Original Research Article

Prevalence and Antimicrobial Susceptibility of *Salmonella spp* isolated from ready-to-eat foods and food handlers in Port Harcourt Metropolis, Nigeria

ABSTRACT

Aim: The aim of this study was to assess the prevalence and antimicrobial susceptibility of *Salmonella spp* isolated from ready-to-eat foods and food handlers in Port Harcourt Metropolis, Nigeria.

Study design: A cross-sectional study

Place and Duration of Study: This study was conducted in selected in selected places in Port Harcourt, between November 2019 and June 2021.

Methodology: A total of 350 food specimens and 230 food handlers were collected and the social demographic information were collected using a questionnaire survey. The samples were analysed for contamination with *Salmonella* species using conventional. *Salmonella* species were isolated from samples using *Salmonella-Shigella* agar (SSA), Xylose-lysine desoxycholate agar (XLD), MacConkey agar (MA), Blood agar (BA) after pre-enrichment and enrichment method has been done using peptone water broth and selenite cysteine. *Salmonella* Chromogenic medium (SCM) was also used to ~~confirmed~~confirm the isolate. Antibiotic susceptibility patterns of the *Salmonella* isolates were determined using Kirby Bauer disk diffusion method. The following street vended food were analysed: White rice/stew, Jollof rice, Rice/beans stew, Porridge beans, Beans/stew, Moi Moi, Africa salad and Roasted plantain.

Results: The prevalence of *Salmonella* species in the street vended foods was 8.2% and 4.8% among the food handlers. However, there was no statistically significant difference in the proportion of *Salmonella* growth observed in street vended food and food handlers (P= 0.2900). The isolates from street vended foods and handlers were susceptible to Sulfamethioxazole/Trimetoprin and Meroprene and resistant to Amoxiclave, Ceftiaxone, Ampicillin, Cefotaxine, Ceftazidine, Levofloxacin and Tetracycline.

Conclusion: *Salmonella* isolates identified from the samples and their handlers showed susceptibility to ciprofloxacin, erythromycin, gentamicin, meropenem, sulfamethroxazole/Trimethoprim with more isolates being sensitive to meropenem. However, they were resistant to amoxiclav, ceftazidine, cefotaxime, levofloxacin and ceftriaxone.

Keywords: Prevalence, Salmonella spp, foods, food handlers, Port Harcourt Metropolis, Nigeria

1. INTRODUCTION

Foodborne illness occurs after consumption of contaminated foods containing microorganisms and their toxin [1]. *Salmonella* is recognized as one of the most common causes of food borne infection worldwide, resulting in millions of infections and significant human death annually [2,3]. Some street foods may be considered as carriers of *Salmonella* species and represents a significant share of the attributed sources of Salmonellosis in

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humans. The widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practice used in the food industries have been a significant problem in public health [4].
Salmonella infections in food and food handlers are seen as a major public health problem [5]. *Salmonella* species are responsible for an estimated 93.8 million cases of food borne diseases in humans and an average of 155,000 deaths annually worldwide [6,7].
Most infections are due to ingestion of food contaminated by animal and human faeces. A food handler is anyone, through their work activities has a direct contact with food during any of its phases until it reaches the final consumer. *Salmonella* serotypes can be divided into two main groups typhoidal and nontyphoidal [8]. Nontyphoidal serotypes are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and are zoonotic, meaning they can be transferred from animal to humans. Typhoidal serotypes include *Salmonella* typhi and *Salmonella* paratyphi A, which are adapted to humans and do not occur in other animals [9].

