

Antimicrobial Resistance and Plasmid Profiles of *Salmonella enterica* Serovars from Different Sources in Lagos, Nigeria

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Abstract

Introduction: *Salmonella enterica* Serovars remains one of the leading pathogens that cause diarrhoea and bloodstream infections in developing countries. The emergence of multidrug resistant (MDR) *Salmonella* has become a serious problem globally. This study investigated the antibiotic resistance and plasmid profiles of *Salmonella* isolates from different sources. **Methods:** Seventy-three samples comprised of clinical (30), hand swab (15), food (10) and water (18) were analyzed bacteriologically. *Salmonella* isolates were identified and characterized by standard procedures. Isolates were subjected to antimicrobial susceptibility testing and were further screened for plasmid DNA by standard methods. **Results:** A total of 27 *Salmonella* isolates made up of 5 (18.5%) *S. typhi*, 6 (22.2%) *S. enteritidis*, 9 (33.3%) *S. typhimurium*, 5 (18.5%) *S. choleraesuis*, and 1 (3.7%) each of *S. arizonae* and *S. vichow* were obtained in this study. All the isolates developed resistance to three or more antibiotics evaluated. Four distinct resistance profiles: TetAmpCol, TetAmpColCot, TetAmpColCip and TetAmpColCotCip were recorded with 63% of the isolates exhibiting resistance profile TetAmpColCot. Specifically 23 of 27 (85.2%) of the isolates harboured plasmid DNA comprised of 12 distinct plasmid profiles of different sizes ranging from 3.2 kb to 30.2 kb. *Salmonella* isolates of the same species from different sources differed in plasmid profile. Plasmid profile was found to show good discriminatory capability compared to antibiotics resistance profile. **Conclusion:** This study revealed that both resistance antibiogram and plasmid profile are still viable epidemiological tools for tracing the source of *Salmonella* isolates. A need for prudent use of antibiotics is suggested.

Keywords

Salmonella, Resistant, Antibiogram, Plasmid, Epidemiology, Disease

1. Introduction

The genus *Salmonella* belongs to the family *Enterobacteriaceae*. They are facultative anaerobic rods. *Salmonella* is mostly motile except *Salmonella pollorum* and *Salmonella galarum*, non-spore forming, Gram-negative bacterium [1]. There are currently over 2587 serotypes of *Salmonella*, responsible for salmonellosis, grouped into two basic species: *Salmonella enterica* and *Salmonella bongori* [2] [3] [4]. *Salmonella* spp. is mainly transmitted by the fecal-oral route mainly through contaminated food or water [4]. Outbreaks of *Salmonella*-associated diseases are usually associated with contaminated water and the ingestion of contaminated food of animal origin like, fishes, poultry, meat and milk [5] [6] [7].

Salmonella enterica is an important cause of human morbidity and mortality worldwide [8] [9] [7]. *Salmonella enterica* Serovar Typhi is the etiological agent of typhoid fever while non-typhoid *Salmonella* species (NTS) are associated with gastroenteritis and invasive infection in children, the elderly and immune compromised patients; these include: *Salmonella typhimurium*, *Salmonella enteritidis* amongst other serovars of *Salmonella enterica* that affect both human and animal, of which *S. typhi* affects human only [10] [11]. Both *Salmonella typhi* and NTS are among the most frequent pathogens causing blood stream infections (BSI) in tropical low-resource settings [11]. The highest incidence and prevalence of *Salmonella* infection worldwide occur in Asia region [8] [12] [13], mainly in south and southeast Asia, where isolates show high rate of antibiotic resistance [13] [14] [15]. It is estimated that each year there are approximately 21.6 million cases of typhoid fever which result in 200,000 deaths worldwide [16]. However, the incidence of cases and death has been greatly increased by combination of poor sanitation and hygiene, unavailability of vaccines and high cost of effective antimicrobial chemotherapy [16].

