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Stool or blood culture? A search for a gold standard for isolation of salmonella typhi from patients with clinical symptoms of enteric fever in Bauchi state tertiary hospital

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Abstract

Introduction: *Salmonella typhi* (*S. typhi*) infection is endemic in Nigeria. In recent time, blood culture and stool were the most common diagnostic means of confirming salmonella infection in humans, since it is based on culture for isolation, identification among others. Although, there is lack of reliable and standard method of culture for its isolation. This study aimed to determine the gold standard between blood and stool for the cultural isolation of *S. typhi*.

Methods: The study used laboratory-based cross-sectional study; patients who presented symptoms of enteric fever visiting Abubakar Tafawa Balewa University Teaching Hospital **(**ATBUTH) during the period of the study were recruited. Informed consent of volunteers and guardians were obtained and in addition confidentially of the results were ensured. A total of 150 patients samples were collected and cultured including adults and children. The bacterial isolated were identified by gram's reaction and biochemical characteristics.

Result: Out of 150 samples examined, 7 (4.1%) tested positive for *S. typhi* comprising of 5(71.4%) culture from blood and 2(28.6%) culture from stool. The result of this study shows a significant mean difference (t-value = 2.95, p-value = 0.026) between Blood culture and stool culture at 5% level of significance. The culture from blood was found to be more sensitive than the culture from stool. It is therefore recommended gold standard for S. typhi isolation.

Conclusion: The study revealed cultures from blood specimen's yielded highest number of *S.typhi* isolates when related to culture from stool samples. Therefore, culture from blood sample should be of priority in the isolation of *S. typhi* for easy identification of the bacteria. Culture from blood sample remains the gold standard method for isolation of *S. typhi* when it comes to blood or stool sample.

Keywords: Blood; Stool; *Salmonella Typhi*; Gold standard

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1 Introduction

Typhoid fever which is also known as enteric fever is an acute febrile illness that is caused by the bacterial *Salmonella enteric* serovar *typhi*. *S. typhi* is a Gram-negative bacterium that infects the intestinal tract of man and can enter blood circulation too [1]. In 2017, an estimated 10.9 million cases of typhoid fever and 116,800 deaths were attributed to *Salmonella enterica* serotype *typhi* globally [2]. Infection occurs in all age group with higher incidence and more variable clinical presentation in children [3]. Human beings are the only reservoir and host for typhoid fever, the disease is transmitted by feacally contaminated water and food in endemic areas, especially by carriers handling food [3]. Typhoid fever has an important socio-economic impact, so accurate diagnosis of the disease at an early stage is important not for etiological diagnosis but also for identifying individuals that may serve as potential carrier who may be responsible for acute typhoid fever outbreak [4]

Culturing the bacteria from body fluids is the definitive test for the diagnosis of typhoid fever [5]. The culture of *S. typhi* can be done from many body fluids such as blood, stool, bone marrow, urine, rose spot extracts, duodenal and aspirates [4]. The objective of the study was to search for gold standard between blood and stool culture in isolation of *Salmonella typhi* for the benefit of accurate diagnosis of typhoid fever in Tertiary Hospital, Bauchi State of Nigeria.

2 Material and methods

2.1 Study Population

The study population, were both male and female young and adult patients who attended the General out patients Department of Abubakar Tafawa Balewa University Bauchi, Nigeria, with the clinical symptoms of enteric fever [6] as diagnosed by the attending physicians. These patients did not include those who were on antibiotics about two weeks prior to specimen's collection.

2.2 Ethical Clearance

Ethical approval was obtained from the ethical committee of Abubakar Tafawa Balewa University Teaching Hospital. Informed consents were also obtained from all the participants.

Abubakar Tafawa Balewa University Teaching Hospital.

2.3 Sample Collection

Two different samples (blood and stool) were collected from 150 patients attending Abubakar Tafawa Balewa University Teaching Hospital showing symptoms of enteric fever resulting to a total of 150 samples obtained. All patents were instructed on how to collect appropriate specimens. Blood samples were collected from patients using blood sample bottles by the clinician while stool specimens were collected using a sterile, clean, , wide necked container with tight fitting lid. All the specimens were taken to the laboratory for analysis without delay [7].

2.4 Sample Processing

2.4.1 Blood culture

Using a sterile syringe and needle, 4ml of whole blood from the patient was collected and dispensed into 20ml tetrathionate. The mixture was incubated overnight at 37^0C for 18-24hrs. Tubes that show turbidity were sub-cultured each from each of the containers unto freshly prepared and dried Salmonella-Shigella agar (SSA), MacConkey agar, Brilliant Green agar and incubated at 37 °C for 18-24 hours. The sub culturing was done three times before conclusion that there is no growth [8].

