Extended Beta-Lactamase (ESBL) Producing *Salmonella typhi* from Presumptive Typhoid Patients in Nasarawa State, Nigeria

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ARTICLE INFO

Article No.: 050918069

Type: Research

DOI: 10.15580/GJEPH.2018.3.050918069

Submitted: 09/05/2018

Accepted: 03/06/2018

Published: 15/06/2018

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Keywords:

Prevalence, β -lactamase, *Salmonella*,
antibiogram, resistance

ABSTRACT

This study was designed to determine the prevalence and antibiogram profile of extended spectrum β -lactamase producing *Salmonella typhi* from stool samples of symptomatic and asymptomatic typhoid patients from three selected hospitals in Nasarawa State, Nigeria. Four hundred and fifty stool samples were collected from hospitals in Doma, Lafia and Obi and isolation done using standard culture and serological techniques. Double disc diffusion technique was employed to determine isolates' capacity to produce β -lactamase and antibiogram profile carried out. Results showed that 76(28.00%) of the samples collected were positive for *Salmonella species*. Prevalence of *S. typhi* in symptomatic patients was 13.77%, while asymptomatic patients had 5.33%, and a significant difference with a p value of 0.129 obtained between symptomatic and asymptomatic patients. Prevalence of *S. typhi* in symptomatic patients with regard to sex showed that male patients were more susceptible to the isolates in Doma and Obi with values of 8(11.43%) and 11(15.28%) respectively, while female had 7(5.26%) and 7(5.60%) in Doma and Obi respectively. Patients aged between 18-29 years recorded 27(6.00%) prevalence rate as the highest, while age group 66-77 recorded 5(1.11%) prevalence throughout the duration of study. Isolates with the capacity of producing β -lactamase enzymes were 5(1.11%) in symptomatic patients and 1(0.22%) in asymptomatic patients. Antibiogram profile showed that ESBL-producers were sensitive to Augmentin and imipenem at 100%, while non ESBL-producers were sensitive to Augmentin at 100%, ceftriaxone at 85.86% and 100% resistant to imipenem and ampicillin. The study concluded that beta-lactamases with the capacity of conferring multidrug resistance were found in *Salmonella typhi* species isolated from willing patients in hospitals in Nasarawa State, Nigeria.

INTRODUCTION

Salmonella species are obligate pathogens found all over the world causing mild to lethal infections, and contributing to economic cost losses in the form of therapy and treatment (Mark *et al.*, 2017; Alocilja *et al.*, 2003). Species *Salmonella enterica typhi* causing typhoid fever and others are described as being responsible for some food borne illnesses (Cardinale *et al.*, 2009). High incidences of typhoid fever have been reported in enteric fever endemic areas with *Salmonella enterica serovar typhi* implicated as the main causal pathogen (Okonko *et al.*, 2010; Parry, 2006).

Typhoid fever is treated using antibiotics, though resistance to different antibiotics have been variously reported in community and hospital environments. Resistant and continued spread of antibiotic resistant strains of bacteria and particularly Extended Spectrum Beta-lactamases (ESBLs) producing bacteria is now a global threat (Ranjbar, 2010; Naser 2009; Literacka *et al.*, 2009). ESBLs are enzymes that attack and deactivate beta-lactam antibiotics (penicillins, cephalosporins, aztreonam and carbapenems). Increasing prevalence of resistant *Salmonella typhi* has been attributed to increasing use of antibiotics with the resultant prolonged therapy (Marathe *et al.*, 2012), and resistance in ESBLs is due to the production of beta-lactamases which are plasmid mediated with the capacity of lateral transfer from bacteria to bacteria among the Enterobacteriaceae (Bradford, 2001).

Typhoid fever disease is of public health importance affecting people of all walks of life in Nigeria and cases of outbreaks have been reported in some states including Nasarawa state. Disease burden due to typhoid fever is on the increase in some part of the world, while reductions have been recorded in some places (Marks *et al.*, 2017; Crump *et al.*, 2004). In Nigeria, due to lack of reliable epidemiological data it is difficult to ascertain the burden of illness though reports from studies in Kano, Niger, Lagos, Owerri, Abeokuta, and Jos in Nigeria had prevalence rates of 13%, 67.8%, 25.9%, 42%, 80.1%, and 42% respectively which were generally high (Adogo *et al.*, 2015; Akinyemi *et al.*, 2015; Ukaegbu, 2014; Opara *et al.*, 2014; Abdullahi *et al.*, 2010; Okonko *et al.*, 2010). The aim of this study was to evaluate the prevalence of *Salmonella* producing ESBLs strains by phenotypic methods and their profile of drug resistance amongst patients.