In Nigeria, the antibiotics mostly readily available for treatment of typhoid and other *Salmonella* related diseases are Chloramphenicol, Ampicillin, and Tetracycline, third generation Cephalosporin, Gentamycin, Quinolones and Trimethoprim/Sulfamethoxazole [17] [18]. Recently multi-drug resistant (MDR) strains have emerged presumably due to the extensive use of antimicrobial both in human and animals husbandry [19] [20] [21]. In veterinary medicine for instance, antibiotics are being used in livestock production, disease prevention and as growth-promoting feed additives [5] [22]. The use of antibiotics in animals' production disrupts the normal flora of the intestine; prolongs faecal shedding of these organisms into the environment and emergence of antibiotics-resistant *Salmonella* strains [14] [23]. MDR in *Salmonella* is a cause of great concern in

both clinical and veterinary medicine, as it may limit the therapeutic options available for the treatment of *Salmonella*-associated diseases [24] [25] [26]. Resistance had been carried out on plasmids or the chromosomes of resistant bacteria [27]. Plasmids have been a major factor in the spreading antibiotic resistance between bacteria [28] [29]. Under antibiotic selective pressure, resistant-plasmids (R-plasmids) spread resistance makers between both homologous and heterologous bacterial communities [16]. Resistance to chloramphenicol, ampicillin and trimethoprim are generally plasmid encoded, although chromosomal resistance to ciprofloxacin which is associated with mutation at *gyrA*, *gyrB* and *parC* has been reported [20]. An epidemic MDR strain *S. typhimurium* phage type 104 (DT104) has been identified as a major cause of salmonellosis in humans and animals in Europe and the United States [24] [27].

The increasing usage of antibiotics against *Salmonella* infections and other infectious diseases, which has continually affected the clinical outcomes, necessitated a need to carry out antimicrobial resistance surveillance study and plasmid profiles on *Salmonella* species from different sources in some communities in Lagos, Nigeria.

2. Methods

2.1. Scope of Study and Sample Definition

This study was conducted between June and September, 2014 in the department of microbiology, Lagos State University, Ojo and Nigeria Institute of Medical Research, Yaba, Lagos. A total of 73 samples comprising of 45 clinical samples (20 fecal, 15 hand swab and 10 blood) and 28 environmental samples (10 sachet water, 10 food and 8 well water) were aseptically collected within Badagry and Ojo Local Government Areas of Lagos State following standard procedures.

2.2. Bacteriological Examination of Samples from Different Sources

2.2.1. Stool Samples

A sterile wire loop was used to pick each stool sample collected and inoculated into selenite cysteine broth, then from the broth into petri dishes containing MacConkey agar (**Oxoid, UK**) using streak plate method. The inoculated plates were incubated at 37°C for 18 - 24 hours. All isolates were characterized using standard techniques [30].

2.2.2. Blood Samples

Standard 0.5% bile salt broth containing 5 g of sodium taurocholate in 1 litre of sterile nutrient broth (pH 7.6) and autoclaved at 108°C for 15 minutes, was used as enrichment broth. Approximately 3.0 ml of each blood sample was inoculated into 27 ml. of the broth and incubation was at 37°C for 18 to 24 hours. Sub-cultured daily for 1 week onto *Salmonella-Shigella* agar (**S-S agar M108, Himedia, Mumbai**) was performed using freshly prepared medium every 3 days. Colonies were observed and isolates were characterized using standard

techniques [30].

2.2.3. Food Samples

One gram analytical unit of each sample (white rice, jollof rice and meat) aseptically obtained from vendors was weighed out and was transferred into a sterile blender jar containing 9 ml of peptone water and blended to give a suitable food homogenate. Serial dilutions of each sample were carried out. The pour plate technique was used for plating out the appropriate dilutions of sample (10^{-2} and 10^{-3}). Molten MacConkey agar cooled to 45°C was dispensed over with the sample in each plate. Immediately, the sample and the molten agar were thoroughly and uniformly mixed by a gentle alternate rotation of the plates on the work benches. The solidified agar plate were inverted and incubated at 37°C for 24 - 48 hours. After incubation, colonies developing on plates with morphological characteristic of *Salmonella* were randomly picked and further inoculated on *Salmonella-Shigella* Agar (S-S agar M108, Himedia, Mumbai) to obtain a pure culture of *Salmonella* isolates. All isolates were characterized using standard techniques [30].