2.4.2 Stool culture

The purudent or mucoid parts of the stool samples were picked using a sterile wire loop and inoculated into selenile F broth medium and then incubated at 37^0C overnight (18-24hrs). The broth cultures were sub-cultured in SSA, and MacConkey agar (MCA). The SSA, and MCA plates were incubated overnight at 37 ºc [8].

2.4.3 Bacteria Identification

The suspected colonies were characterized morphologically using Gram's staining method according to the method described by Merchant and Packer, (1967). For carbohydrate fermentation tests, triple sugar iron agar (TSIA) slant

reaction, hydrogen sulphide production, urease test, Indole reaction tests and citrate utilizing test were carried out for identification of suspected *Salmonella* according to the methods described by Sur *et al.* 2007 [8].

3 Results

Table 1: shows the numbers of samples collected for each of the specimen and the numbers of *S. typhi* isolated from each of the specimen. A total number of 150 sample where collected out of which blood is 118(78.7) and stool 32(21.3), 5 samples from blood shows growth confirmed to be *S. typhi* while only 2 sample from stool shows growth of *S. typhi.*

Table 1 *Salmonella typhi* isolates from blood and stool samples

Specimen	**No. of Sample (%)**	**No. of positive for *S. typhi* (%)**
Blood	118(78.7)	5(71.4)
Stool	32(21.3)	2(28.6)
Total	150(100)	7(4.7)

Table 2, shows the morphological, cultural and biochemical characteristics of *Salmonella typhi.* For the positive isolates, on MCA it shows pale, colorless, transparent, smooth and round colonies while on SSA its shows a characteristic opaque, translucent, smooth and raised with black center colonies. The positive isolates shows no reaction with indole, urease but utilze citrate where color changed from green blue. For carbohydrate using triple sugar iron, only glucose was fermented leaving lactose and sucrose unfermented.

Table 2 Morphological, cultural and biochemical characteristics of *Salmonella typhi* isoted from blood and stool samples

Colonial characteristics						
MAC			**SSA**			
Pale, colorless, transparent,Smooth and round colony			Opaque, translucent, smooth and raised, colorless colonies with black center			
Biochemical Test						
Glu	Lac	Suc	H2S	Indole	Citrate	Urase
+	-	-	+	-	+	-

Key: MaC=MacConkey, SSA=Salmonella-Shigella agar, Dex= Dextrose, Mal= Maltose, Lac= Lactose, Suc= Sucrose, Mann= mannose, += Positive, -=Negative TSI= Tripple Sugar Iron Agar

Table 3, shows the prevalence of *S. typhi* and other bacteria isolated. *S. typhi* 7(4.6). Additionally, *E. coli* 40(26.7), *K. pneumoniae* 17(11.3), *S. aureus* 17(11.3), *S. epidermitis* 12(8), *S. pyogens* 7(4.6), *P. mirabilis* 6(4*), P. aeruginosa* 5(3.3), *S. enterica 1*2(8.0), *K. oxytoca* 4(2.7), *P. vulgaris 4*(2.7) were isolated.

Table 3 Prevalence of *Salmonella typhi* and other bacteria in both blood and stool samples

Bacteria	Blood	Stool	Total (%)
E. coli	40	0	40(26.7)
K. oxytoca	3	1	4(2.7)
K. pneumonia	17	0	17(11.3)
P. vulgaris	4	0	4(2.7)
P. mirabilis	6	0	6(4.0)
P. aeruginosa	5	0	5(3.3)
P. vulgaris	2	0	2(1.3)
S. enterica	2	0	2(1.3)
S. typhi	5	2	7(4.6)
S. aureus	17	0	17(11.3)
S. epidermitis	10	2	12(8.0)
S. pyogens	7	0	7(4.6)
Total (%)	118(78.7)	32(21.3)	150(100)

Table 4, shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) computed for the two specimens. More growth from blood culture were regarded as the True Positives and all other growth with blood culture negative for *S. typhi* as the True Negatives.

Table 4 Sensitivity, Specificity, Positive predictive value and Negative predictive value of

Stool culture				
	Positive	**Negative**	**PPV**	**NPV**
				(95%CI)
Blood culture positive	5	1	83.3%	50.0%
Blood culture negative	2	2		
	Sensitivity	71.4%	Specificity	66.7

Key: PPV= positive Predictive Value, NPV= Negative Predictive Value

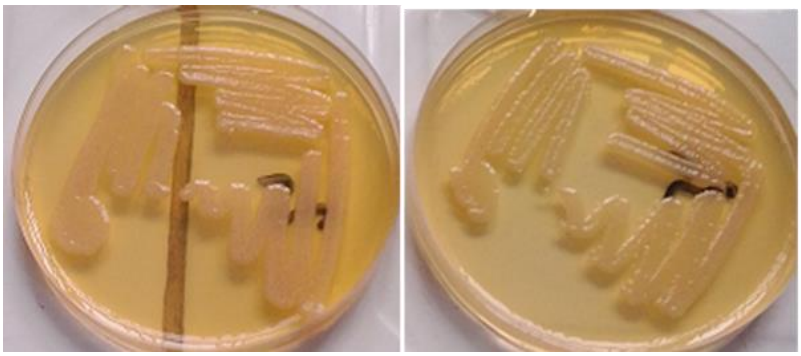

Figure 1 Examination of growth of *Salmonella typhi* isolated from blood and stool specimen on nutrient agar

