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Epidermology of *Salmonella Enterica (Typhi)*, in the Bori Community of Khana Lga, Rivers State, Nigeria

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ABSTRACT

Early morning urine, stool and blood samples were collected from 1000 randomized persons at four different sites within the period of six months and screened for presence of *Salmonella entericatyphi*. Attention was given to clinical patients as well as healthy persons, particularly student community. Bori General Hospital and Sita-Esther Diagnostic Laboratories, Bori, were considered to access the clinical patients. Birabi Memorial Grammar School and Ken SaroWiwa Polytechnic, Bori, were considered for the healthy persons. Analysis were conducted on seven hundred samples from clinical patients, five hundred from Bori General Hospital and two hundred from Sita-Esther Diagnostic Laboratories, Bori, whereas three hundred samples from healthy people were also screened, one hundred from Birabi Memorial Grammar School and two hundred from Ken SaroWiwa Polytechnic, Bori. Comparative analysis of results indicated 60% Positive for blood, 30% Positive for Urine and 40% Positive for Stool. Evaluation of results show that 60% of the total population tested positive for *Salmenella enterica typhi* by serologicalfebrile antigen, but only 40% had evident visible growth of bacterial colonies, identified as *salmonella typhi*, in stool. The differences in results from blood, stool and urine from the various samples are likely due to patients being on a recent or current antibiotic treatment in general, or not being able to properly collect the Early morning Urine as directed, in case of Urine samples. High level of antibiotic potential(300mm), were seen in Augmentin, Ciprofloxacin and Chloramphenicol.

Introduction

Typhoid is a life threatening and highly infectious multi-systemic disease caused by the *Salmonellae enterica*serovar*typhi*, a bacterial pathogen. Typhoid, a disease condition often referred to as typhoid fever or enteric fever is often characterized with a high internal fever following the ingestion of the bacterial pathogen, *Salmonella enterica*serovar*typhi*in contaminated food or water (Hussein,et. al, 2001). Typhoid is an acute infectious illness, and infected persons can further distribute the illness by way of poor hygiene to other susceptible persons most commonly, by stool contamination of surrounding water and food materials (Ashraf, et. al, 2013., Meseret, et.al,2014). Typhoid infection is generally followed with enteric fever which could be generalized in acute conditions. Disease condition is observable in many cases with malaise, severe headache, with general weakness. These are followed by serious bowel disorders, constipation, diarrhoea, loss of appetite, nausea and at times joint pains (Broek, et.al, 2013; David, 2003).

Aetiological agent for Typhoid disease is the bacterium *Salmonella enteritica*, serotype *typhi* of the family *Enterobacteriaciae*. Serovar*typhi*, also known as Serotype *typhi*, and simply addressed as *Salmonella typhi*, a member of the *Enterobacteriaciae* family. *Salmonella enterica* serotype *typhi is* a Gram negative bacillus. It is a motile, non-lactose fermenting and facultative anaerobe. In the Laboratory, identification can be attained by growth on MacConkey and Eosin Methylene blue agar (EMB) (Emmeluth et al.,2009). *Salmonella enterica* serotype *typhi*is generally known as enteric fever, because the bacterium can be commonly found in the host as intestinal resident pathogen, where it affects the enteric domain of the gastro intestinal tract of its human host. Although, it has been isolated from spleen, liver and blood tissues of its human host (David, 2003; Emmeluth et al.,2009). *Salmonella typhi* was originally isolated in the year 1880 by Karl Joseph Eberth, a doctor and student of Rudolf Virchow, who discovered the bacillus in the abdominal lymph nodes and spleen in 1879, but published his discovery in 1880. This discovery was thereafter verified by Robert Koch and other team of German and English bacteriologists of the time (David, 2003; Emmeluth et al., 2009; Brands et al.,2006). *Salmonella typhi* inhibits lymphatic tissues of the small intestine, liver, spleen and even the human host blood tissues. Untreated typhoid fever cases have been reported to associate with global population mortality of 12-30% annually, whereas treated cases have been reported with survival rate of 99% (David,2003; Stephen et al.,2008).