2.2.4. Environmental Water Samples

The multiple tube fermentation technique as described by Mackie and McCartney [31]. Briefly, varying quantities of each of the water samples were added to bottle containing broth (Oxoid, UK) of different strengths, with an inverted Durham tube in each bottle. The bottles were incubated under aerobic condition at 37°C for 18 - 24 hours. For each test batch sterile water was used as control. After overnight incubation, subcultures from the positive broth were made onto *Salmonella-Shigella* Agar (SSA) (Oxoid, UK) and MacConkey Agar (Oxoid, UK) followed by subculturing on non-lactose fermenting colonies on triple sugar iron agar (TSIA) (Oxoid, UK). The inoculated media were incubated aerobically at 37°C for 18 - 24 hours. All the isolates obtained were identified by standard methods (30). Further identification was by using API 20E (Espania) was used. Somatic and flagella antigens of isolated *Salmonella* species were performed using polyvalent antisera (Wellcome Diagnostic, UK).

2.2.5. Hand Swab

The left and right hand palms of the subjects (mainly among final year students of department of microbiology, Lagos State University) with the help of sterile cotton buds, soaked in 0.85% saline solution from define (fixed) area on the palm before and after hand washing. Hands were washed thoroughly with water and soap in their usual (regular) manner. These swabs were added into saline solutions of various dilutions under aseptic conditions and 0.2 mL of from each dilution was inoculated on sterilized MacConkey agar (Oxoid, UK) plate which was uniformly spread and incubated at 37°C for 24 h. After Incubation, numbers of CFU were counted and different types of colonies were identified. Colonies were further subcultured on *Salmonella-Shigella* agar (**S-S agar M108, Himedia, Mumbai**) for the presence of non-lactose fermenting *Salmonella* spp. All isolates

were characterized using standard techniques [32]

2.3. Antibiogram of *Salmonella* Isolates

Sensitivity of the pure culture of *Salmonella* isolates to different antibiotics was determined using the Kirby-Bauer disc diffusion technique and interpreted based on the guidelines of the Clinical and Laboratory Standards [33]. Discs used contained the following antibacterial agents: Ampicilin (AMP) (10 µg), Cotrimoxazole (Cot) (30 µg), Colistin (COL) (10 µg), Ciprofloxacin (CIP) (5 µg), Tetracyclin (TET) (30 µg) Ceftriaxone (CRO) (30 µg), Cefotaxime (CTX) (30 µg), Cephalothin (CEF) (30 µg), Imipenem (IMP) (10 µg), Tetracyclin (TET) (30 µg), Nitrofloxacilin (NIT) (10 µg) and Gentamicin (GEN) (10 µg). Mueller-Hinton (MH) agar plates were swabbed with cells from the bacteria stock solution, already adjusted to the 0.5 Mac-Farland's turbidity standard. The discs were thereafter, carefully layered on the agar and incubated at 37°C for 24 H.

2.4. Plasmid DNA Isolation from *Salmonella* Isolates

Plasmid extraction was performed on all the isolates. It was done by using TENS-Mini Prep as described by [34]. Overnight culture (1.5 ml) was micro-centrifuged for one minute to pellet the cells. The supernatant was gently decanted, leaving 50 - 100 µl together with cell pellet and vortex at high speed to re-suspend cells completely. TENS (300 µl) was then added, mixed by inverting tubes 3 - 5 times until the mixture becomes sticky. Potassium acetate (3.0 M, 150 µl, pH 5.2) was then added and vortexed to mix completely. The mixture was micro-centrifuged at 10,000 rpm for 10 min to pellet cell debris and chromosomal DNA. The supernatant was then transferred into fresh tube and mixed well with 900 µl of ice-cold absolute ethanol. The mixture was micro-centrifuged for 10 minutes to pellet plasmid DNA, (white pellet was observed). The supernatant was discarded, pellet rinsed twice with 1 ml of 70% ethanol and dried. The pellet was re-suspended in 20 - 40 µl of 10 mM Tris 0.1 mM EDTA buffer (pH 7.6), and stored at -4°C until further analysis.

2.5. Gel Electrophoresis Preparation

Immediately before loading the gel mix, 8 µl of the plasmid DNA sample was prepared with 2 µl of loading buffer (0.05 M EDTA, 20% Ficoll, 0.25% bromophenol blue, in H₂O). A horizontal electrophoresis apparatus was used, mini-gels was prepared as follows: about 25 ml of 0.8% - 1.0% low electroendosmosis (EEO) agarose in TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.0025 M EDTA) was poured on a 10 × 7 cm glass slide. Depending on the comb used, up to 14 samples can be run. The same TBE buffer is used as electrophoresis buffer. Usually the electrophoretic separation was done at 60 voltages for 6 hours. For visualization of DNA bands and photography, the intercalating dye ethidium bromide was used (proper care was taken as it is a carcinogenic compound). One drop of a 10 mg/ml stock solution was added to the staining tray containing

water and the gel. The tray was covered with a lid. After staining for 30 min DNA bands were made visible under short UV light. A fundamental description of the application of agarose gel electrophoresis is as described [35].

