1 *Original Research Article*

2 Antimicrobial Resistant Genes in Non-typhoidal 3 *Salmonella* Species in Food and Stool Samples 4 in Port Harcourt, Rivers State, Nigeria

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14 **ABSTRACT**

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Background: Non-typhoidal *Salmonella* (NTS) species are the major cause of foodborne infection.

Aim: To analyze antimicrobial resistant genes found in non-typhoidal *Salmonella* species in food and stool samples in Port Harcourt using molecular methods.

Study Design: This was a cross-sectional study with simple randomized sampling.

Methodology: In this study, 12 *Salmonella*-positive isolates gotten from food and stool samples in Port Harcourt were subjected to antimicrobial sensitivity testing using disc diffusion method of the commonly prescribed antibiotics in the city. Molecular techniques were employed to determine the presence of TET-W and CTX-M resistant genes, and also phylogenetic relationships.

Results: Out of 12 isolates [7 (3.3%) food samples and 5 (2.4%) stool samples], all (100.0%) were sensitive to ofloxacin, imipenem, levofloxacin, and gentamicin. High sensitivity was also observed to be ciprofloxacin (92.0%) and ceftriaxone (75.0%). However, complete resistance (100.0%) was recorded against tetracycline and cefotaxime. Resistance to cefpodoxime-proxetil (16.7%) and amikacin (8.3%) was exclusive to food isolates. Clinical isolates showed 20.0% resistance compared to 23.0% in food isolates, with none being sensitive to all antibiotics tested. Resistance patterns by antibiotic class indicated 0.0% resistance to fluoroquinolones, 4.2% to aminoglycosides, 25.0% to beta-lactams, and 100.0% to tetracycline. Multiple Antibiotic Resistance (MAR) analysis revealed that 75.0% of isolates had MAR indices >0.2, significantly higher ($p = .0143$) than those with MAR <0.2. Notably, all isolates with MAR indices >0.2 were obtained from Obio/Akpor. PCR assays confirmed the presence of resistance genes, with TET-W detected in all tested stool and food isolates, while CTX-M was found in 25.0% of food isolates but not in stool isolates.

Conclusion: There was a total resistance to tetracycline antibiotics. More antimicrobial resistance was noted in food samples than clinical samples which could have arisen from the use of antibiotics in the agricultural activities or improper handling of food by vendors.

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17 *Keywords: Non-typhoidal Salmonella, antimicrobial resistance, foodborne disease*

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1. INTRODUCTION

Ten percent of the world's population suffers from foodborne infections, which also cause 33 million deaths annually [1]. The high prevalence of *Salmonella enterica* is of great public health concern due to their detection in several foods, including fish, poultry, vegetables, and shellfish [2, 3]. This trend in *Salmonella* infection has been attributed to poor or lack of proper sanitary procedures in the food chain (conservation, transit, processing, and marketing) [3, 4]. Typhi and Paratyphi are common serovars of *S. enterica* that cause severe systemic typhoid fever in humans [5]. However, other species of *Salmonella* cause gastroenteritis which are often referred to as nontyphoidal *Salmonella* (NTS). The serovars belonging to this category include *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis [5, 6].

There are estimated 1.3 billion cases of acute gastroenteritis per year, resulting in 3 million deaths [7]. Africa has a high mortality rate, with 4,100 deaths annually, or 320 deaths per 100,000 people [8]. Variations in serovar fitness, virulence factors, and host vulnerability are linked to the disease's severity and prognosis [9]. The evolution of resistance in these pathogens to antimicrobials poses a worldwide risk to the health of both humans and animals. This resistance development further led to difficulty in clinical management of the patients infected with antibiotic-resistant bacteria with increased likelihood of these resistant diseases spreading [10, 11]. This raises serious alarm as a significant portion of the antibiotic-resistant *Salmonella* has been obtained from eating tainted food that originated from animals, putting human health at risk and driving up medical expenses [12, 13]. Antimicrobial-resistant infections are expected to cause 10 million fatalities globally by 2050, according to some experts [14].

