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(RESEARCH ARTICLE)

Phenotypic characterization of *Salmonella typhi* isolated from febrile and diarrhea patients in Bauchi, Nigeria

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Abstract

Salmonella typhi infection occur in most endemic areas. Patients suspected of typhoid fever and diarrhea attending health facilities in Bauchi metropolis were the population studied. 384 stool and blood specimens were collected. The aim of the study was to investigate *Salmonella typhi* using phenotypic analysis to determine whether the organism pose significance attributes among age group or gender and it is specific with fever and diarrhea in Bauchi metropolis, Bauchi, Nigeria. Blood and stool specimens were first enriched in tetrathionate and selenite F broth respectively before sub-cultured on selective medium while identification was conducted with some biochemical analysis. Of the 384 blood and stool specimens screened, 178(46.4 %) yielded significant bacterial growth, while 206(53.6 %) showed no evidence of bacterial growths. *Salmonella typhi* accounted for 6.2 % of the total bacteria isolated while other Enterobacteriaceae accounted for 93.8 %. Distribution of *Salmonella typhi* were insignificant in the selected health facility using Cochran-Mantel's analysis with male and female at (P=0.827), and (P=0.866) in blood and stool specimens respectively. Age groups also shows insignificant attributes to *Salmonella typhi* investigation at (P=0.44). It continues to maintain a mainstream focus of difficulty to isolate the organism via culture despite the selective medium used for the study. Therefore, screening and identification for *Salmonella typhi* at phenotypic level still pose a problem in many Health facility. The research has considerably shown the adverse variability of *Salmonella typhi* from both samples collected among research inclusions.

Keywords: *Salmonella typhi*; Phenotypic; Blood; Stool; Bauchi

1. Introduction

Salmonella typhi infection occurs in most industrialized nations and developing countries at high frequency and is an important public health concern worldwide [1]. In Africa, it has an estimated crude incidence of 362 cases per 100,000 individuals annually [2]. In most endemic areas, approximately 90 % of enteric fever and gastroenteritis are typhoidal/non typhoidal and caused about 216,500 deaths among children and young adults worldwide [3-4]. The agent of typhoid fever causes serious health problem in developing countries due to their unsuitable sewage treatments, poor standards of hygiene and unavailability of potable drinking water [5]. It is mostly encountered in tropical and sub-tropical countries including Nigeria where it constitutes significant sources of morbidities and mortalities [6].

The incidence of typhoid salmonellosis (which is caused by *Salmonella enterica typhi*) is increasing worldwide, causing millions of infections and many deaths in the human population each year.

Salmonella enterica serovar Typhi are gram-negative rods, non-spore forming, motile and microscopic living creatures. They are oxidase-negative, catalase-positive, non-lactose fermenters, producing acid from D-glucose usually at times

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accompanied with the production of carbon dioxide and some utilize citrate as a sole carbon source [7]. Phenotypic characterization via culture still remains the goal standard for identification of *Salmonella typhi*. The research will provide a baseline for phenotypic characterization of *Salmonella typhi* from blood and stool specimen of febrile and diarrhea patients attending hospital facility.

2. Materials and methods

2.1. Collection of samples

Blood were drawn aseptically using 5 ml syringe and needle [8]. About 2 ml of blood were collected from patients. The blood was aseptically transferred to 8ml tetrathionate broth tube and incubated. Tubes that shows no turbidity were kept for 48, 72 hours respectively. Each tube was carefully labeled with the patient's number already indicated in the research consent form [8].

For collection of stool specimen, the subjects were provided with clean wide-mouthed containers and about 2 g of the stool were transferred unto tubes containing 8ml of Selenite F Broth and was incubated at 37 ^{o}C for 24 hours [8-9].

2.2. Microbiological analysis

2.2.1. Processing of blood specimens

The collected blood and tetrathionate broth were incubated overnight at 37 ºc for 18 to 24 hours. Tubes that shows turbidity were sub-cultured each from each of the containers unto freshly prepared and dried Salmonella-Shigella agar (SSA) and MacConkey agar (Biotec) and incubated at 37 °C.

2.2.2. Processing of stool specimens

The stool samples were first inoculated into the enrichment medium (selenite–F broth) and after incubation for 24 hours at 37 ºC each was sub-cultured unto Salmonella-Shigella agar and MacConkey agar (MCA). The SSA and MCA plates were incubated overnight at 37 ºC and examined for growth [9].

2.3. Characterization of isolates

2.3.1. Sugar fermentation tests

Nutrient broth cultures were prepared. Bijou bottles containing the basal medium and appropriate prepared carbohydrate (mannitol, maltose, dulcitol, sucrose and glucose) were inoculated with drop of the nutrient broth suspension of the test isolate and were loosely capped and incubated at 35 ºC overnight. Each was observed for change in colour from amber to red and for gas production (in the medium filled inverted Durham tube) [10-12].