3. Results

Twenty-seven *Salmonella* isolates made up of six *Salmonella enterica* Serovars: *S. typhi*, *S. typhimurium*, *S. choleraesuis*, *S. enteritidis*, *S. virchow* and *S. arizonae* were recorded from 73 samples from different sources in this study (Table 1). Of these, 11 isolates were from 20 faecal samples, 7 from 10 food samples, 3 from 8 well waters and 2 isolates each from 10, 10 and 15 sachet water, blood and hand swab respectively. The different serovars isolated from different sources was as shown in Table 1.

Eleven different antibiotics were tested against 27 *Salmonella* isolates identified. All the isolates (100%) were resistant to at least three different antibiotics TET, AMP and COT. Also about 70% (n = 19) of the *Salmonella* isolates were resistant to four antibiotics TET, AMP, COL and COT. It is interesting to note that *S. Typhi* 02 and 04 had the highest resistance rate, with resistance to five different antibiotics TET, AMP, COL, COT and CIP. However, about 14% of the isolates developed resistance to CIP (Table 2).

Four distinct resistance profiles; **A** (TetAmpCol), **B** (TetAmpColCot), **C** (TetAmpColCip) and **D** (TetAmpColCotCip) were observed from the 27 *Salmonella* isolates evaluated. Resistance profile B was most prevalent with 17 *Salmonella* isolates exhibiting the trait, followed by profile A (six isolates). Two isolates each, exhibited resistance profiles C and D. It was also observed that some *Salmonella* species exhibited more than one resistance profile, for instance *S. enteritidis* isolated from different sources had 3 resistance profiles A, B and C (Table 3).

Twenty-three out of the 27 *Salmonella* isolates harboured plasmid DNA ranging in size from 3.2 kb - 30.2 kb. We observed 12 distinct plasmid profiles among the isolates from different sources (Table 4 and Figures 1-3).

Table 1. *Salmonella serovarss* from different samples analyzed.

Sources	Number of samples collected	Number of positive samples	<i>Salmonella isolates</i>					
			<i>S. typhi</i>	<i>S. enteritidis</i>	<i>S. typhimurium</i>	<i>S. choleraesuis</i>	<i>S. virchow</i>	<i>S. arizonae</i>
Food	10	7	2	2	3	0	0	0
Faeces	20	11	3	2	1	3	1	1
Water-sachet	10	2	0	1	1	0	0	0
Well-water	8	3	0	1	2	0	0	0
Blood	10	2	0	0	0	2	0	0
Hand swab	15	2	0	0	2	0	0	0
Total	73	27	5	6	9	5	1	1

Table 2. Summary of antibiotics resistance of *salmonella* isolates.

<i>Salmonella</i> ser.(n = 27)	TET (30 µg)	AMP (10 µg)	COT (30 µg)	COL (10 µg)	CIP (5 µg)	CRO, CEF, CTX, IMP,
<i>S. typhi</i> (5)	5 (18.5%)	5 (18.5%)	4 (14.8)	5 (18.5)	3 (11.1)	None
<i>S. enteritidis</i> (6)	6 (22.2%)	6 (22.2%)	4 (14.8)	6 (22.2%)	1 (3.7)	None
<i>S. typhimurium</i> (9)	9 (33.3)	9 (33.3)	7 (25.9)	9 (33.3)	None	None
<i>S. choleraesuis</i> (5)	5 (18.5%)	5 (18.5%)	3 (11.1)	5 (18.5%)	None	None
<i>S. arizonae</i> (1)	1 (3.7)	1 (3.7)	1 (3.7)	1 (3.7)	None	None
<i>S. vichow</i> (1)	1 (3.7)	1 (3.7)	None	1 (3.7)	None	None

Key: Ampicilin (AMP) (10 µg), Cotrimoxazole (Cot) (30 µg), Colistin (COL) (10 µg), Ciprofloxacin (CIP) (5 µg) and Tetracyclin (TET) (30 µg), Ceftriaxone (CRO) (30 µg), Cefotaxime (CTX) (30 µg), Cephalothin (CEF) (30 µg), Imipenem (IMP) (10 µg), Tetracyclin (TET) (30 µg), Nitrofloxacine (NIT) (10 µg) and Gentamicin (GEN) (10 µg).