The adoption of antibiotics in animal husbandry in several regions in the world has further increased the chances of the spread of resistant strains of infectious agents. *Salmonella* species which develop resistance to extended-spectrum cephalosporins have been discovered from chickens. The consumption of these poultry products could potentially cause resistant infection [15, 16]. Resistant *Salmonella* infection has been connected to higher fatality and poorer clinical outcomes [17]. Based on the laws and initiatives put in place to combat illness, different nations have different reporting prevalence rates of salmonellosis. Despite the growing number of reports of non-typhoidal *Salmonella*, a particularly invasive, multidrug-resistant strain in Sub-Saharan Africa, surveillance, documentation, and reporting of salmonellosis are frequently inadequate and dispersed over the African region [4,18,19]. Developing effective control methods requires a thorough understanding of the molecular epidemiology and antibiotic resistance profiles of *Salmonella* isolates, particularly in nations like Nigeria where the disease is highly prevalent [18,19].

Determining the antimicrobial profile of this disease is crucial in order to identify the most effective medications for treating salmonellosis, which is a disease that affects people of all demographics. Currently, there is paucity of data on this NTS in Nigeria. As a follow up to a previous demographic study on the prevalence of NTS in food and clinical samples [20], this study evaluated the antimicrobial profile of non-typhoidal *Salmonella* as well as evaluating the antimicrobial resistance genes of this pathogen. Laboratories in Nigeria hardly perform susceptibility testing on positive *Salmonella* stool cultures leading to misuse of antibiotics in treatment, therefore, it is justifiable that this study will provide salient data on antibiotic regimen in the treatment of salmonellosis. This research brings to light the prevalence of *Salmonella* in locally made food.

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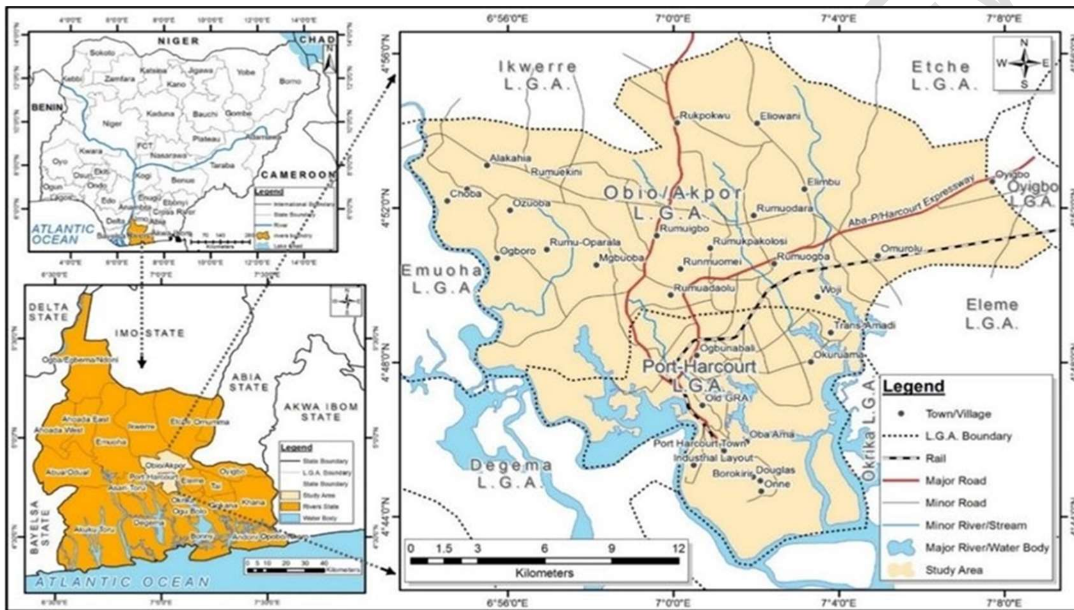
2. MATERIAL AND METHODS

2.1 Study Area

The study was carried out in Port Harcourt of Rivers State located in the oil rich Niger Delta of Nigeria. Port Harcourt is situated within latitude 4°49´27" N and longitude 7°2´1 " E with an estimated population of 3,480,101 and a land area of 369 km^2. The study worked on isolating NTS species from food and clinical samples. Hence, the food samples were obtained from street food vendors within Port Harcourt metropolis. The clinical samples were obtained from the Rivers State University Teaching Hospital (RSUTH) and University of Port Harcourt Teaching Hospital (UPTH), others were collected from medical laboratories and identified individuals presenting with gastroenteritis in the Port Harcourt metropolis.