Infection with nontyphoidal serotypes of *Salmonella* generally results in food poisoning and usually occurs when a person ingests foods that contain high concentration of the bacteria 10^5/cfu/g. Infants and young children are much more susceptible to infection, easily achieved by ingesting a small number of bacteria. In infants, infection through inhalation of bacteria-laden dust is possible. In developed countries, nontyphoidal serotypes present mostly as gastrointestinal disease, in sub-Saharan Africa, these serotypes can create a major problem in bloodstream infections, and are the most commonly isolated bacteria from the blood of those presenting with fever [10]. Bloodstream infections caused by nontyphoidal *Salmonella*e in Africa were reported in 2012 to have a case fatality rate of 20 – 25%. Most cases of invasive nontyphoidal *Salmonella* infection (iNTS) are caused by *Salmonella enterica* typhimurium or *Salmonella enterica* enteritidis [11]. A new form of *Salmonella* typhimurium (ST313) emerged in the southeast of the African continent 75 years ago, followed by a second wave which came out of central Africa 18 years later.This second wave of iNTS possibly originated in the Congo Basin, and early in the event picked up a gene that made it resistant to the antibiotic chloramphenicol. This created the need to use expensive antimicrobial drugs in areas of Africa that were very poor, making treatment difficult [1]. The increased prevalence of iNTS in sub-Saharan Africa compared to other regions is thought to be due to the large proportion of the African population with some degree of immune suppression or impairment due to the burden of HIV, malaria, and malnutrition, especially in children. The genetic makeup of iNTS is evolving into a more typhoid-like bacterium, able to efficiently spread around the human body. Symptoms are reported to be diverse, including fever, hepatosplenomegaly, and respiratory symptoms, often with an absence of gastrointestinal symptoms [12].

Typhoid fever is caused by *Salmonella* serotypes which are strictly adapted to humans or higher primates, these include *Salmonella* typhi, Paratyphi A, Paratyphi B, and Paratyphi C [8]. In the systemic form of the disease, *Salmonellae* pass through the lymphatic system of the intestine into the blood of the patients (typhoid form) and are carried to various organs (liver, spleen, kidneys) to form secondary foci [12]. Endotoxins first act on the vascular and nervous apparatus, resulting in increased permeability and decreased tone of the vessels, upset of thermal regulation, and vomiting and diarrhoea. In severe forms of the disease, enough liquid and electrolytes are lost to upset the water-salt metabolism, decrease the circulating blood volume and arterial pressure, and cause hypovolemic shock. All food handlers are required to observe proper hygiene and sanitation methods when working with food [12]. The food - handlers also refer to people that directly touch open food as part of their work. [1]. The consumption of contaminated foods may result in illness, also referred to as food-borne disease. Such diseases remain a major public health problem globally, but particularly in developing countries due to difficulties in securing optimal hygienic food

handling practices. An estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food [9]. Reliable statistics on food-borne diseases are not available due to poor or non-existent reporting systems in most developing countries.

Rising drug resistance is caused mainly by use of antimicrobials in humans and other animals, and spread of resistant strains between the two [13]. Considering the level of patronage of street foods in Nigeria; and the close interaction between animals, plants and man, antibiotic resistant organisms may pose dangers to humans through the food chain or zoonotic infection and precipitate a similar pattern of resistance in man [14]. Most cases of salmonellosis in human samples are the consequence of consuming contaminated food mostly prepared by street food vendors [15]. Contaminated street foods are among the important sources for food-borne infection or outbreak than from any other animal or food products [16,17,18]. The aim of this study was to assess the prevalence and antimicrobial susceptibility of *Salmonella spp* isolated from ready-to-eat foods and food handlers in Port Harcourt Metropolis, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in fourteen different locations in Obio Akpor and Port Harcourt Local Government Areas of Rivers State, Nigeria. Both areas have an estimated population of 1,029,578 persons. Obio-Akpor is bounded by, Oyigbo and Eleme to the East, Ikwerre and Etche to the north, and Emohua to the west. Obio-Akpor is located between latitudes 4^{O}45'N and 4^{O}60'N and longitudes 6^{O}50'E and 8^{O}00'E and Port Harcourt LGA is located between latitudes 4^{O}84'N and 4^{O}99N and longitudes 7^{O}01E and 9^{O}21E (Figure 1). It is one of the major centers of economic activities in Rivers State. Obio-Akpor LGA covers an area of 260 km^2 and its population was stated at 464,789 in the 2006 census and was projected at 649,600 by 2019. Port Harcourt LGA covers an area of 198 km^2 and its population was stated at 564,789 for the 2006 census and was projected at 749,600 by 2019.

The major occupation of the people in these areas are farming, trading and White-collar jobs. Its proximity to Aba, the biggest trading and commercial city in Nigeria noted for the high proliferation of local manufacturers of clothing, foot wares and a vast array of both household items and machinery may explain the high level of trading activities in both local government areas. Both LGAs are made up of heterogeneous communities with people from different tribes, culture and religion

2.2 Determination of Sample Size

The Street vended food and handlers sample sizes were determined using the equation as described by Okafor and Ogugua [19]. The prevalence rates of 33.5% [19], and 17.2% (Oghenevo *et al* [20] for the two sample types respectively, were used to determine the sample size using the formular below:

$$N = \frac{PQ}{\left(\frac{E}{Z}\right)^2},$$

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A total sample of 342.7 and 219 respectively were gotten from the calculations.