Table 3. Resistance profile of *Salmonella* isolates obtained from the study.

type	Resistance pattern	<i>Salmonella</i> isolates						TOTAL
		S.T	S.TP	S.E	S.CH	S.V	S.A.	
A	TetAmpCol	0	2	1	2	1	0	6
B	TetAmpColCot	2	7	4	3	0	1	17
C	TetAmpColCip	1	0	1	0	0	0	2
D	TetAmpColCotCip	2	0	0	0	0	0	2
	TOTAL	5	9	6	5	1	1	27

KEYS: Ampicilin (AMP) (10 µg), Cotrimoxazole (Cot) (30 µg), Colistin (COL) (10 µg), Ciprofloxacin (CIP) (5 µg) and Tetracyclin (TET) (30 µg). S. T (*S. typhi*), S. TP (*S. typhimurium*), S. CH (*S. choleraesuis*), S.V (*S. vichow*), S. E (*S. enteritidis*), S. A (*S. arizonae*).

It was observed from this study that, 17 different *Salmonella* isolates showed the same resistance profile, TetAmpCol, which were of 9 different plasmid profiles ranging from 3.2 kb to 30.2 kb size. For instance, *S. typhimurium* 04 and 05 which were isolated from food with the same resistance profile TetAmpColCot, but harboured different plasmid sizes of 5.0 kb and 30.2 kb respectively. Also *S. Typhi* 02 and 04 isolated from faecal sample with the same resistance profile, TetAmpCol, possessed different plasmid profiles in which *S. typhi* 02 possess 2 different plasmid sizes of 17.5 kb and 30.2 kb; and *S. typhi* 04 had 3 different plasmid sizes of 21.5 kb, 25 kb and 30.2 kb.

It was also observed that some *Salmonella* isolates of the same species from diverse sources possessed different plasmid profiles. For instance, *S. choleraesuis* 01 (blood) and *S. choleraesuis* 04 (faeces) possessed the same resistance profile, TetAmpCol that differed in plasmid profiles in the sense that *S. choleraesuis* 01 (blood) had 2 plasmids (3.2 kb and 7.2 kb) while *S. choleraesuis* 04 (faeces) possessed only one plasmid of 30.2 kb (**Table 4**).

4. Discussion

Salmonellosis caused by strains of *Salmonella* (both typhoidal and non-typhoidal) is a serious infection that poses problems for treatment due to its MDR nature

Table 4. Antimicrobial resistance and Plasmid profiles of *Salmonella* isolates from different sources.

<i>Salmonella</i> isolates	Sources	Resistance pattern	Plasmid profile	Approx. plasmid size	No of plasmids isolated
<i>S. typhi</i> 01	Food	TET, AMP, COT, COL	A	25, 30.2	2
<i>S. typhi</i> 03	Faeces	TET, AMP, COT, COL	B	30.2	1
<i>S. enteritidis</i> 01	Food	TET, AMP, COT, COL	A	25, 30.2	2
<i>S. enteritidis</i> 04	Well-water	TET, AMP, COT, COL	C	7.2, 21.5	2
<i>S. enteritidis</i> 03	Faeces	TET, AMP, COT, COL	B	30.2	1
<i>S. typhimurium</i> 04	Food	TET, AMP, COT, COL	D	25	1
<i>S. enteritidis</i> 05	Faeces	TET, AMP, COT, COL	E	5.0	1
<i>S. typhimurium</i> 05	Food	TET, AMP, COT, COL	A	25, 30.2	2
<i>S. typhimurium</i> 06	Food	TET, AMP, COT, COL	F	0	0
<i>S. typhimurium</i> 01	Well-water	TET, AMP, COT, COL	F	0	0
<i>S. typhimurium</i> 02	Well-water	TET, AMP, COT, COL	F	0	0
<i>S. typhimurium</i> 09	Faeces	TET, AMP, COT, COL	G	21.5, 25	2
<i>S. typhimurium</i> 07	Hand-swab	TET, AMP, COT, COL	A	25, 30.2	2
<i>S. choleraesuis</i> 03	Faeces	TET, AMP, COT, COL	B	30.2	1
<i>S. choleraesuis</i> 05	Faeces	TET, AMP, COT, COL	B	30.2	1
<i>S. choleraesuis</i> 02	Blood	TET, AMP, COT, COL	H	7.5	1
<i>S. arizonae</i>	Faeces	TET, AMP, COT, COL	I	15.0	1
<i>S. typhi</i> 05	Food	TET, AMP, COL, CIP	F	0	0
<i>S. enteritidis</i> 02	Faeces	TET, AMP, COL, CIP	J	17.5, 30.2	2
<i>S. typhi</i> 02	Faeces	TET, AMP, COL, COT, CIP	J	17.5, 30.2	2
<i>S. typhi</i> 04	Faeces	TET, AMP, COL, COT, CIP	K	21.2, 25, 30.2	3
<i>S. enteritidis</i> 06	Water-sachet	TET, AMP, COL	L	3.2, 7.2	2
<i>S. typhimurium</i> 03	Water-sachet	TET, AMP, COL	M	5.0, 7.2	2
<i>S. typhimurium</i> 08	Hand-swab	TET, AMP, COL	G	21.5, 25	2
<i>S. choleraesuis</i> 01	Blood	TET, AMP, COL	L	3.2, 7.2	2
<i>S. choleraesuis</i> 04	Faeces	TET, AMP, COL	B	30.2	1
<i>S. virchow</i>	Faeces	TET, AMP, COL	A	25, 30.2	2