Map 1. Map of Port Harcourt, Rivers State, Nigeria (Adapted from Ogbozige and Toko, [21]).

2.2 Selection Criteria

Individuals with visible sign and symptoms of gastroenteritis such as vomiting, diarrhea, nausea, bloody stool were included in the study. Individuals on any antibiotics were excluded. Foods included in this study were freshly dished. Waste foods were excluded from the study.

2.3 Sample Collection

From December 2022 to November 2023, a cross-sectional approach was utilized to collect stool samples from suspected individuals with gastroenteritis were collected using a vial. Subjects were given clear guidance on how to avoid contamination. The clinical samples were either processed directly or sent to the medical microbiology laboratory of Rivers State

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University where processing took place immediately. Clinical samples that were not immediately cultured were stored in the freezer at -4°C prior to experiments.

2.4 Sample size

The study used a prevalence of 16.3% determined by Akinyemi et al. [22]. Using the Cochran's equation of sample size = $\frac{Z.Z.p.q}{e.e}$, the appropriate sample size given the specified combination of precision, confidence and variability was 209.6. Hence, the sample size adopted for both clinical samples and food samples was 210 each.

2.5 Bacterial Isolation

Stool specimens were prepared in Selenite F Broth and incubated at 37°C for 18 hours. The overnight culture was sub-cultured unto *Salmonella-Shigella* Agar (SSA) and bismuth sulfite agar (BSA) and incubated for 18 hours at 37°C. For the food samples, a 1:10 dilution of each food sample was made by weighing and homogenizing 10 g of the food sample and dissolving it in 90 mL of peptone water. 1 mL of the dissolved sample was transferred aseptically into 10 mL of sterile Selenite F Broth and incubated at 37°C for 24 hours. The overnight culture was sub-cultured unto *Salmonella-Shigella* Agar (SSA) and BSA and incubated for 18 hours at 37°C. In all, twelve isolates were recovered (5 out of 210 stool samples and 7 out of 210 food samples). The isolates recovered were subjected to biochemical tests (triple sugar iron test, Urease and Indole test) and were kept in nutrient agar slants in an incubator for further testing.

2.6 Antimicrobial Susceptibility profiling

Antimicrobial susceptibility test was done using the Kirby-Bauer disk diffusion technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [23]. Five mL tryptic soya broth (OXOID, England) was inoculated with test isolates and incubated at 35°C for 4 hours. Culture of each isolate was compared with 0.5 McFarland turbidity standards. Using swabs, isolates were inoculated on Mueller-Hinton agar, and the inoculated plates were allowed to dry for 30 minutes at room temperature. The isolates were tested for their susceptibility to fourteen (14) antimicrobial agents with the following disc contents; tetracycline (30 µg), amoxicillin/clavulanic acid (30 µg), ciprofloxacin (5 µg), gentamicin (120 µg), ofloxacin (5 µg), ceftriaxone (30 µg), cefpodoxime-proxetil (10 µg), cefuroxime (30 µg), cefotaxime (30µg), ceftazidime (30 µg), imipenem (30 µg), levofloxacin (10 µg), cefepime (30 µg), and amikacin (30 µg). Muller-Hinton agar cultures were coated with antibiotic-impregnated discs, which were then incubated for 20 hours at 37°C. Using the CLSI interpretive chart, the widths of the zones of inhibition were measured to the nearest millimeter and categorized as resistant, moderate, or susceptible [24].