2.3.2. Urease test

The test organisms were inoculated heavily on the entire slope surface of the urea agar slants prepared in caped tubes. The tubes were placed in racks and incubated at 37 ºC up to 48 hours. Tubes were thereafter examined for change of colour from plain to pink [9].

2.3.3. Indole test

Inoculate a tube containing 5 ml of the tryptone/tryptophan medium with the suspected colony. Incubate at 37 °C for 24 h. After incubation and 1 ml of the Kovacs reagent was added. The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction [9].

2.3.4. Hydrogen sulphide production

Test organisms were inoculated into the triple sugar iron agar slants contained in test tubes. These were incubated at 35- 37 °C for up to 48 hours. After incubation, the TSI agar media were checked for blackening and change in colour from amber to red at the bottom of the tube [9].

2.3.5. Serological identification (Serotyping)

Suspected colonies were picked and sub-cultured unto moist nutrient agar slopes in MacCartney bottles. These were incubated for minimum of 4 hours. One to two loopfuls of the agar cultures were mixed with normal saline on clean

microscope slides to form a paste. A drop each of the O and H polyvalent sera were added and further mixed with the organisms on the slide. Positive results were indicated by visible agglutination within 30 seconds. Slide tests were repeated for the positive cultures using single factor sera [13-15].

2.3.6. Microbact kit examination

Serological confirmed isolates were subjected to Salmonella microbact kit and result were obtain in percentages [9].

3. Results and discussion

A total of 384 patients were recruited to the study at three health facilities from June 2016 to November 2016. One hundred and fifty-seven patients (40.9 %) and 227 equivalents to (59.1 %) were males and females enrolled in the three health facilities accounting to three simultaneous health factors of fever (231), diarrhea (117) and fever/diarrhea (36) equivalent to 60.2 %, 30.5 % and 9.3 % respectively. The patient's age ranged from 1 year to 70 years with a mean of 38.7 and standard deviation of 19. Out of 384 participants, 122 were obtained from ATBUTH, 207 from Primary health care facility of Tashan Babiye and 55 from NIIMA Consultants respectively (Table 1). The mean age of the females was significantly different from the mean age of the males. There was no significant difference in the age of the participants across gender ($X2$= 0.57 df =383).

Table 1 Distribution of subjects based on age and sex in the three selected hospitals in Bauchi

Age group	Health facilities																		
	ATBUTH (n=122)						BABIYE (n=207)						NIIMA (n=55)						
	Male			Female			Male			Female			Male			Female			Total
	F	D	F/D	F	D	F/D	F	D	F/D	F	D	F/D	F	D	F/D	F	D	F/D	
1-10	3	8	1	11	15	2	16	18	1	37	26	9	14	2	1	6	4	1	175
11-20	7	6	2	9	4	1	22	5	2	15	3	3	7	0	0	3	1	3	93
21-30	6	2	0	13	3	0	4	1	2	3	1	2	3	0	0	1	0	1	42
31-40	1	0	0	2	3	0	1	0	0	4	4	0	3	0	0	0	0	0	18
41-50	3	1	0	1	1	0	0	4	0	1	0	0	0	0	0	1	0	0	12
51-60	2	0	0	3	0	1	0	1	0	11	1	0	1	0	0	0	0	0	20
61-70	3	0	2	3	3	0	1	0	0	7	0	2	1	0	0	2	0	0	24
Total	25	17	5	42	29	4	44	29	5	78	35	16	29	2	1	13	5	5	384

Key; n= number of specimen, F – Febrile, D – Diarrhoeic, F/D – Febrile and Diarrhoeic, ATBUTH- Abubakar Tafawa Balewa University Teaching Hospital, BABIYE- District clinic Tashan Babiye, NIIMA- Niima consultant Hospital

Salmonella typhi continues to be a burden in most developing countries. Poor diagnosis has contributed to the deteriorating conditions as typhoid fever have common signs and symptoms similar to those of other common febrile illnesses. The blood culture and stool culture which are methods commonly used for the diagnosis of typhoid fever were done simultaneously to increase the probability and accuracy of detecting typhoid fever cases (Table 1). It is necessary to note that the use of the Widal test has been banned in many countries but it is still widely used in most health centers [16], [17] due to it unreliability [18].