MATERIALS AND METHODS

a Study area

The study was carried out in three Hospitals in Obi, Doma, and Lafia, Nasarawa State. The major occupation of the people is farming, while others are civil servants and petty traders. While the hospitals in Obi and Doma are government general hospitals serving two local government council areas, Dalhatu Araf Specialist Hospital (DASH) in Lafia the state capital is a referral centre, and the most equipped government hospital in the state with different units and departments.

b Sample collection

Sterile stool samples were collected as described by Cheesbough, (2010). Samples were collected twice weekly from September to November 2017. Inclusion and exclusion criteria was carried out by including all typhoid patients (symptomatic and asymptomatic) attending the hospitals and who willingly gave consent, while excluding those who declined to give consent.

c Isolation and identification of *Salmonella*

Isolation of *Salmonella* species was carried out using selenite F enrichment broth and incubated at 35 °C for 10 h before re-inoculation onto *Salmonella-Shigella* agars (SSA) at 35 °C for a day. Colonies (pink) with black centres (except *S. paratyphi* A, whose colonies do not have black centres) were sub-cultured to obtain pure isolates. Cultural characteristics and biochemical tests such as Gram staining, indole test, citrate utilization, catalase and urease tests, and MR-VP test (Methyl Red/Voges-Proskauer reaction) were carried out as described by Cheesbough (2011) for the identification of the isolates.

d Serological identification of *Salmonella typhi*

Serological identification of the isolates were as described by the methods of Guibourdenche *et al.* (2010). Serotyping was based on the agglutination of bacteria with specific sera to identify variants of the somatic (O) and flagella (H) antigens.

e Determination of multiple antibiotics resistance (MAR) index

The MAR Index was determined using the modified methods of Krumperman (1983) and Paul *et al.* (1997). MAR is calculated from the result of the antibiotic susceptibility test thus;

$$\text{MAR Index} = \frac{\text{No. of antibiotics to which isolate is resistant}}{\text{Total no. of antibiotics tested}}$$

f Detection of β -lactamase producing species of *Salmonella typhi*

Iodometric method of Catlin (1975) was used in the detection of β -lactamase producing species of *Salmonella typhi*. One hundred microliter of penicillin solution was dispensed into a well of microtitre plate, colonies of the suspected isolates were then emulsified into the solution. Two drops of starch was added, and incubated between 30-60 min after which 1 drop of iodine was added. Formation of blue solution that disappeared within 10 min showed the isolate was β lactamase positive.

g Phenotypic confirmatory test

The modified methods of NCCLS (2002) was employed in the phenotypic confirmatory combination disc diffusion test. A disc of ceftazidime (30 μ g) alone and ceftazidime + clavulanic acid (30 μ g/10 μ g) were placed at a distance of 25 mm, center to center, on a MHA plate inoculated with a bacterial

suspension of 0.5 McFarland turbidity standards and incubated overnight at 37 °C. An increase in the inhibition zone diameter of 5 mm for a combination disc versus ceftazidime disc alone confirmed ESBL production.

h Statistical analysis

Statistical computation of data obtained were performed using Microsoft excel™ 2010 and graph prism tab version 7.4 for analysis of Chi-square test and analysis of variance (ANOVA) to compare the results.

RESULTS

a Prevalence of *Salmonella typhi* in stool samples

Out of the 450 stools sample collected and analysed (250 from symptomatic and 200 from asymptomatic patients) as shown in Table 1, 76 samples were positive for *Salmonella typhi* at the prevalence rate of 16.89 % (24.4% for symptomatic and 7.50% for asymptomatic patients). There were no statistical difference at $p > 0.05$ between the two groups.

Table 1: Prevalence of *Salmonella typhi* in stool samples of patients

Sampling Period	Symptomatic patients N = 250 n = 61	Asymptomatic patients N = 200 n = 15
Week 1	8(13.11)	2(13.13)
Week 2	4(6.19)	1(6.67)
Week 3	9(14.75)	4(26.67)
Week 4	7(11.48)	0(0.00)
Week 5	6(8.84)	3(20.00)
Week 6	10(16.39)	2(13.13)
Week 7	9(14.75)	1(6.67)
Week 8	8(13.11)	2(13.13)
Total	61(24.4%)	15(7.50%)

b Prevalence of *S. typhi* in relation to different risk factors

Prevalence according to gender showed that of the patients that were positive for the presence of *S. typhi*, 41 were males and 35 females as shown in Table 2. Male patients with *S. typhi* were 53.95%, DASH - 16(21.05%), DOMA - 14(18.42%) and OBI - 11(14.47%), while the total prevalence in the female patient was 35(46.05%); DASH - 21(27.63%), DOMA - 7 (9.21%), and OBI - 7(9.21%). According to the age groupings, the highest prevalence was within the age group 18 - 29 years, with 12, 6, and 4 symptomatic patients in DASH, OBI and DOMA respectively. The prevalence was low in the age group 54 - 77 and 66 - 77 years. There