2.7 Determination of Multiple Antibiotic Resistance (MAR) Index

For the determination of the MAR index, the formular MAR = $\frac{a}{b}$ was used, where 'a' represents the number of antibiotics to which the test isolate showed resistance and 'b' represents the total number of antibiotics to which the test isolate was subjected to for susceptibility.

2.8 Molecular Analysis

2.8.1 DNA Extraction

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131 Logarithmic phase cultures (1.5 mL) were harvested by centrifugation at 10,000 rpm for 5
132 minutes. The bacterial DNA was extracted using the HotSHOT DNA Extraction Kit (Qiagen)
133 according to manufacturer's instructions. The extracted DNA was collected and quantified
134 using Nanodrop before storage at -20°C until further use.

135 **2.8.2 Detection Bacteria 16S rRNA and Antimicrobial Resistance Genes**

136 Conventional PCR was performed to amplify the variable region (V4) of the 16S rRNA gene
137 using primers in **Table 1**. 20 µL PCR reaction mixture (4 µL of 5x master mix, 0.4 µL of each
138 10 µM 16S rRNA, TET-W and CTX-M primers (to achieve a final concentration of 0.2 µM),
139 13.2 µL of molecular-grade water, and 2 µL of extracted DNA. PCR amplification was
140 performed using the Applied Biosystems PCR Thermal Cycler 9700 with the following
141 conditions: 5 min at 94°C; 35 cycles of 40 s at 94°C, 40 s at 58°C, and 1 minute 30 s at
142 72°C; 5 minutes at 72°C. This was followed by gel electrophoresis where a 2% agarose gel
143 was prepared by dissolving 0.6g agarose powder in 30 mL 1X Tris-Borate-EDTA (TBE)
144 buffer. Safe Green dye (30 µL) was added for visibility. The gel, with pre-placed wells for
145 PCR products, solidified, and the comb was removed. PCR products (4 µL loaded buffers
146 each) and a marker lane were loaded into the wells. Electrophoresis was conducted, and the
147 gel was visualized under a UV transilluminator. Positive bacterial samples were expected to
148 show a band size around 292 bp following 16S rRNA PCR, 168 bp (TET-W), and 255 bp
149 (CTX-M).

150 **Table 1: List of Primers used for PCR Amplification**

Target	Forward	Reverse
16S rRNA	5'-GTGYCAGCMGCCGCGGTAA-3	5'-GGACTACNVGGGTWTCTAAT-3'
TET-W	5`-GAGAGCCTGCTATATGCCAGC-3	5`-GGGCGTATCCACAATGTTAAC-3`
CTX-M	5`-GTGATACCACTTCACCTC-3`	5`-AGTAAGTGACCAGAATCAG-3`

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152 **Data Analyses**

153 The results were presented as percentages, Mean±SD, photographs and bar charts. The
154 statistical package used was GraphPad Prism. The statistical tools used for the analyses
155 were Chi-square and t-test. The statistical significance was considered at 95% confidence
156 interval.
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158 ## 3. RESULTS
159
160 **3.1 Antimicrobial Susceptibility Patterns of the Isolates**

161 *Salmonella* isolates showed either sensitive, resistant or intermediate susceptibility to test
162 antibiotics as shown in **Table 2**. Out of the 12 isolates obtained 7 (3.3%) were recorded for
163 food samples and 5 (2.4%) stool samples. The highest rate of resistance to tetracycline was
164 12 (100.0%) and cefotaxime 12 (100.0%) were recorded. Five (42.0%) of the isolates were
165 resistant to amoxicillin + clavulanic acid while three (25.0%) were resistant to cefuroxime.
166 Resistance to cefpodoxime-proxetil (16.7%) and Amikacin (8.3%) were associated only with
167 food isolates (two out of seven for cefpodoxime- proxetil and one out of seven for amikacin).
168 Overall, there was a 20% resistance in the clinical isolates compared to 23% resistance
169 among the food isolates. In terms of antibiotic classes, the isolates showed no resistance to
170 the fluoroquinolones (0.0%), carbapenems (0.0%), but the highest resistance 100% against
171 tetracycline (**Fig. 1**).

