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Antibiotic resistance and Molecular characterization of *Salmonella* in diarrhoeal patients' faeces in south-western Nigeria

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Abstract
The study reports the prevalence of multiple antibiotic resistance (MAR) and molecular chacterization of resistance (*BlaCTX* and *GyrA*) genes in *Salmonella* recovered from stool samples of diarrhoeal patients in Ile-Ife, Osun state, Nigeria.
Salmonella was cultured on molten Salmonella and Shigella agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) plate at 37^0C. Susceptibility of isolates to antibiotics was done on Mueller Hinton (Himedia lab Ltd, Vadhani) by disk diffusion technique. Detection of plasmid DNA in multiple antibiotic resistant isolates was carried out by alkali lysis (TENS) method and resistance genes (*BlaCTX* and *GyrA*) were detected by the polymerase chain reaction. A total of 69 *Salmonella* (*S.*Typhimurium(82.6 %), *S.*Typhi(10.1 %) and *S.*Paratyphi*A* (7.3 %)) were cultured from 187 diarrhoeal stool samples analysed. Resistance was mostly to nitrofurantoin (100%), ceftriazone (97.2 %), and gentamicin (94.2 %) among others. Sixty seven (97.1 %) of the *Salmonella* isolates were resistant to at least two different classes of antibiotics with 32 antibiotypes. Multiple plasmids of molecular weights (1.46 - 23.13 kbp) and resistance genes (*GyrA*-282 bp, *blaCTX*-480 bp*)* were detected in the representative MAR isolates. The prevalence of MAR *Salmonella* in diarrhoeal patients' stool samples is high in the study area with attendant public health and economic loss consequences.
Keywords:*Salmonella*, diarrhoea, antibiotic resistance, plasmid, resistance genes

1.0 Introduction

Salmonellae are Gram-negative, rod-shaped, non-spore forming, and facultative anaerobic bacteria which cause gastroenteritis characterized by nausea, vomiting and diarrhoea (CDC, 2008). *Salmonella* has been recovered from the intestinal tract of a wide range of warm and cold-blooded animals including fish, reptiles, birds, and mammals (Cox, 1999). Diarrhoeal symptoms include stomach cramps, fever, loss of appetite, stomach upset, weight loss, dehydration, bloody stool, mucoid stool or stool with pus (Rimawa*et al*., 2001; Donna and Lindsay, 2002). The most commonly isolated diarrhoegenic pathogens include *Escherichia coli*, Rotavirus, *Salmonella* sp., *Shigella* sp., *Campylobacter jejuni*, *Entamoebahistolytica*, and *Giardia lamblia*.The main sources of salmonellosis in humans are food animals and their products such as raw eggs, poultry meat and pork (Hald*et al.*, 2003).*Salmonella* epidemics may occur among infants in pediatric wards, immune-compromised individuals, elderly people and others. Socio-demographic factors (age, education, income etc.), environmental and sanitation factors (poor access to a good water source and poor sanitation) and climatic factors (rainfall, temperature and humidity) are thought to be related to incidence and spatial distribution of diarrhoea (WHO, 2007). The frequency and gravity of these epidemics are affected by hygienic conditions, malnutrition, and the excessive use of antibiotics that select for multiresistant strains (CDC, 2008). Diarrhoea is one of the most important causes of illness and death all over the World, particularly among infants and young children ((WHO, 2007). It has been reported that five million people especially children under the age of five years die of diarrhoea annually in Nigeria (UNICEF/WHO, 2009).
Over the years, misuse of antibiotics has caused selection, emergence and dissemination of antibiotic resistant *Salmonella* sp. with attendant public health and economic loss consequences (WHO, 1998). There are extremely limited data on the overall health implications of antibiotic resistance in diarrhoeal treatment in Ile-Ife, hence the probable under-estimation of the disease burden in the area. The study reports antibiotic resistance profile and characterization of *Salmonella* in diarrhoeal patients' faeces in Ile-Ife, Nigeria.