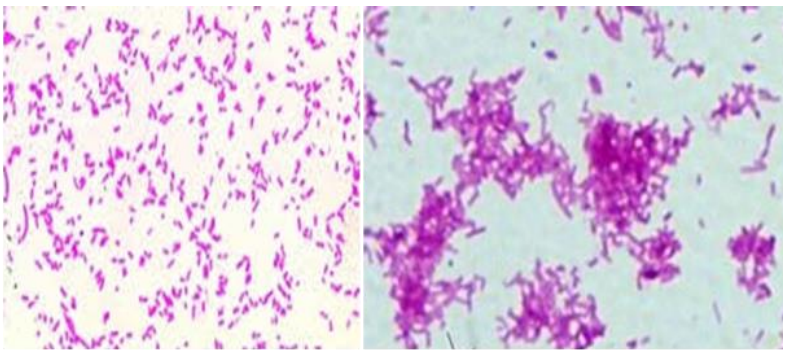

Figure 2 Morphological Examination of *Salmonella typhi* isolated from blood and stool respectively

4 Discussion

Enteric fever is a major health problem in developing Countries,the laboratory diagnosis of the causative agent is arduous though, in clinical settings its diagnosis is mostly dependent on isolation of the *Salmonella typhi* from different specimen which called for culture method. In this study, a total of 150 samples were taken from patients (Adults 18years and above and Children 1-14 years) attending Abubakar Tafawa Balewa University Teaching Hospital Bauchi (ATBUTH) at the period of this study. The study evaluated the result obtained from blood and stool culture method. The 150 samples analyzed showed different growth characteristic and a total of 7 were confirmed to be *Salmonella typhi*. Confirmation of 7 positive cultures indicating overall prevalence of 4.6%.

The result showed 71.4% positive for blood culture while 28.6% showed positive for stool culture. The results agree with the findings of the work of Melita (2011) [9] and Kabir *et al*. (2007) [10] who also reported high positive results of *Salmonella typhi* from blood samples. This study also agrees with the study of Okonko *et al.* (2010) [11] where they reported less number of *salmonella typhi* from stool samples than the blood samples.

Comparing blood and stool culture generally, the blood culture remains the Gold standard method for the detection of salmonella infection because of its high level of specificity; however, it could be useful in resource limited areas where laboratory capacity is limited alongside with authentic clinical investigations World Health Organization (2006) [12].

In table 2, 78.7% of growth of different bacterial were isolated from blood culture while only 31. 3% growth where isolated from stool. Blood culture method becomes standard method for isolation of *Salmonella typhi*. Blood is normally a sterile environment, so detection of bacteria in the blood is an indication of systemic infection. Culture of blood is the most sensitive method for detection of bacteremia (Lin *et al*., 2010).

Table 3 shows that 5 out of the 7 confirmed positive culture is from blood and only 2 were negative for blood while 2 were positive for stool culture and 5 negative for stool culture. From the study it was observed that the *S. typhi* is more sensitive when blood is used as culture specimen than when stool is used and more specific when blood is used than when stool is used.

The stool culture is somewhat specific, but cannot be reliable; but the blood culture is sensitive and should be the gold standard for the isolation of salmonella. The sensitivity tells how likely the blood culture will be positive with 71.4% sensitivity blood and the specificity tells how likely the culture will be negative when the blood culture confirms negative with 66.7% specificity. The positive predictive value shows that a positive blood culture could indicate the presence of *S. typhi*, while the negative predictive value indicating that a negative blood culture can still indicate the presence or absent of *S. typhi.*

5 Conclusion

The study revealed cultures from blood specimens yielded highest number of *Salmonella typhi* isolates when related to culture from stool samples. Therefore, culture from blood sample should be of priority in the isolation of *S. typhi* for easy identification of the bacteria. Culture from blood sample remains the gold standard method for isolation of *S. typhi* when it comes to blood or stool.

Recommendations

- The clinician should be encouraged to request for blood as sample for easy isolation of *S. typhi* for diagnosis of enteric fever.
- The number of patient's specimens for study and more samples apart such as bone marrow should be included in future studies.
- PCR and other modern identification procedures should be explore for proper identification of the isolates from different samples.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicting interests

Statement of ethical approval

The study was approved by the Abubakar Tafawa Balewa University Teaching Hospital Ethical Committee (REC. 0027/2021). and confirmed consent was obtained from the each patient.

Statement of informed consent

Confirmed consent was obtained from each patient.

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