172 **Table 2. Antimicrobial susceptibility in percentages of *Salmonella* isolates.**

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Antibiotic Class	Antibiotic	N = 12 Sensitive (%)	Intermediate (%)	Resistant (%)
Carbapenems	Imipenem (R ≤ 19, I = 14 – 15, S ≥ 16)	12 (100)	0 (0)	0 (0)
Cephalosporins	Amoxicillin-Clavulanic acid (R ≤ 13, I = 14 – 17, S ≥ 18)	1 (8.3)	6 (50.0)	5 (41.7)
	Cefotaxime (R ≤ 14, I = 15 – 22, S ≥ 23)	0 (0.0)	0 (0.0)	12 (100)
	Cefpodoxime-Proxetil (R ≤ 14, I = 15 – 22, S ≥ 23)	3 (25.0)	7 (58.3)	2 (16.7)
	Ceftazidime (R ≤ 14, I = 15 – 17, S ≥ 18)	1 (8.3)	9 (75.0)	2 (16.7)
	Cefepime (R ≤ 14, I = 15 – 17, S ≥ 18)	8 (66.7)	4 (33.3)	0 (0)
	Cefuroxime (R ≤ 14, I = 15 – 22, S ≥ 23)	5 (41.7)	4 (33.3)	3 (25.0)
	Ceftriaxone (R ≤ 13, I = 14 – 20, S ≥ 21)	9 (75)	3 (25)	0 (0)
Aminoglycosides	Amikacin (R ≤ 14, I = 15 – 16, S ≥ 17)	8 (66.7)	3 (25.0)	1 (8.3)
	Gentamicin (R ≤ 17, I = 18 – 19, S ≥ 20)	12 (100)	0 (0)	0 (0)
Tetracyclines	Tetracycline (R ≤ 14, I = 15 - 18, S ≥ 19)	0 (0)	0 (0)	12 (100)
Fluoroquinolones	Ofloxacin (R ≤ 12, I = 13 – 15, S ≥ 16)	12 (100)	0 (0)	0 (0)
	Ciprofloxacin (R ≤ 15, I = 16 – 20, S ≥ 21)	11 (91.7)	1 (8.3)	0 (0)
	Levofloxacin (R ≤ 13, I = 14 – 16, S ≥ 17)	12 (100)	0 (0)	0 (0)

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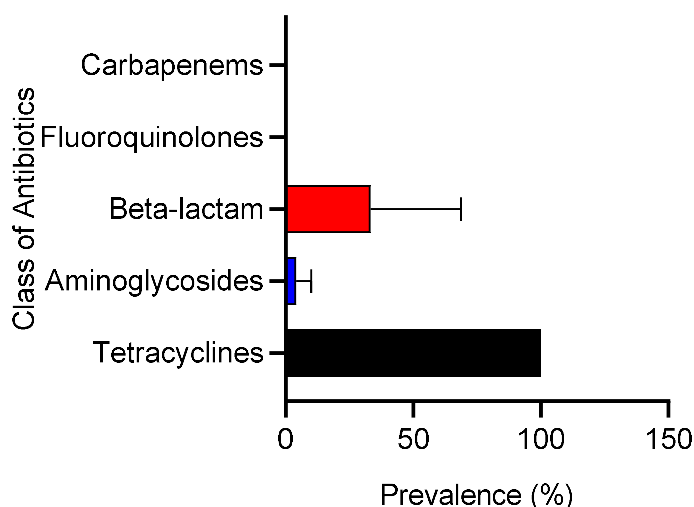

Fig.1. Prevalence of Resistance by Antibiotic Classes

3.2 Multiple Antibiotic Resistance (MAR) Index of the Isolates

The MAR indices analysis of the isolated bacteria revealed that 9 isolates (75%) had MAR indices greater than 0.2 which were significantly higher (p = .0143) than those less than 0.2 3 (25%). The *Salmonella* isolates which had MAR indices greater than 0.2 were mostly *Salmonella* isolates obtained from food samples 6 (66.7%). Notably, all the isolates with MAR indices greater than 0.2 were isolated from only one of the local government areas, Obio/Akpor 9 (100%) (**Table 3**).