2.0 Materials and Methods

2.1Collection and bacteriological analysis of samples

One hundred and eighty seven (187) diarrhoeal stool samples were collected from patients (children and adults) between April, 2011 and March, 2012 at ObafemiAwolowo University Teaching Hospitals Complex (OAUTHC) and other Health Institutions in Ile-Ife (Latitude 7^0 28' 00''N; Longitude 4^0 34' 00''E; Altitude 286 m), Osun State, Nigeria. Stool samples of apparently healthy individuals without diarrhoea were used as control. Samples were collected in clean universal sampling bottles and immediately transported to the Department of

Microbiology laboratory for bacteriological analysis. Stool samples were first enriched in Selenite F broth at 37^0C and cultured by spreading on *Salmonella* and *Shigella* (SS) agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) plates using a calibrated inoculating loop. The plates were then incubated aerobically at 37°C for 18 to 24 hours.

Colonies with a presumptive Salmonella morphology on SS agar were identified by biochemical tests which include; determination of glucose and lactose; gas formation and the production of ferrous sulfite from sodium thiosulfate in Kligler agar; the presence of cytochrome oxidase; citrate utilization; urea hydrolysis in Christensen's agar; the presence of phenylalanine deaminase; the liberation of indole; acid production from sugar fermentation; the decarboxylation of lysine, ornithine, and arginine; and the utilization of malonate as the sole carbon source. These were compared to Bergey's Manual of Determinative Bacteriology (2001).

2.2Antibiotic susceptibility of isolates

Susceptibility of *Salmonella* to antibiotics was carried out using Kirby-Bauer's disk diffusion method. The disk containing the antibiotics (Fondisk, Lagos, Nigeria); (augmentin (30µg), amoxicillin (25µg), nitrofurantoin (200µg) pefloxacin (5 µg), tetracycline (30 µg), ciprofloxacin (10µg), ofloxacin (5µg), ceftriazone (30 µg), gentamicin (10 µg) and cotrimoxazole(25 µg) were firmly placed on Mueller-Hinton agar (HIMEDIA lab. Ltd Vadhani) plates previously seeded with standardized inoculums (10^6 CFU/ml). The plates were incubated at 37^0C for 24 h and the diameter of the zone of inhibition was measured to the nearest millimeter with a transparent ruler, and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2009).

2.3Plasmid Analysis

Plasmid DNA extraction was carried out on the representative MAR isolates following the alkaline lysis (TENS-Tris 25mM, EDTA 10mM, NaOH O.1N and SDS 0.5%) (Sigma products)) method of Kraft *et al*. (1988) and separated by 0.8% (w/v) agarose gel in Tris-acetate-EDTA buffer containing ethidium bromide (20 ml of 50 X TAE and 6.0 µl of 10 µg/ml ethidium bromide per litre). Hind III DNA marker (Biomerx Lab, Hamburg, Germany) was used as control. Plasmid DNA fragments were visualized by UV light illuminator and photographed with a Leicaflex SL-camera.

2.4Detection of resistance genes by polymerase chain reaction

The representative *Salmonella* isolates that were resistant mostly to beta lactam and fluoroquinolones were screened for *BlaCTX* (480 bp) and*GyrA* (282 bp) genes, respectively using polymerase chain reaction.

DNA extraction in *Salmonella* was done by suspending a colony from an overnight culture plate on *Salmonella* and *Shigella* agar plate in 200 µl of distilled water. After vortexing, the suspension was boiled for 5 min, and 50 µl of the supernatant was collected after centrifuging for 10 min at 14,000 rpm. The primers *GyrA*- forward - CGTTGGTGACGTAATCGG; reverse - CCGTACCGT CAT AGT TAT and *BlaCTX*- forward- ATGTGCAGYACCAGT AAR GTKATG GC; reverse- TGGGTR AAR TAR GTSACC AGA AYCAGCGG were employed for the detection of *GyrA* and *BlaCTX* genes, respectively. Amplification reactions were carried out in a volume of 25 µl of a PCR mixture containing 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP; 0.2 µl primer 1,0.2 µl primer 2, 1.5 µl of genomic DNA and 0.1 µl of Taq polymerase..