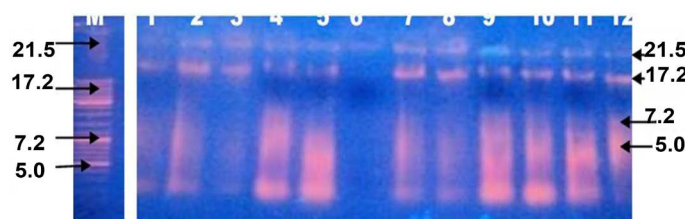


Figure 1. Plasmid profile gel analysis of *Salmonella* isolates; M: Hind III digested λ DNA (DNA Maker (Inqaba Biotech, South Africa) + 10 kb DNA markers); Lane 1: *Salmonella typhi*-01 (food); Lane 2: *Salmonella enteritidis*-01 (food); Lane 3: *Salmonella enteritidis*-02 (faeces); Lane 4: *Salmonella enteritidis*-03 (faeces); Lane 5: *Salmonella enteritidis*-04 (food); Lane 6: *Salmonella enteritidis*-05 (well-water); Lane 7: *Salmonella typhi*-01 (food); Lane 8: *Salmonella enteritidis*-06 (water-sachet); Lane 9: *Salmonella typhi*-02 (faeces); Lane 10: *Salmonella typhi*-03 (faeces); Lane 11: *Salmonella typhi*-04 (faeces); Lane 12: *Salmonella enteritidis*-01 (food); Lane 13: *Salmonella enteritidis*-02 (faeces).

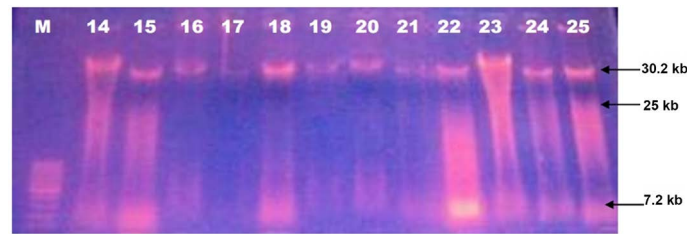


Figure 2. Plasmid profile gel analysis of *Salmonella* isolates; M: Hind III digested I DNA (DNA Maker (Inqaba Biotech, South Africa) + 10 kb DNA markers); Lane 14: *Salmonella typhi*-04 (faeces); Lane 15: *Salmonella typhimurium*-03 (water-sachet); Lane 16: *Salmonella typhimurium*-04 (food); Lane 17: *Salmonella typhimurium*-05 (food); Lane 18: *Salmonella typhimurium*-05 (food); Lane 19: *Salmonella typhimurium*-07 (hand swab); Lane 20: *Salmonella typhimurium*-08 (hand swab); Lane 21: *Salmonella typhimurium*-09 (faeces); Lane 22: *Salmonella arizonae* (faeces); Lane 23: *Salmonella choleraesuis* (blood); Lane 24: *Salmonella choleraesuis* (blood); Lane 25: *Salmonella choleraesuis* (faeces).