were no significant difference when compared with the prevalence of *Salmonella typhi* according to age group at $p > 0.039$. A comparison of the prevalence rate based on the patients' occupation (Table 2) showed that students were the highest symptomatic patients with a prevalence of 20(32.78%), while farmers recorded the highest number of cases 6(40%) in asymptomatic patients. Civil servant had the lowest prevalence for both symptomatic and asymptomatic patients with 6(9.83%) and 1(6.66%) respectively. The prevalence values obtained according to the different occupations were significantly different at $p = 0.129$. The study recorded 6 cases of ESBL-producers 5(8.19%) isolates were from symptomatic patients and 1(7.17%) from asymptomatic patient. Symptomatic patients with non ESBL producing *Salmonella*

typhi obtained prevalence of 56(91.8%), while 14(93.33%) prevalence was recorded from the

asymptomatic patients as shown in Table 2.

Table 2: Prevalence of *Salmonella typhi* and ESBL-producers in patients

	DASH		DOMA		OBI	
Gender Prevalence (%)						
Sex	Male	Female	Male	Female	Male	Female
<i>S. typhi</i> in patients	16 (8.33%)	21 (10.94%)	14 (10.50%)	7 (5.26%)	11 (8.80%)	7 (5.60%)
<i>S. typhi</i> in symptomatic patients	13 (22.81%)	19 (33.33%)	6 (10.53%)	4 (7.02%)	10 (17.54%)	5 (8.77%)
Age Group Prevalence (%)						
Group	Sym	Asym	Sym	Asym	Sym	Asym
6 -17	6	1	3	0	4	0
18 -29	12	2	6	1	4	2
30 -41	3	2	5	1	3	2
42 -53	2	1	2	0	2	1
54 -65	2	0	1	0	2	1
66 -77	2	0	0	2	1	0
Total	27	6	17	4	16	6
Occupational Prevalence (%)	Symptomatic patients		Asymptomatic patients			
Students	20		5			
Farmers	10		6			
Civil servants	6		1			
Business women	10		1			
House wives	15		2			
Prevalence of Extended Spectrum β-lactamase (ESBL) producing <i>Salmonella typhi</i> (%)						
Isolates	Symptomatic patients N = 61		Asymptomatic patients N = 15			
ESBL producer	5 (8.19 %)		1 (7.17 %)			
Non ESBL producer	56 (91.80 %)		14 (93.33 %)			

Sym = Symptomatic patients; Asym = Asymptomatic patients

c Antibiogram analysis

Table 3 summarised the antibiogram profile of the isolates regarded as either ESBL producer or non-EBLS producers. The EBLs producers showed 100 % resistance to ampicillin and ceftazidime, 83.33% to ciprofloxacin, while levofloxacin, ofloxacin, and ceftriazone

recorded 50 % resistance. Resistance was higher in ESBL-producers than non-ESBL producer as shown in Table 3. Most of the isolates resisted more than three classes of antibiotic. The Multiple Antibiotic Resistance Indices was higher in ESBL-producing isolates than the non ESBL-producers.

Table 3: Antibiogram of non- and ESBL producing *Salmonella typhi*

Antibiotics	Non-ESBL producer N = 70 n (%)	ESBL producer N = 6 n (%)
Ofloxacin	18(28.57%)	3(50.00%)
Augmentin	0(0.00%)	0(0.00%)
Ciprofloxacin	39(55.71%)	5(83.33%)
Ampicillin	70(100%)	6(100%)
Ceftianzone	15(21.43%)	5(50.00%)
Imipenem	70(100%)	0(0.00%)
Levofloxacin	34(48.57%)	3(50.00%)
Cefotaxime	12(17.14%)	2(33.33%)
Ceftazidime	40(57.14%)	6(100%)

n = number of resistance cases

DISCUSSION

Typhoid fever is common in most developing nations of the world due to poor sanitation and lack of access to clean drinking water. The situation have become worse with the emergence of multidrug resistance which makes treatment difficult (Crump and Kirk, 2015). In this study, 450 stool samples were collected and analysed. Of which 75 cases of *Salmonella typhi* was recorded. The sample were collected from two category of patients. Two hundred and fifty samples were collected from those that have symptoms of the disease (symptomatic), while 200 samples were collected from those that came to the hospital for other complications and did not have symptoms of typhoid fever. The prevalence rate was determined to be 16.18 % which was lower compared to reports of studies carried out in other parts of the Nigeria. A prevalence of 67.80 % was obtained in a research carried out in Minna by Adogo *et al.* (2015), 42 % in Jos by Ukaegbu (2014) and in Kano, Abdullahi *et al.* (2010) obtained a prevalence of 13 % which was close to the value recorded in this study. Variation noted in the prevalence rate were due to source of water, way of living and level of education. Other reasons are differences in diagnostic methods used, as it has been reported that sensitivity of stool culture is lower compared to blood culture and molecular methods (Ochai, 2010); and the level of awareness which might explain the lower prevalence since about 45% of those that took part in the research were asymptomatic.