The PCR reaction was performed in the DNA thermocycler (Eppendorf Mastercycler, USA) programmed for optimum conditions as follows: an initial denaturation at 95^0C for 3 minutes, 30 cycles of denaturation at 95 ^{0}C for 30 seconds, annealing temperatures of 42 ^{0}C for*GyrA* and 62 ^{0}C for *blaCTX* gene for 60 seconds, elongation at 72 ^{0}C for 60 seconds and final 10 minutes of extension period at 72 ^{0}C. The amplified PCR products alongside DNA standard molecular weight size marker (hind lll; 100bp) was subjected to electrophoresis at 80 volts in horizontal gels containing 1.2 % agarose with Tris-borate buffer (45 mMTris borate, 1mM EDTA). The gel was stained with ethidium bromide and visualized with ultra-violet illumination and photographed. *Salmonella enterica*serovarTyphimurium was used as positive control.

2.5 Statistical analysis

Statistical analysis was performed using T-test and Pearson correlation index at $P < 0.05$ with statistical software SPSS Data Editor Version 16. P value of < 0.05 was considered statistically significant for all the comparisons.

3.0 Results

Sixty-nine S*almonella* (*Salmonella* Typhimurium*(57)*, *Salmonella* Typhi(7), *Salmonella* ParatyphiA (5) were obtained from 187 diarrhoeal stool samples analysed.

Table 1 shows the resistance profile of the isolates to various antibiotics employed. The isolates showed high levels of resistance to nitrofurantoin (100 %), ceftriazone (89.9 %) and augmentin (55.1 %). The increasing order of occurrence of resistance to the antibiotics was GEN <OFL<PFX<CPX< TET < COT <AMX< AUG <CEF< NIT. There was a significant statistical difference in the incidence of antibiotic resistance among the *Salmonella* isolates ($p < 0.05$).

Table 1. Antibiotic resistance profile of the *Salmonella* isolates

Antibiotics	Resistance (%)
Amoxicillin	52.2
Augumentin	55.1
Ceftriazone	89.9
Ciprofloxacin	26.1
Cotrimoxazole	44.9
Gentamicin	23.2
Nitrofurantoin	100
Ofloxacin	24.6
Pefloxacin	26
Tetracycline	37.7

Sixty seven (97.1 %) of the *Salmonella* isolates were multiple antibiotic resistant to at least two different classes of antibiotics with 32 antibiotypes (Table 2). Resistance to combinations of four antibiotics (25.0 %)) was the highest with "CRO, NIT" featuring predominantly in the antibiotypes (Table 2).
AUG-augmentin,AMX-amoxicillin, NIT-nitrofurantoin, PFX- pefloxacin, TET-tetracycline, CPX-ciprofloxacin, OFL-ofloxacin, CRO-ceftriazone, GEN- gentamicin, COT-cotrimoxazole; *S*. Typhimurium-1, 2, 4, 6, 8-10, 12-22, 24-28, 30, 31, 33-35, 37-39, 41-45, 47, 48, 50-62, 64-69; *S*.Typhi-3, 29, 32, 36, 40, 49, 63; *S*. Paratyphi-5, 7, 11, 23, , 46
The estimated molecular weights of plasmid DNA in *Salmonella*and their resistance to individual antibiotics used is presented in table 3. Eighteen *S*. Typhimuriumhaboured multiple plasmids with molecular weights of 1.46-23.13kbp and *S*. Typhi (2) had single plasmid with molecular weight of 23.13kbp.

