis and/or improper treatment. Analysis of patient medical records in hospitals in zaria as well as responses distributed to the public in the same environment showed that Widal serological test is virtually the only diagnostic tool used to confirm clinical symptoms presented by typhoid suspected patients in Zaria and other major towns in Northern Nigeria. This is because it yields results within a few hours (9). This work report on the significance of Widal serological diagnostic test in relationship to the cultural method on blood samples from presumptively diagnosed typhoid patients in Zaria with a view of assessing the proper role of serological test in typhoid diagnosis in this environment

MATERIALS AND METHODS

Blood Sample Collection

Blood samples of patient presumptively diagnosed for typhoid fever by physicians and referred for Widal serological test, were obtained from four laboratories where Widal serological test were being performed; two hospitals with equipped microbiology laboratories and two private medical laboratories, all located within Zaria town of Kaduna State, Nigeria. The blood samples were aseptically collected into sterile bijou bottles containing sodium citrate anti-coagulant solution (10), mixed and stored in cold packs for transport to the laboratories for culturing. A total of 315 blood samples (one sample from each patient) were collected for analysis

Serological Test

Widal serological diagnosis was carried at the four collection centers by staffs of the respective laboratories. The tube agglutination method in which various dilutions of patient's serum are mixed with drops of either O or H-antigen of *Salm. Typhi, salm. Paratyphi* A, *Salm. Paratyphi* B or *Salm paratyphi* C (11) was employed in all the four laboratories. The titre values obtained in these laboratories were collected and compared with the corresponding results of the bacteriological cultures. Stained Bacterial Antigen suspensions used in this test were products of Biotec Laboratories (Suffolk, UK) and Antec Diagnostic Products, also of U.k.

Preparation of Bacteriological Culture Media

For the culturing, isolation and eventual identification of organisms from the blood samples, various bacteriological media were used. Bismuth sulfite agar, Koser citrate, MRVP broth, Peptone water, Salmonella-Shigella agar an tetrathionate broth media were Oxoid products (Oxoid Ltd, Basingstoke, England). Casein peptone soya peptone(CASO) broth and agar were obtained from Biotec Laboratories (Surrey, U. K.). Urea broth was from Difco Laboratories (Detroit, USA).

Appropriate quantities of the dehydrated media were reconstituted in freshly distilled water, distributed in desired amounts and sterilized as specified by the manufacturers. The sterilized media were stored in refrigerator until required.

Bacteriological Examination

Collected blood samples were cultured into sterile peptone water, for tetrathionate broths. Growth from these broth cultures were sub-cultured onto surfaces of selective and differential agar media of Bismuth sulfite, Deoxycholate citrate, MacConkey and Salmonella Shigella, incubated at 37^0C for 24-48 hours. Where necessary, growths were also inoculated onto other selective and diagnostic media such as Pseudocel (for Pseudomonas aeruginosa) and Kligler iron ager media. Biochemical test were carried out as recommended in some monographs and textbooks (12,13). Eventual identification of the various isolates were achieved by comparing the morphological characteristics of resulting growths (microscopic and macroscopic) and their biochemical profiles with thos stated in the individual media monograph in the Oxoid manual and literatures (1,11,14,15).

RESULTS

Bacteriological culturing of blood samples from the 315 presumptive diagnosed typhoid patients yielded organisms in 237 samples. Of these numbers, 112 of them were Salmonella organisms (Table 1). The relationship between antigen titre values and isolation of Salmonella spp and other organisms from typhoid patients is also illustrated in this table. Generally, higher proportions of organisms were isolated at the higher O-antigen titre values. For example, at O-antigen titre value of 1:20, only 6 of the 54 samples yielded organisms (i.e., 11%) compared with 107 organisms out of 108 blood samples at titre value of 1:160. Similarly, percentage of samples in which Salmonella organisms were isolated increased with increasing O antigen tire values. For example, percentage of blood sample in which salmonella organisms were isolated were 5.6%, 23.64%, 42.42% and 50% at titre values of 1:20, 1:40, 1:80 and 1:60 respectively. Analysis of the data based on the flagella (H) antigen showed similar trend with the O-antigen. Highest percentages of Salmonella organisms were obtained at H-antigen titre values of 1:80 and 1:60 (6.3% at 1:20 and 29% at 1;40, compared with 49% at 1:80 and at 1:160).

Analysis of other organisms isolated at the different 0_antigen titre values shows that organisms mostly belonging to the Enterobacteriaceae, pseudomonas and Streptococcus Families were also isolated at O-antigen titre values normally considered as indicative of typhoid infection (Table 2). Of the 35 other non-Enterobacteriaceae organisms (27 of them were sugar fermenting and 8 others non-sugar fermenting gram negative organisms), 29 were isolated at high 0-antigen titre values.

Table 3 shows that the percentage of samples in which Salmonella organisms were isolated, varied significantly from one diagnostic laboratory to another: 58% in center A (a 50-bed hospital in Samaru, Zaria) to 31.3% in center D (a private medical laboratory). He variation in the distribution of isolates among the various centers is more obvious at relatively high O-antigen titre values. For example, at O-antigen titre value of 1:160,

83.3% samples screened in Center A yielded Salmonella organisms, which dramatically dropped to 47% in center C.