Comparing the prevalence rate of symptomatic and asymptomatic patients of which no statistical significant difference was observed implies that anybody once exposed to high dose of *Salmonella typhi* could be infected, while manifestation of symptoms depends on other factors (Chatterjee *et al.*, 2012). The prevalence rate of 7.5% was recorded among the symptomatic patients in this study showing that there were carriers of the disease in the population who will continue to spread the disease without showing symptoms of the disease. This poses a great challenge on the fight against typhoid fever and calls for urgent attention in other to identify such carrier and treat them.

The research work excluded the age group less than five years old because there was no reasonable matched age group for the asymptomatic patients during the research. This was done to avoid bias. Age group 18 – 29 presented the highest prevalence. There was a statistical significant differences comparing the age groups and the prevalence obtained ($p < 0.05$). This findings disagreed with the work done in Kaduna by Bobai *et al.* (2015) who obtained the highest prevalence in children less than 10 years old. Another study designed to check the risk factors of the disease showed that the prevalence was higher in children (Saddiqui *et al.*, 2008). Reasons adduced for high prevalence in the age group 18 -29 in this study may be that high proportion in that class were students who eat what is available as compared to those who boil their water and carefully prepare their own food (Barbara and Sarah., 2011). The challenge of poverty,

drought, and scarcity of food in the Northern part of Nigeria also predisposes people to eating anything that is available.

The prevalence of *Salmonella typhi* was higher among male participants in the study though statistics showed no significant differences between the genders. Findings in this study contrasted report published by Okwonkwo *et al.* (2010), who obtained higher prevalence in female patients than in male patients. The reason for the higher prevalence observed in male may be that most women stayed home because of religious obligations, while their male counterparts are always outside and are more likely to eat all manner of junks foods. Research have it that one infected cook can infect hundreds of her customer as in the case of Typhoid Mary (Fontana *et al.*, 2003). Unhygienic handling and preparation of food is a key risk factor (Al-Khatib *et al.*, 2004).

Occupation of the patients has effect on the number of *Salmonella typhi* cases recorded since there was significant statistical difference between them. This implies that the rate of infection depends on the day to day activities of the patients (Crump, 2015). Some occupation tend to have higher risk of exposure than others as reported by Fontana *et al.* (2003); and the study showed that farmers were predisposed due to life style and level of awareness about the disease (Ram *et al.*, 2008).

Contaminated water is a higher risk factor of the disease in the study area. The odd ratio between the symptomatic and the asymptomatic was above 1 implying that the source of drinking water is an independent risk factor. High odd ratio was also seen among those who have taken antibiotic before (OR = 2.91) meaning that the disease is an independent factor and indiscriminate use of antibiotics is a key risk factor, and is responsible for antibiotic resistance (Barbara and Sarah., 2011).

The study recorded cases of *Salmonella typhi* among asymptomatic patients 15(7.5%) which showed the population have a good number of carriers without visible symptoms of typhoid fever making them potential reservoirs. ESBL-producing *Salmonella typhi* were detected in the study area with the capacity to produce the enzyme beta-lactamase. The prevalence obtained for these resistant isolates agreed with the study carried out in Calabar by Oghenevo *et al.* (2016) who identified ESBL producer among *Salmonella typhi* and disagreed with the report of Casmir *et al.* (2014) who recorded zero prevalence of ESBL-producing *S. typhi*. *Salmonella typhi* resistance to multiple antibiotics have been documented (Adanbara *et al.*, 2012). This study confirms multiple antibiotics resistance of *Salmonella typhi*, while ESBL-producing *S. typhi* showed more resistant pattern than non EBLs producers. The ESBL-producing *S. typhi* with 83.33% resistance for ciprofloxacin and 100% to Ceftrazidime and ampicillin agreed with the study of Adanbara *et al.* (2012).

CONCLUSION

The study concluded that *S. typhi* prevalence was relatively high from the 3 hospitals where samples were collected and was higher in male than female patients. Isolates with capacity to produce β -lactamase were obtained which calls for concern since the enzyme confers beta-lactamase resistance factor on bacteria, and can be transferred into other bacterial species. Isolates of *Salmonella typhi* producing beta-lactamase presented multi drug resistant pattern to antibiotics tested against them; especially ampicillin, ceftriaxone and ceftriaxime. While discouraging self-medication, the study implore the government to provide clean and safe drinking water to the people.

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