Table 2. Multiple antibiotic resistance patterns of the *Salmonella* isolates

Antibiotic class	MAR patterns	Number of isolates	Total occurrence (%)	*Salmonella* isolate code	Totals cod (%)
2	AMX,NIT	1 (1.5)	16 (23.5)	Sal 40	16 (23.5)
	AUG, NIT	1 (1.5)		Sal 29	
	CRO, NIT	14 (20.9)		Sal 5, 7, 8, 13, 38, 39, 41, 4, 44, 43,44, 47, 49, 53, 54, 65	
3	AMX, CRO, NIT	6 (8.9)	14 (20.6)	Sal 2, 3, 17, 48, 56, 66	14 (20.6)
	AUG, CRO, NIT	5 (7.5)		Sal 6, 30, 45, 58, 61	
	AUG, NIT, COT	1 (1.5)		Sal 11	
	CRO, NIT, TET	1 (1.5)		Sal 12	
	AUG, NIT, GEN	1 (1.5)		Sal 25	
4	CRO,NIT,COT,TET	1 (1.5)	17 (25.0)	Sal 9	17 (25.0)
	CRO,NIT,AMX, TET	1 (1.5)		Sal 15	
	CRO,NIT,COT,AMX	1 (1.5)		Sal 18	
	AUG,CRO,NIT,COT	5 (7.5)		Sal 19, 24, 35, 60, 63	
	CRO,NIT,GEN,COT	1 (1.5)		Sal 26	
	CRO,NIT,AMX,PFX	1 (1.5		Sal 34	
	CRO,NIT,AMX,OFL	2 (2.9)			
	AUG,NIT,COT,TET	1 (1.5)		Sal 46, 55	
	AUG, CRO,COT,TET	1 (1.5)		Sal 28	
	AUG,NIT,GEN,COT	2 (2.9)		Sal 23	
	AUG,CRO,NIT,TET	1 (1.5)		Sal 42,43	
				Sal 67	
5	AUG,CRO,NIT,COT,TET	1 (1.5)	4 (5.9)	Sal 37	4 (5.9)
	AUG,CRO,NIT,COT,OFL	1 (1.5)		Sal 50	
	AUG, CRO,NIT,TET,CPX	1 (1.5)		Sal 57	
	AUG,CRO,NIT,TET,OFL	1 (1.5)		Sal 52	
6	AUG,CRO,NIT,GEN,COT,TET	1 (1.5)	5 (7.4)	Sal 36,42, 69, 16,4	5 (7.4)
	AUG,CRO,NIT,GEN,COT,OFL	1 (1.5)		Sal 20	
	AUG,CRO,NIT,COT,TET,OFL	3 (4.5)		Sal 51, 31, 21	
7	CRO,NIT,GEN,COT,TET,AMX, OFL	1 (1.5)	12 (17.6)	Sal 59	12 (17.6)
	AUG,CRO,NIT,GEN,TET,COT,OFL	1 (1.5)		Sal 44	
	AUG,CRO,NIT,GEN,COT,TET,OFL	10 (14.9)		Sal 36, 32, 10, 1, 14, 22, 27,33,64, 68	

Table 3. Estimated molecular weights of plasmid DNA in *Salmonella*

Salmonella species isolate code	Number of Plasmid	Estimated molecular sizes of plasmid (kb)	Resistance to general antibiotics employed
Sal 1	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 9	3	23.13, 1.60, 1.46	$CRO^{R}NIT^{R}COT^{R}TET^{R}$
Sal 10	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 19	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}COT^{R}$
Sal 20	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}PFX^{R}$
Sal 22	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 25	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}COT^{R}$
Sal 26	3	23.13, 1.60, 1.46	$CRO^{R}NIT^{R}GEN^{R}COT^{R}$
Sal 27	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 31	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}COT^{R}AMX^{R}TET^{R}$
Sal 32	1	23.13	$AUG^{R}CRO^{R}NIT^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 33	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 35	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}COT^{R}AMX^{R}TET^{R}$
Sal 36	1	23.13	$CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 42	3	23.13, 1.60, 1.46	$AUG^{R}NIT^{R}GEN^{R}COT^{R}AMX^{R}$
Sal 50	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 62	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}AMX^{R}TET^{R}$
Sal 64	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 66	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}CPX^{R}TET^{R}PFX^{R}$
Sal 69	3	23.13, 1.60, 1.46	$AUG^{R}CRO^{R}NIT^{R}GEN^{R}COT^{R}OFL^{R}AMX^{R}TET^{R}PFX^{R}$

AUG-augmentin,AMX-amoxicillin, NIT-nitrofurantoin, PFX- pefloxacin, TET-tetracycline, CPX-ciprofloxacin, OFL-ofloxacin, CRO-ceftriazone, GEN- gentamicin, COT-cotrimoxazole; *S.* Typhimurium-1, 9, 10, 19, 20, 22, 25, 26, 27, 31, 33, 35, 42, 50, 62, 64, 66, 69; *S.*Typhi - 32, 36