Table 2 Identification of *Salmonella typhi* based on cultural, morphological and biochemical characterization

No. of Sample	**Media used**	**Change in broth**	**Cultural examination**	**Total positive samples in hospitals for *S. typhi*/other org.**				**Positive/negative result**		
			Positive *S. typhi*	**Positive for others**	**ATBUTH**	**BABIYE**	**NIIMA**			
384	MCA,DCA,SSA, NA, TTB, SFB	Turbidity	11 (384)	167 (384)	03/40	07/123	01/4	178/206 (384)		
Cultural and Morphological characteristics										
Sources of isolates	**Colonial Characteristics**			**Serology**						
	MCA		**SSA**	**Poly O**	**Poly H**					
	Pale, colourless, smooth,transparent and raised colony		Opaque, translucent, colourless, smooth and round colonies	+	+					
Biochemical Test										
Isolates	**Carbohydrate fermentation test**					**Indole**	**Citrate**	**Urease**	**TSI**	**Micobact kit**
	Dex	Mal	Lac	Suc	Mann					
Escherchia coli	+	+	+	D	+	+	-	-	+	
Klebsiella spp.	+	+	+	-	+	-	+	+	+	
Shigella spp.	+	+	-	-	+	D	-	-	+	
Salmonella spp	+	+	-	-	+	-	D	-	R/Y	76 %
Salmonella typhi	+	+	-	-	+	-	+	-	R/Y	82 %
Staphylococcus aureus	Catalase+	Coagulase+								

KEY: MCA=MacConkey, DCA=Deoxychocolate citrate, SSA=Salmonella –shigella agar, NA=Nutrient agar, TTB= Tetrathionate broth, SFB= Selenite F broth, DEX=Dextrose, Mal= Maltose, Lac= Lactose, Suc=Sucrose, Mann= Mannitol, +=positive, -=negative, org=organisms

The findings indicate that febrile, diarrheic and febrile/diarrheic patients suspected of typhoid fever cases reported were diagnosed using the Widal test. However, the same patient's blood or stool culture did not confirm *S. typhi* isolate. In these particular patients, whose widal test was reactive, other bacterial organisms were isolated thus emphasizing the unspecific nature of the Widal test. A single widal test, using the slide test technique, is commonly used in the three health facilities where the study was carried out, for the diagnosis of typhoid bacteria (Table 2). Though widely used in such resource limited settings, it is not reliable and may produce false-positive results thus leading to over-diagnosis of typhoid fever. Its performance is affected by cross-reactions with other bacterial pathogens, previous immunization with *Salmonella* antigen, non-Salmonella infections such as malaria, Shigellosis, entero-toxigenic *E. coli* and other infectious organism that lead to an increase in the O antibodies [19]. Both gram negative and gram-positive bacteria were obtained from the clinical samples. All the isolates obtained from stool samples were all gram-negative bacteria while those from blood samples were a mixture of both gram positive and negative bacteria. Some blood cultures and stool cultures confirmed positive results for *S. typhi* from the study participants who showed signs and symptoms of typhoid fever infection. The specific bacterial pathogens isolated from the blood cultures include *S. aureus*, which correspond to the research of [20] among different sample group (Table 2).

Table 3 Distribution of *Salmonella typhi* and other Bacterial organism in stool and blood specimen of subject based on gender

Gender	Patient	Stool (n = 135)		Blood (n = 249)		Positive/ Negative
		S. typhi	Other Bacteria	*S. typhi*	Others Bacteria	
Male	Febrile (98)	-	30	1	13	
	Diarrhoeic (48)	2	23	-	5	
	Febrile/diarrhoeic (11)	1	4	-	-	
Total	157	3	57	1	18	79/78
P (0.827) [a]						
Female	Febrile(133)	1	43	2	14	
	Diarrhoeic (69)	2	27	-	6	
	Febrile/diarrhoeic(25)	1	1	1	1	
Total	227	4	71	3	21	99/128
P (0.866) [a]						

Key: S. typhi= *Salmonella typhi*, Other Bacterial organism= *Escherichia coli*, Klebsiella species, Shigella species, Salmonella species, *Staphylococcus aureus*; a= insignificance.

4. Conclusion

Age and sex variation among the patients attending the selected health facilities shows high level of fever and diarraoea among the females of paediatric ages and the lowest was observed among the productive age range of 41-50 respectively. Despite the aforementioned case, it poses no significance to *Salmonella typhi* infection among the subjects. *Escherichia coli* was found to be the highest isolated organism from the stool specimen as it constitutes the zone of adaptation of many enteric organisms. The blood specimen continues to maintain a sterility status with mostly *S. aureus* as a predominant organism isolated from the suspected blood specimen respectively. The study had shown not all febrile case is related to typhoid inclusions in medical prognosis.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There's no what so ever any conflict of interest we are giving the outfit full right to publication of this work.

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