Host-pathogen interaction is unique with typhoid fever infection as it demonstrates host specificity to human alone (Stefenia et al.,2011; Stefenia et al.,2012). Apart from being human host specific, *Salmonellartyphi* has also shown other unique attributes of life-long persistent infection in convalescent carriers (Stefenia et al.,2011). *Salmonella typhi* particularly exhibits with human host specificity. By this characteristic, *Salmonella typhi* is a strict human pathogen and responsible for the severe human systemic infection known commonly as typhoid fever, reported globally (Christopher, et. al, 2002; Stefania and Jorge, 2012).

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Typhoid fever in children and young people has been reported more common than in the adults (Hohmann,2013). Typhoid fever is a worldwide disease, although it is not so common in industrialized countries (Suman et al.,2008), but has been found most prevalent among people with high compromised lifestyle in sanitation and hygiene due to poverty and overcrowding, resulting to an annual global infection rate of about 17 million people, and an estimate death toll of more than 200,000 persons (Suman et al.,2008; Stefenia et al.,2011; Stefania et al.,2012).

Incidence estimate reported in non-epidemic circumstances suggest that more than one thousand persons fall victim to typhoid fever infection annually in the regions of South-central Asia, South-east Asia and South Africa, and in the United States of America an approximate of 200 to 300 cases are reported annually, 80% of which emanate from travellers coming in from countries reported for high endemic typhoid fever (Lynch et al.,2010).

Bori Community, in Khana Local Government Area of Rivers State, Nigeria, is an urban city, constituted of people from all over Nigeria and beyond. The community is located at a strategic economic centre in the Niger Delta of Nigeria, connecting to Opobo, Andoni and Gokana, Rivers State, and situated very proximal to Abia and Akwa-Ibom States of Nigeria. It is highly multi ethnical following its economic constitution of Bori mega market and location of Ken SaroWiwa Polytechnic, a tertiary education institution. With this status, Bori community has influx of visitors and strangers from all over Nigeria and beyond coming in as travellers, traders and students, apart from the indigenous people of Ogoni. However, no outstanding health facility to monitor the health status of people coming into the city either as travellers or resident is functional for now, and as a result, surveillance of health status of the people living within the Bori community became necessary.

This project is carried out as a way to verify the presence of *Salmonella typhi* infection within the area. It is also aimed at evaluating the rate of occurrences of Salmonella infection in the society as to verify if the community is prone to typhoid fever, and if there is the likely threat of Salmonella typhi epidemic within the Bori community.

Methodology

Clinical diagnosis of *Salmonella typhi* is laboratory based; either by WIDAL agglutination reaction, a presumptive serological test, or by isolation of bacterial cells in pure cultures, made from sample cultures (AACC,2013). Samples of blood, urine and stool were collected from 1000 randomized persons and analysed within the period of six months in the Bori community. The antigen-antibody, blood serological (WIDAL agglutination) technique and the culture technique for Urine and stool were employed, for evidence and isolation of *Salmonella typhi* (Nguyen et al.,2004; AACC,2013).

Early morning urine, Stool and blood samples were collected from 1000 randomized persons at four different locations within the period of six months and screened for presence of *Salmonella entericatyphi*. Attention was given to clinical patients as well as healthy persons, particularly student community. Bori General Hospital and Sita-Esther Diagnostic Laboratories, Bori, were considered to access the clinical patients. Birabi Memorial Grammar School and Ken SaroWiwa Polytechnic, Bori, were considered for the healthy persons. Analysis were conducted on seven hundred samples from clinical patients, five hundred from Bori General Hospital and two hundred from Sita-Esther Diagnostic Laboratories, Bori, whereas three hundred samples from healthy people were also screened, one hundred from Birabi Memorial Grammar School and two hundred from Ken SaroWiwa Polytechnic, Bori. Prerequisite was given to clinical patients in number of samples against the healthy communities. Reason was for diagnostic purposes to the clinical patients as well.