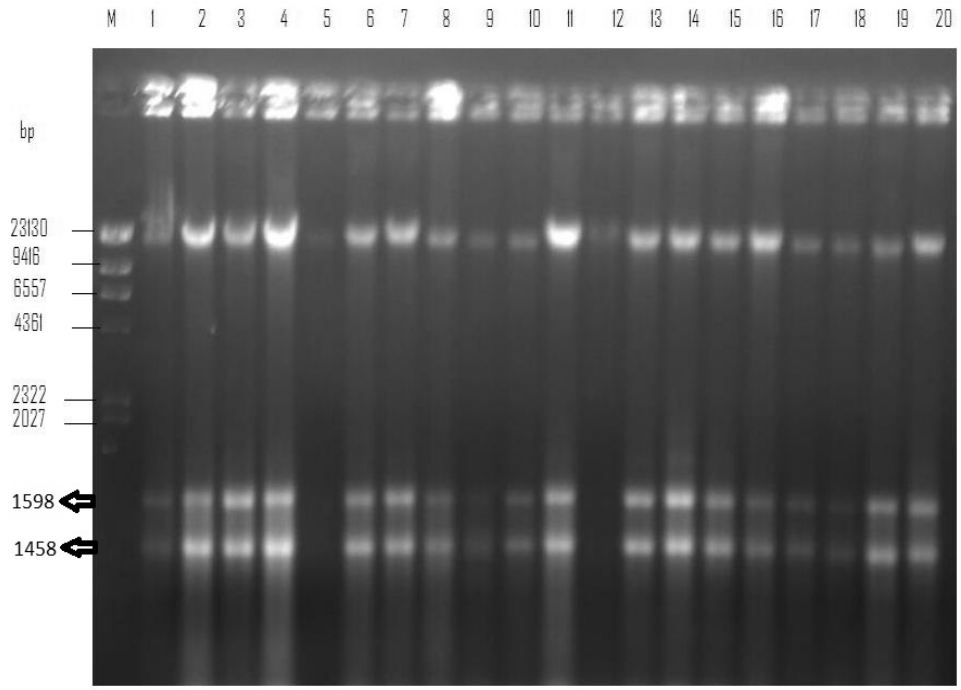

Figure 1 shows the agarose gel electrophoresis of the various plasmid DNA bands isolated from the representatives MAR *Salmonella* isolates and their molecular weights.
Figure 1. Agarose gel electrophoresis of plasmid DNA in multiple antibiotic resistant *Salmonella* isolates. Lane M= DNA marker (HIND III digest); Lane 1 = Sal 1; Lane 2 = Sal 42; Lane 3 = Sal 33; Lane 4 = Sal 64; Lane 5 = Sal 36; Lane 6 = Sal 27; Lane 7 = Sal 10; Lane 8 = Sal 20; Lane 9 = Sal 62; Lane 10 = Sal 31; Lane 11 = Sal 69; Lane 12 = Sal 32; Lane 13 = Sal 22; Lane 14 = Sal 35; Lane 15 = Sal 66; Lane 16 = Sal 9; Lane 17 = Sal 26; Lane 18 = Sal 19; Lane 19 = Sal 25; Lane 20 = Sal 50.

The molecular detection of *BlaCTX*(480 bp) and *GyrA* (282 bp) genes in 20 representatives *Salmonella* profiled is shown in Table 3. The *BlaCTX*(480 bp) gene was haboured by *Salmonella* Typhimurium (18), *S*. Typhi (1) and *S*. Paratyphi A (1). Meanwhile, all the representative isolates had *GyrA* (282 bp) gene.
The agarose gel electrophoreses showing the amplification of resistance (*GyrA* and *BlaCTX*) genes in the representatives MAR *Salmonella* isolates are depicted by figures 2 and 3, respectively. All the representative isolates had *GyrA* gene (282 bp) (Fig 2) while 4 had *BlaCTX* gene (480 bp) (Fig. 3).