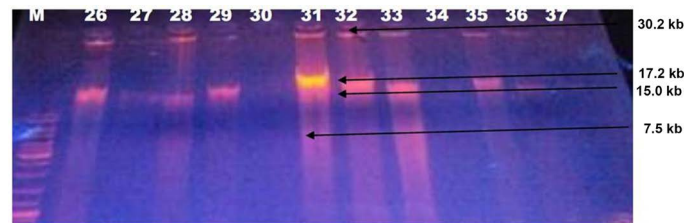


Figure 3. Plasmid profile gel analysis of *Salmonella* isolates; M: Hind III digested I DNA (DNA Maker (Inqaba Biotech, South Africa) + 10 kb DNA markers); Lane 26: *Salmonella choleraesuis* (faeces); Lane 27: *Salmonella choleraesuis* (faeces); Lane 28: *Salmonella virchow* (faeces); Lane 29: *Salmonella virchow* (faeces); Lane 30: *Salmonella enteritidis*-05 (well water); Lane 31: *Salmonella typhimurium*-07 (hand swab); Lane 32: *Salmonella typhimurium*-08 (hand swab); Lane 33: *Salmonella choleraesuis* (blood); Lane 34: *Salmonella typhimurium*-09 (faeces); Lane 35: *Salmonella typhimurium*-03 (water-sachet); Lane 36: *Salmonella enteritidis*-06 (water-sachet); Lane 37: *Salmonella typhi*-04 (faeces).

in many parts of the world. Drug resistance is a global crisis affecting human health, livestock, and the economy [36] with significant adverse impact on clinical outcomes and high cost of treatment [7].

In this study, twenty-seven (27) *Salmonella* strains which included 11 (15.1%) from faecal sample, 7 (9.6%) food, 3 (4.1%) well-water and 2 (2.7%) each from blood, sachet-water and hand-swab samples were identified. The isolation of *Salmonella* strains from faecal and food samples indicates that the pathogen is prevalent and endemic in our locality. Food and water remain the major vehicles of transmission of Salmonellosis [37]. Similar observations were recorded in Ado-Ekiti, Southwest Nigeria [37]. Owoseni and Onilude [38] also isolated different enteric bacteria pathogens including *Salmonella enterica* from food samples in four different West African countries (Nigeria, Togo, Ghana and Benin Republic). In this study, nine (33.3%) *S. typhimurium*, the most prevalence se-

rotype was isolated from all the sources evaluated. *Salmonella typhimurium* had been documented to be the leading cause of non-typhoidal salmonellosis globally [39]. Other *Salmonella* serovars recorded in this study are *S. enteritidis*, *S. choleraesuis*, *S. arizonae* etc. The implication of this is the continual horizontal spread of non-typhoidal salmonellosis within Lagos metropolis. The present study shared similarity with the previous report by Akinyemi *et al.* [40] where different bacteria genera including *Salmonella* particularly *S. typhimurium* were isolated from vended foods in Lagos. Also, similar observation of contaminated well-water and vended foods were recorded by Umaru *et al.*, [41] in Taraba State Northwest Nigeria. The detection of these strains or other enteric bacterial pathogens most especially in water and food is an indication of poor quality control being maintained by some sachet-water producing companies and food vendors as well as handlers of these products thus posing a potential threat to public health. Therefore, an urgent attention from National Agency for Food and Drugs Administrations and Control (NAFDAC) is needed for quality standardizations check.

Nevertheless, it should be noted that *Salmonella choleraesuis* was isolated from the blood samples of patient diagnosed of typhoid fever. Isolation of invasive non-typhoidal *Salmonella* had been reported in Kenya [42] [43]