Blood analysis

Venous blood were collected from the patients and separated by centrifugation to obtain serum. Sera from test persons were labelled accordingly in separate test tubes and kept in rack for analysis. WIDAL agglutination technique was adopted for blood tests. Using sterile Pasteur dropping pipettes, one to two drops of each sera were separately made in two rolls, at four spots on a clean micro-titre tile (8 spots altogether). Equal volume of the Salmonella febrile antigens of (Typhi: [O/H] and Paratyphi: [AO/AH][BO/BH] and Paratyphi:[CO/CH]) were added, mixed well and rocked gently. Reading was taken in about 10-20 minutes.

Urine analysis

Urine culture plates were prepared from the non-centrifuged and undiluted urine samples, using standard wire loop of 0.05ml volume, and inoculating a loop full of sample unto fresh plates of Cystine-lactose-electrolyte deficient (CLED) agar and MacConkey agar (Baker and Silverton, 1985; Brooks et al.,2004). The inocula of all samples were spread-plated and finally were incubated at 370C for 24hours. Pure bacterial isolates were obtained and identified based on cultural, morphological and biochemical characteristics (Boyd and Hoerl, 1977; Sneath et al.,1986; Cheesbrough,2006).

Stool analysis

Stool samples were collected in sterile universal bottles by patients. Instructions on how to aseptically collect the samples were given before sampling. Stool samples were plated by streaking upon surfaces of fresh plates of MacConkey and Salmonella-Shigella agar, labelled and incubated at 370C for 24hours. Pure bacterial isolates were obtained and identified by biochemical tests.

Antibiotic susceptibility tests

Antibiotic susceptibility tests were carried out by the disc diffusion technique (Bauer et.al, 1996). Overnight 18hours test cultures were spread over Mueller-Hinton agar plates and incubated at 370C.

Results and Discussion

Results for evidence of infection with *Salmonella typhi* in blood sera of tested blood samples and frequency of occurrence of the bacterial isolates of *Salmonella typhi* recovered in Urine and Stool samples are as illustrated in tables 1, 3 and 4. Biochemical tests table 2, and table 5 for Antibiotic susceptibility tests. Table1 shows evidence of a 60% positive reaction with evidence of agglutination in 60 samples, and a 40% non-agglutination, or negative reaction. Urine (Table3), revealed significant growth of *Salmonella typhi* in 30% of total samples. Whereas, stool results in table 4, indicated the significant growth of 40% of the total samples with bacterial colonies confirmed as *Salmonella typhi*. It is however evident that some of the certain of the test persons, especially the clinical Patients mentioned that they were undergoing antibiotic therapies at various points. Table 5 indicated that antibiotic potential of Ciprofloxacin, Augmentin and Chloramphenicol were very high over other drugs.

Table 1. WIDAL AGGLUTINATION REACTIONT: Serological test for evidence of Salmonella typhi and paratyphi Antibodies, using Salmonella FebrileAntigens forty phiandpara typhi.

Sample site	Positive	Negative	% Positive	% Negative
Sita-Esther Lab.	125	75	12.5	7.5
General Hospital.	355	145	35.5	14.5
Ken-Poly.,Bori.	85	115	8.5	11.5
BMGS, Bori.	35	65	3.5	6.5
Total	**600**	**400**	**60**	**40**

KEY: No Agglutination or reaction= Negative
Agglutination or reaction = Positive
Grand total Screened = 1000 persons
Sita-Esther Diagnostic Laboratories =200 persons
Bori General Hospital = 500 persons
Ken SaroWiwa Polytechnic = 200 persons
Birabi Memorial Grammar School = 100 persons

Table 2. Biochemical, Motility and Gram's Reaction Tests for identification of (Salmonella typhi) Bacterial isolates at the various sites.