Table 3. Distribution of the Isolates based on MAR Index

		Sample Types		Geographical Locations		
MAR Index	**Total of Isolates (%)**	**Food (%)**	**Stool (%)**	**Obio/Akpor Isolates (%)**	**Ikwerre Isolates (%)**	**PHALGA Isolates (%)**
< 0.2	3 (25)	1 (33.3)	2 (66.7)	3 (100)	0 (0)	0 (0)
> 0.2	9 (75)	6 (66.7)	3 (33.3)	9 (100)	0 (0)	0 (0)
p-value	**.0143**	**.0075**	.5271	**.0143**	-	-

Key: MAR= Multiple Antibiotic Resistance

3.3 Confirmation of 16s Ribosomal RNA of Bacterial Isolates

PCR technique successfully detected the V4 region of the 16S rRNA gene specific to *Salmonella* species in the samples. **Figure 2** shows the gel electrophoresis picture of 6 representatives of the 12 isolates, and they could be confirmed to be 292 bp band size which was expected. This validates the specificity of the PCR assay for bacteria species identification.

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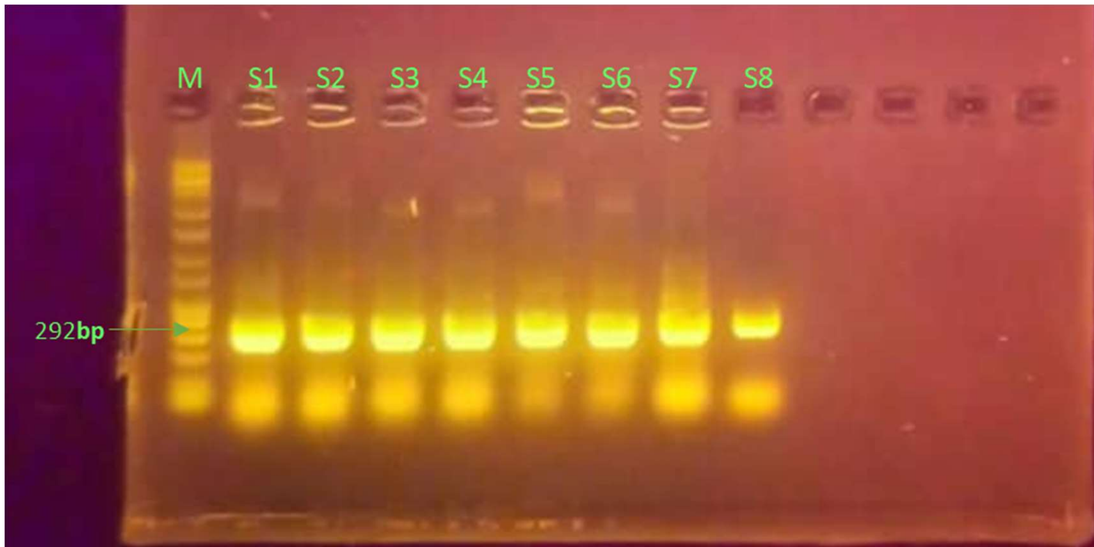

197 **Fig. 2. Bands Generated from 16S rRNA Amplification.** Lane M is 1 kb Ladder, S1-6 are
198 culture samples positive for bacteria while S7-8 are control samples.
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200 ### 3.4 Antimicrobial Resistance Genes Detection