High antibiotic resistance rate was found in all the twenty-seven (27) *Salmonella* strains isolated across different sources in this study as most were resistant to 3 or more antibiotics. All the 27 *Salmonella* isolates were resistant to tetracycline ampicillin and colistin and 66.67% of the isolates were resistant to cotrimoxazole. This result was consistent with the study conducted in Abakaliki, Nigeria and that of the study conducted in Ibadan, Southwest, Nigeria where majority of the *Salmonella* isolated from faeces, sachet-water and bore-hole water were MDR; chloramphenicol, tetracyclin, ampicillins, sulfomothazole and trimethoprim [44] [45]. It is pertinent to note that four (4) distinct resistance profiles: A (TetAmpCol), B (TetAmpColCot), C (TetAmpColCip) and D (TetAmpColCotCip), with resistant profile B being the most frequently encountered profile occurring in 17 (62.9%) *Salmonella* isolates. Resistance profiles C and D contained ciprofloxacin resistance phenotypic trait of *Salmonella* isolates particularly *S. typhi* and *S. enteritidis*. It was observed that four *Salmonella* strains were involved in the resistance pattern with *S. typhi* and *S. enteritidis* possessing resistance pattern C, while, two *S. typhi* strains isolated from faecal samples possessed resistance pattern D (TetAmpColCotCip). However, some *Salmonella* species have more than one resistance profile, for instance *S. enteritidis* isolated from different sources had 3 resistance profiles A, B and C (Table 4) thus, limiting the discriminating capacity of antibiotic resistance profile as a typing tool for epidemiological purpose. Ammari *et al.* [46] observed that 9 out of the 16 *Salmonella* isolates identified exhibited the same resistance patterns.

Twenty-three (85.2%) out of the 27 *Salmonella* isolates harboured one or more plasmid DNA ranging in size from 3.2 kb - 30.2 kb exhibiting 12 plasmid

profiles.. For instance, profiles A, C, G, J, K, L and M were made up of plasmid sizes (25, 30.2) kb, (7.2, 21.5) kb, (21.5, 25) kb, (17.5, 30.2) kb, (21.2, 25, 30.2) kb, (3.2, 7.2) kb and (5.0, 7.2) kb respectively. However, some profiles were made up of single plasmid size, these include, profiles B, D, E, H and I with 30.2 kb, 25 kb, 5.0 kb, 7.5 kb and 15.0 kb respectively. The largest profile clustered was profile B made up of 6 (22%) *Salmonella* isolates with two plasmids size of 25 kb and 30 kb. It is interesting to note that, 17 different *Salmonella* isolates showed the same resistance profile TetAmpCol with 9 different plasmid profiles ranging from 3.2 kb to 30.2 kb in size. For instance, *S. typhimurium* 04 and 05 isolated from food were found to possess the same resistance profile of B (TetAmpColCot), but different plasmid DNA sizes of 5.0 kb and 30.2 kb respectively. Similarly, *S. typhi* 02 and 04 isolated from faecal samples with the same resistance profile of A (TetAmpCol), possessed different plasmid profiles of 17.5 kb and 30.2 kb for *S. typhi* 02 and 21.5 kb, 25 kb and 30.2 kb for *S. typhi* 04. This means that, organisms of the same species isolated from different sources may differ in plasmid profile. Furthermore, *S. choleraesuis* 01 (blood) and *S. choleraesuis* 04 (faeces) possessed the same resistance profile A (TetAmpCol) but differed in plasmid profile in that *S. choleraesuis* 01 from blood sample harboured 2 plasmids of 3.2 kb and 7.2 kb in sizes while *S. choleraesuis* 04 (faeces) possessed only one plasmid of 30.2 kb. This result indicated that, plasmid profile has a good discriminating tool of characterizing *Salmonella* isolates compared to antibiotic resistance profiles for epidemiological purpose. This finding was in agreement with several reports published elsewhere [47] [48] [49] [50] on the better discriminatory ability of plasmid profile than antimicrobial resistance profile. Nevertheless, plasmid profiling has its limitation as it could be lost or gain and could also be inserted into transposon and chromosomes of pathogenic organisms [16] [50], which also limits its capability as a good tool for tracking the actual cause of disease outbreak.

Generally, this study revealed that all the *Salmonella* isolates were MDR and most of which harboured plasmid DNA of different sizes ranging from 3.2 kb - 30.2 kb. This study did not ascertain whether or not plasmids were responsible for resistant in *Salmonella* isolates, as plasmid curing was not performed a limitation that is worthwhile. Plasmid profile exhibited high discriminatory capability compared with antimicrobial resistance profile with low discriminatory capability. It provides important information regarding the dissemination of antimicrobial resistance properties and emphasized the need to exploring molecular typing methods in *Salmonella* epidemiology and surveillance, and to determine whether the resistance possessed by these organisms are plasmid mediated. Further studies on gene sequencing and expression are essential for better infection control.

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