Code	Gram's	Morph	Motil	Indole	Met.red	V-Pros	Oxidas	Catalas	Isolate
Sita-Esth-Bacilli	+	-	+	-	-	+	-	+	*Salmonella typhi*
Hospital-Bacilli	+	-	+	-	-	+	-	+	*Salmonella typhi*
KEN Poly -Bacilli	+	-	+		-	-	+		*Salmonella typhi*
BMGS-Bacilli	+	-	+	-	-	+	-	+	*Salmonella typhi*

KEY: -=Negative or No reaction
+ = Positive or Reaction
Bacilli = Rod shaped
Morph =Morphology
Motil =Motility
Catalas =Catalase
Met.red =Methyl Red
V-Pros = Voges-Proskauer
Oxidas = Oxidase

Table 3. Frequency of Bacterial isolates in Urine samples.

Sample site	Positive	Negative	% Positive	% Negative
Sita-Esther Lab.	66	134	12.5	7.5
General Hospital.	198	302	35.5	14.5
Ken-Poly., Bori.	20	180	8.5	11.5
BMGS, Bori.	16	84	3.5	6.5
Total	**300**	**700**	**30**	**70**

Grand total Screened = 1000 persons
Sita-Esther Diagnostic Laboratories =200 persons
Bori General Hospital = 500 persons
Ken SaroWiwa Polytechnic = 200 persons
Birabi Memorial Grammar School = 100 persons

Table 4. Frequency of Bacterial isolates in Stool samples.

Sample site	Positive	Negative	% Positive	% Negative
Sita-Esther Lab.	85	115	8.5	11.5
General Hospital.	260	240	26	24
Ken-Poly.,Bori.	30	170	3	17
BMGS, Bori.	25	75	2.5	7.5
Total	**400**	**600**	**40**	**60**

Table 5:Antibiotic zone of inhibition were measured in Millimeters(mm)

Antibiotic susceptibility test	CPR	AGU	ERY	CHL	SPR	PEF	TRV
Sita-Esther Diagnostic Laboratories	30	30	20	30	10	20	10
Bori General Hospital	30	30	10	30	10	20	10
Ken SaroWiwa Polytechnic	30	30	20	30	10	20	10
Birabi Memorial Grammar School	30	30	20	30	10	20	10

From the results, it is evident that *Salmonella typhi* infection is prevalent in the Bori Community. Results from Urine samples showed evidence of 30%growth of bacterial isolates confirmed as Salmonella typhi, Stool 40%and result from serological test to survey presence of Salmonella typhi and paratyphi antibodies revealed a scaring 60% positive samples out of one thousand random samples.

The antimicrobial susceptibility tests were performed by the disc diffusion method (Bauer et al., 1996). Overnight cultures of test organisms were incubated at 37 and spread over the surface of Mueller-Hinton agar plates using a sterile cotton swab (Selvamohan et al., 2012) and allowed to dry for 2 to 5 minutes. Antibiotic discs such as Ciprofloxacin, Augumentin, Sparfloxacin, Peflacin, Tarivid, Rifampicilin and Erythromycin were aseptically placed onto the agar using sterile forceps and incubated at 37 for 24hours (CLSI, 2006). Interpretation of results was based on the zones of inhibition, susceptible or resistant (Smith, 2004; Cheesbrough, 2006).

Treatment of proven disease conditions has posed untold difficulties during many clinical attempts, especially in patients with history of chronic disease conditions. Combined treatment of Chloramphenicol and Aureomycin or Ampicillin has been found very useful at many instances (Suman et al.,2008). However, most broad spectrum antibiotic drugs applicable for use against both Gram-negative and Gram-positive bacteria, such as Ciprofloxacin, Augmentin, Ofloxacin, Peflacin and Ceporex have been found very useful at various long-term treatments (emedicine.medscape.com).

In most of the industrialized and developed nations where adequate clinical diagnostic facilities exist, patients are screened for laboratory based evidence of typhoid fever before treatments are given. Three negative stool cultures and one negative Antigen blood test have been recommended for a proof of true negative result before or after treatments (Suman et al.,2008).

Following this research, it is my request that the attention of Government agencies, concerned citizens and particularly Public Health Care, be drawn to especially the public and commercial water supply facilities and ready-to-eat food supplies within the Bori Community, as epidemics of Typhoid fever could very likely become the fate of the generality of the resident population.

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