201 PCR assays targeting TET-W and CTX-M resistance genes resulted in specific amplicons of
202 the expected sizes. Gel electrophoresis confirmed the presence of these resistance genes in
203 the bacterial cultures, providing insights into their antibiotic resistance profiles (**Figure 3**).
204 The TET-W primers amplified six of 168 bp amplicons while the CTX-M primers amplified a
205 255 bp product. While all the tested *Salmonella* isolates 2 (100%) from the stool samples
206 used showed TET-W genes, none 0 (0%) showed a CTX-M gene. On the other hand, all the
207 *Salmonella* assessed isolates 4 (100%) from food samples also showed the presence of
208 TET-W but only one 1 (25%) showed CTX-M gene (Table 4).
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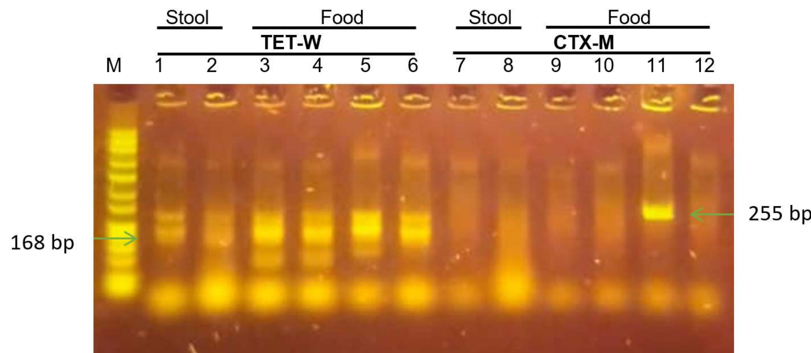

212 **Plate 1. Representative bands that are positive for TET-W and CTX-M antimicrobial**
213 **genes.** Lane M is 1 kb Ladder, S1-6 are culture isolates amplified with TET-W primers while
214 S1b-6b were amplified with CTX-M primers.
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Table 4. Prevalence of Antimicrobial Resistant Genes in NTS species.

Sample	CTX-M n (%)	TET-W n (%)
Stool	0 (0)	2 (100)
Food	1 (25)	4 (100)
Total	1 (100)	6 (100)
p-value	.4945	>0.9999

4. DISCUSSION

The high burden of NTS has led to a huge economic burden which has affected the healthcare setting [25]. This study elucidated the prevalence of antibiotics resistance among NTS species among patients and food samples collected from Port Harcourt in Nigeria. In our study, a disturbing trend noted was the complete resistance (100%) to tetracycline and cefotaxime was observed among all isolates. These antibiotics belong to the tetracyclines and third generation cephalosporins respectively. These results align with previous studies by Adedokun *et al*. [20] who reported high resistance of *Salmonella* serovars isolated from food, animals and human samples in Lagos of Nigeria to cefuroxime and ceftazidime. Similarly, Akinyemi et al. [22] reported cefuroxime and ceftazidime resistance in *Salmonella* species from febrile patients. Outside Nigeria, a 2015 study demonstrated high resistance to tetracycline from retail aquaculture products however was lower than those found in this study [27]. In the same country, raw chicken, of commercial broilers exhibited high resistance to cefotaxime and tetracycline. Put together, these studies have shown extensive evidence of increasing resistance of *Salmonella* to tetracycline due to its extensive use in food-producing animals.

In another study in Lagos, Akinyemi *et al*. [22] reported amoxicillin + clavulanic acid, cefuroxime and ceftazidime resistance in *Salmonella* species from febrile patients. A 2015 study in Shanghai, China by Zhang *et al* [27] reported high resistance of *Salmonella* serovars from retail aquaculture products to tetracycline. Similar antibiotic-resistant patterns were observed in a study by Li *et al.* [28] on *Salmonella* serovars isolated from raw chicken carcasses of commercial broilers and spent hens in Tai'an, China, the *Salmonella* isolates showed high resistance to. The resistance observed against amoxicillin-clavulanic acid (42%) and cefuroxime (25%) suggests a potential reduction in the efficacy of these antibiotics for treating *Salmonella* infections, particularly in clinical settings [29]. The observation of intermediate susceptibility among isolates to ceftazidime (66.6%) and cefpodoxime-proxetil (58.3%) suggests a gradual shift towards resistance, which has been previously reported in various surveillance studies [30].