Table 1: Distribution of Organisms isolated from Presumptively diagnosed Typhoid Patients According to O-antigen Titre

O-antigen titre	No of Samples screened	No of samples in which organisms were Isolated	No of samples in which Salmonella spp were Isolated
1:20	54	6	3 (5.6)*
1:40	55	29	13 (23.10
1:80	98	95	42 (42.4)
1:160	108	107	54 (50.0)
Total	315	237	112

***Figures in parenthesis represent the percentage of the isolates that were identified as* Salmonella spp.**

Table 2: Distribution of Non-Salmonella Organisms from Blood Samples of Suspected typhoid Patients According

Isolated Organisms	No of antigen 1:20	Organisms titre Values 1:40	Isolated of: 1:80	At O- 1:160
A. Gram Negative Rods				
I. **Enterobacteriaceae**				
i. Citrobacter spp	0	0	4	1
ii. Enterobacter spp	0	2	3	8
iii. Klebsiella spp	0	1	2	5
iv. Proteus spp	0	0	3	5
v. Seratia spp	0	1	1	3
vi. Shigella spp	0	2	1	2
Non-Enterobacteriaceae				
i. *Ps. aeruginosa*	0	1	3	5
ii. other Pseudomonas spp	0	0	4	2
iii. others (e.g. Acinetobacter, Aetomonas)	1	5	16	13
B. Gram Positive Bacteria				
i. Staphylococci spp	0	0	2	1
ii. Streptococci spp	0	0	6	4
TOTAL	**1**	**12**	**45**	**49**

Table 3: Distribution of Organisms Isolated from Presumptively Diagnosed Typhoid Patients According to Serological Diagnostic Centres.

Diagnostic Centres	No of Samples screened	No of *Salmonella spp* isolated	Percentage of Samples in which *Salmonella spp* were isolated at O-antigen titres of	
			1:80	1:160
A	31	18	72.7	83.3
B	34	9	25.0	56.6
C	151	54	31.0	47.0
D	99	31	55.0	50.0

DISCUSSIONS

The higher percentages of Salmonella organisms isolated at high O – and H –antigen titre values in this study is in agreement with previous findings: in a study on the usefulness of Widal test for diagnosing typhoid fever in Lebanon (16), Widal test was mostly discriminative at O-titre values of at least 1/160, having a sensitivity of 67.9%. Though there is a positive and direct relationships between increasing serum antigen titre values and probability of isolating Salmonella organisms, high serum O-antigen titre value should not be taken alone as indicative of active typhoid infections state, as organisms other than *Salmonella sp.* May be responsible for such infections as shown in this study. It has also been reported that correlation between serological findings and isolation of causative organisms of typhoid fever is often low (1); it is dependent on the stage of infection at which the sample is collected and the type of sample obtained for bacteriological culturing. For example, while *Salmonella sp.* Can be isolated from blood in 90% of cases in the first week of an infection, the probability decreases substantially to about 30-50% in the third week (1, 17). In contrast, the serological titre values continue to rise as long as the infectious organism is not cleared from the body system. Conversely, the frequency of isolation of organism in feaces increases from 40-50% in the first week to 80% in the third week. It should be realized also that O-antigen titre value may be very low (e.g 1:20) in patients infected by *Salmonella sp.* Possessing virulent (Vi) antigens which usually masks the O-antigen and prevent agglutination of such organisms by patient' serum. As observed in this study, in most clinical typhoid infections states, high O-antigen titre values are usually associated with high H-antigen titres, hence such patients are often contagious.

The isolation of non-Salmonella organisms in patient presenting relatively high O- and H-antigen titre values indicates that organisms beside *Salmonella sp.* may also be responsible for elevation of O-antigen titre values. This observation is in agreement with the findings of a similar study carried out in the Eastern part of Nigeria (8). This might be due to the fact that organisms belonging to *Enterobacteriaceae* and *Pseudomonas* Families possess similar outer membranes with Salmonella organisms (18): O-antigen is a lipopolysacchacide polypeptide complex, present in the outer membranes of most gram negative bacteria. It has also been reported that there is cross-reactivity between Salmonella O-antigen and other *Enterobacteriaceae* organisms particularly *Citrobacter, E. coli, Serratia and Enterobacter spp.* (19, 20). Cross-reactivity between O-antigens of *Pseudomonas aeruginosa* and *Salmonella* spp. had also been observed (18). The isolation of organisms which are not negative bacteria and therefore do not possess common outer membrane structures with Salmonella oranissm at high O-angiten titres may not be particularly strange as cross reactivity of O-antigen of Salmonella with *Saccharomysces cerevisiaw* has been reported (18). The presence of *Streptococcus pneumoniae* and *H. influenzae* at high Widal O-antigen titres may be due to the pathological conditions caused by these organisms (20). Even malaria infections is reported to increase Salmonella O-antigen titre values 918). The isolation of non Salmonella organisms from patients presumptively diagnosed for typhoid fever is worrisome as it has been shown that non-typhi Salmonella bacteria can occur with high incidences of morbidity and mortality rates (21).

The wide variation in the distribution of isolates among the various centers and the relatively low correlation between the antigenic titre values and cultural isolation of Salmonella organisms from the blood samples might be attributed to the non-

adherenceof of the laboratory personnel to recommended dianostic procedures. Investigation carried out during the study revealed that in some private medical laboratories, the O-antigen suspensions provided in the Widal test kits were often pooled together for use in the determination of antigen titres, as way of maximizing profit. This could lead to non-specificity in the reaction and may account for the non-isolation of salmonella organisms at the high O-antigen titre values the pooling together of O-antigen suspensions may also be responsible for the differences in the percentage of samples that yielded Salmonella sp. at high O-titre values among the hospitals and medical laboratories where the serological tests were performed.

Widal serological test should as much as possible be complemented with isolation of the causative organisms of typhoid fever. Cultural isolation, biochemical characterization and sero-typing are essential for complete identification of salmonella organisms, since no matter how well-defined a serological laboratory may be, serological procedures do not supercede bacteriological culturing.

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