Table 4. Molecular detection of *BlaCTX* and *GyrA* resistance genes in *Salmonella* isolates

Lanes / Isolates	*BlaCTX* gene (480 bp)	*GyrA* gene (282 bp)
M – Marker	-	-
L_1 *Salmonella*Typhimurium	-	+
L_2 *Salmonella*Typhimurium	+	+
L_3 *Salmonella*Typhimurium	-	+
L_4 *Salmonella*Typhimurium	-	+
L_5 *Salmonella*Typhi	+	+
L_6 *Salmonella*Typhimurium	-	+
L_7 *Salmonella*Typhimurium	-	+
L_8 *Salmonella*Typhimurium	-	+
L_9 *Salmonella*Typhimurium	-	+
L_{10} *Salmonella*Typhimurium	-	+
L_{11} *Salmonella*Paratyphi A	+	+
L_{12} *Salmonella*Typhimurium	-	+
L_{13} *Salmonella*Typhimurium	-	+
L_{14} *Salmonella*Typhimurium	-	+
L_{15} *Salmonella*Typhimurium	+	+
L_{16} *Salmonella*Typhimurium	-	+
L_{17} *Salmonella*Typhimurium	-	+
L_{18} *Salmonella*Typhimurium	-	+
L_{19} *Salmonella*Typhimurium	-	+
L_{20} *Salmonella*Typhimurium	-	+

Key: - = absent; + = present

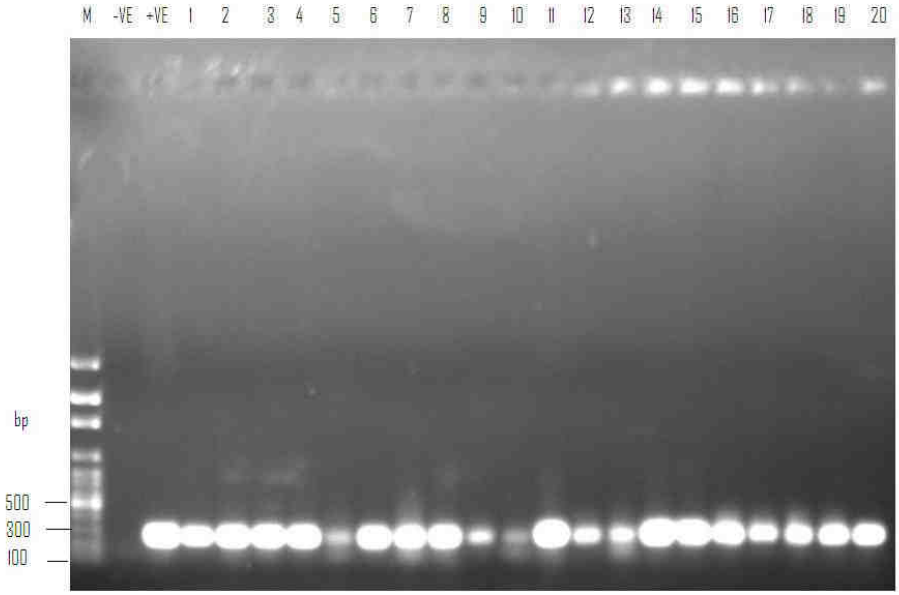

Figure 2. PCR amplification gel of *GyrA* (282 bp) gene in *Salmonella* isolates
Lane M=100bp marker (hind lll); -ve = negative control without DNA; +ve = *Salmonella enterica*serovarTyphimurium (positive control).