Besides these resistance phenotypes, the *Salmonella* isolates also demonstrated some sensitivity patterns to the different classes of antibiotics. Notably, all isolates exhibited complete sensitivity to ofloxacin, imipenem, levofloxacin, and gentamicin, indicating the continued efficacy of fluoroquinolones and carbapenems against *Salmonella* spp. This finding is consistent with previous studies that have reported low resistance rates of *Salmonella* to fluoroquinolones [31]. Additionally, high sensitivity to ciprofloxacin (92.0%) and ceftriaxone (75.0%) suggests that these antibiotics remain viable treatment options in Port Harcourt, as corroborated by recent reports in the literature [31].

In terms of resistance to antibiotic classes, majority of the isolates were resistant to the tetracycline, followed by β-lactam antibiotics then aminoglycoside which is corroborated by the report of Adedokun *et al.* [26] but contrasts that of Pławińska-Czarnak *et al.* [32] who reported a higher resistance to aminoglycoside than β-lactams in *Salmonella* isolated from

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raw meat in Poland. Although increasing resistance of *Salmonella* to quinolones have been reported, in this study majority of the isolates were susceptible to ofloxacin, gentamicin and ciprofloxacin. This difference in susceptibility might be due to the source of isolates since clinical isolates are known to be more resistant to quinolones and most of the isolates in this study were from non-clinical samples.

The Multiple Antibiotic Resistance (MAR) index analysis revealed that 75% of isolates had MAR indices greater than 0.2, indicating exposure to high-risk antibiotic environments [24]. The significantly higher prevalence ($p = 0.0143$) of MAR indices >0.2 among food isolates (66.7%) compared to clinical isolates aligns with previous studies suggesting that food sources may act as reservoirs for resistant *Salmonella* strains [34]. Interestingly, all isolates with MAR indices greater than 0.2 were obtained from the Obio/Akpor local government area, suggesting localized antibiotic selection pressures that may contribute to resistance spread. A recent study corroborated this trend through the demonstration that Obio/Akpor has high prevalence of resistance pathogens [35].

The molecular analysis via PCR further confirmed the presence of resistance genes, providing genetic evidence for the observed phenotypic resistance. The detection of TET-W genes in all stool isolates (100%) and all tested food isolates (100%) strongly supports tetracycline resistance, in agreement with earlier reports identifying TET-W genes in foodborne *Salmonella* [34]. The detection of CTX-M genes in only 25% of food isolates and none from stool samples suggests that extended-spectrum beta-lactamase (ESBL) production is still emerging among these isolates, though continued surveillance is necessary.

These findings have opened the avenue for stringent antibiotic stewardship and enhanced surveillance of antimicrobial resistance in both food and clinical settings. This present study had some important limitations. First, the sampling was carried out over a relatively short period of time. Again, the study could have explored the presence of resistance genes on the plasmid. This is because several studies have shown that plasmids harbor resistance genes as a form of adaptational measure to harsh conditions [36, 37, 38].

5. CONCLUSION

More NTS strains were observed in food samples (3.3%) than stool samples (2.4%). The NTS strains demonstrated 100% resistance to tetracycline and cefotaxime. This phenotypic attribute was confirmed by the molecular detection of TET-W gene in all the bacterial isolates. Also, bacteria exhibiting MAR indices of >0.2 was significantly higher than those having MAR indices of <0.2 which indicates that majority of the bacteria have had prior transient exposure to antibiotics.

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305 **CONSENT**
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307 All authors declare that a written informed consent was obtained from the patient. A copy of
308 the written consent is available for review by the Editorial office of this journal.
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311 **ETHICAL APPROVAL**
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313 All authors hereby declare that all experiments have been examined and approved by the
314 Rivers State Health Research Ethical Committee with REC number RSUTH/REC/202319 in
315 accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.
316
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ABBREVIATIONS

NTS - Non-typhoidal *Salmonella*
RSUTH - Rivers State University Teaching Hospital
UPTH - University of Port Harcourt Teaching Hospital
SSA - *Salmonella Shigella* agar
BSA - Bismuth sulfite agar

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