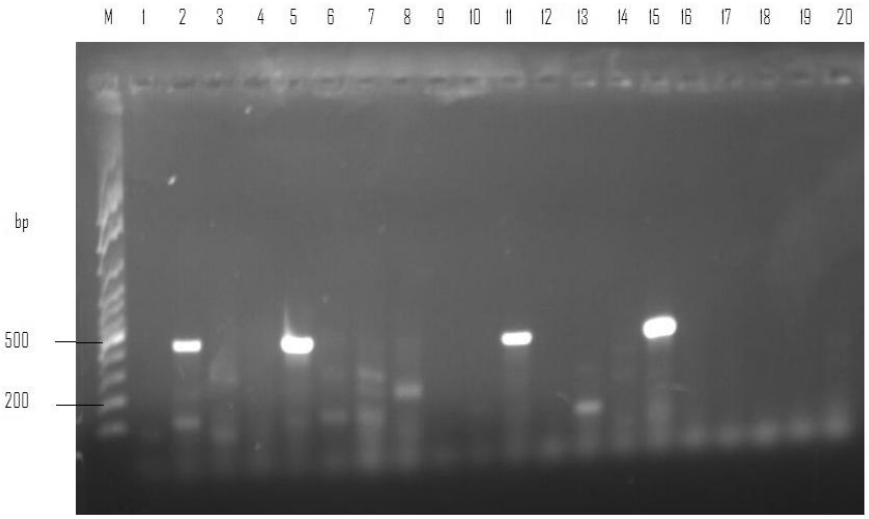

Figure 3. Polymerase chain reaction amplification gel of *BlaCTX* (480 bp) gene
Lanes 1-20: *Salmonella* isolates, Lane M=100bp marker; Lane 2, 5, 11 and 15 were positive for *BlaCTX*.

DISCUSSION

The importance of *Salmonella* in public health is very significant. In this study, 82.6 % of the *Salmonella* recovered were *Salmonella* Typhimurium. This result confirms *Salmonella*Typhimurium as one of the important causal agents of diarrhoea in Ile-Ife. The incidence of *Salmonella* in diarrhoea had been previously reported (Grabow, 1996; Scuderi*et al*., 1996). Of recent, Casmir *et al.* (2014) reported the isolation of *Salmonella* in children with acute gastroenteritis in Abuja, Nigeria. Major outbreaks of human salmonellosis had been attributed to the consumption of contaminated foods in restaurants and institutional kitchen. Undercooked food, slow freezing, and keeping foods for many hours under no refrigeration are considered contributing factors for the emergence of this disease in humans (Costa, 1996). However, the incidence of cases and deaths due to diarrhoea has been greatly increased by a combination of poor sanitation and hygiene, unavailability of vaccines and high cost of effective antimicrobial chemotherapy.

The resistance of the *Salmonella* isolates to the antibiotics tested in this study calls for great concern, as some of the isolates were resistant to beta-lactam antibiotics (augmentin and amoxicillin) and other antibiotics (tetracycline, gentamycin, ciprofloxacin)typically used for the treatment of diarhoea and a number of infectious diseases. Ehinmidu (2003) previously reported resistance by *Salmonella* to beta-lactam antibiotics. In 2001, detection of quinolone resistant isolates was reported from Austria (17 %), France (9 %), Greece (32 %), the Netherlands (6 %) and Portugal (60 %) (EC, 2003).

Resistance, particularly to the commonly available antibiotics poses a major health concern, as alternative therapeutic choices are either unavailable or too expensive to be affordable by most patients. Most of the diarrhoeal patients employed in this study first engaged in self-medication before seeking physician's attention. This act may be responsible for the large number of the multiple antibiotic resistance patterns observed among the isolates in this study. Some of the *Salmonella* isolates were still susceptible to expensive antibiotics like ofloxacin (62.3 %), pefloxacin (60.9 %) and ciprofloxacin (58 %) because these antibiotics are very expensive and not readily available.

The multiple antibiotic resistance commonly observed in most strains in this study may be plasmid mediated as plasmid DNA of large molecular weights were isolated. The first case of diarrhoea due to *Salmonella* carrying plasmid-encoded resistance to chloramphenicol, ampicillin and cotrimoxazole was reported from East and South Asia (Mirza*et al*., 2000). In the present study, all the 69 *Salmonella* isolates were resistant to nitrofurantoin and a greater percentage to tetracycline. Murugkar*et al*. (2005) previously reported the resistance of *Salmonella*Typhimurium to quinolones, gentamicin, chloramphenicol, tetracycline etc.

The report of this study corroborates the findings of Adeshina*et al*. (2010) who reported that multiple antibiotic resistant *Salmonella* isolates haboured plasmid sizes of 23.13 kbp and 0.145 kbp conferring resistance to antibiotics. The detection of *GyrA* gene in all the isolates depicts resistance to fluoroquinolones while *BlaCTX* gene denotes resistance to beta-lactam antibiotics. This could be due to the wide and varying use of different antibiotics by human patients with simultaneous evolution of newer antibiotics that have precipitated into pathogens of multiple antibiotic resistance.

Conclusion: *Salmonella* recovered from the study are multiple antibiotic resistant, harbouring*blaCTX* and *gyrA*genes.

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