However, for the purpose of obtaining precise results in the research work, a total of 580 ready to eat food samples and handlers' samples were collected for the study. Three hundred and fifty ready-to-eat food samples sold by road side vendors were collected for analysis. Consequently, 230 specimens (blood, urine and stool) were also collected from food handlers that met the inclusion criteria. The food samples were collected by simple random sampling from selected locations in Port Harcourt metropolis.

2.3 Eligibility Criteria

2.3.1 Inclusion and Exclusion Criteria

2.3.1.1 Inclusion Criteria for Food Samples
The inclusion criteria for food samples include all the food sold by road side vendors.

2.3.1.2 Exclusion Criteria for Food Samples
The exclusion criteria are homemade prepared food; the restaurant and fast-food areas were also excluded.

2.3.1.3 Inclusion Criteria for Handlers Samples
All participants must be food handlers with or without Clinical evidence of *Salmonella* infections. (Diarrhea, fever, stomach discomforts) within the age range of 14years – 55years. Another group of participants in the inclusion criteria are those who experienced diarrhea for the past 3 weeks, and not on any antibiotic therapy.

2.3.1.4 Exclusion Criteria for Handlers Specimen
Handlers on any form of treatment for Salmonellosis. Individuals who met the inclusion criteria but did not give their consent were excluded from the study. Non consenting handlers are also excluded.

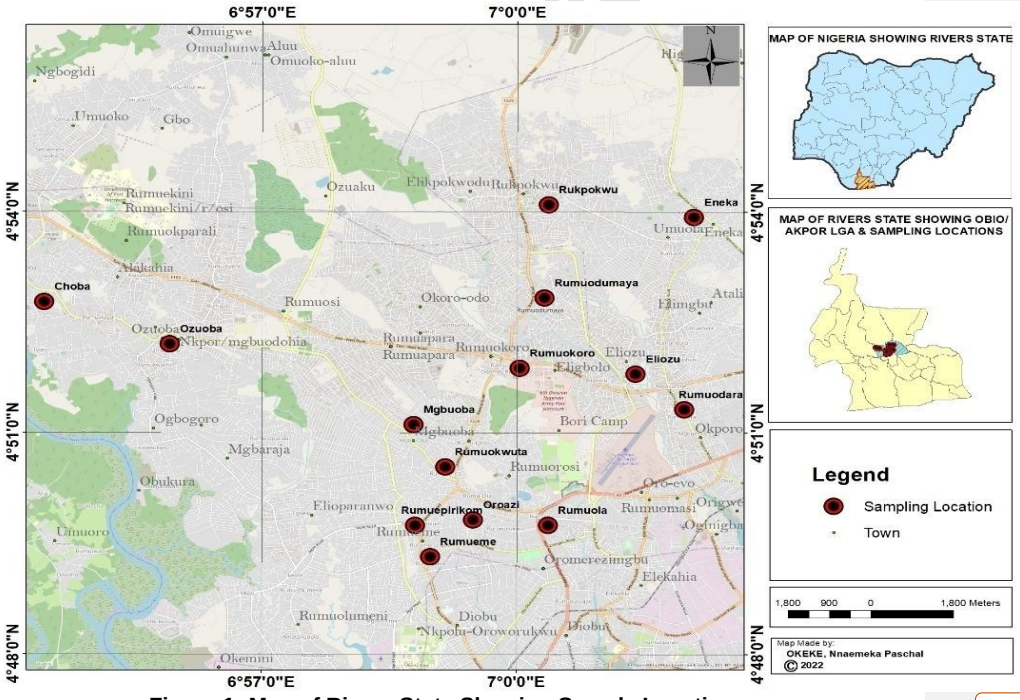

Figure 1: Map of Rivers State Showing Sample Locations.

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2.4 Sample Collection

2.4.1 Ready to eat food samples

White rice and stew, jollof rice, rice and beans with stew, porridge beans, white beans and stew, Abacha (African salad), moi-moi and roasted plantain, were bought from food vendors at different locations in plastic plates sterilized with 70% ethanol and placed in cooler with ice pack. All collected food samples were subjected separately and transferred within 4h~~r~~ in a cooler with ice pack for bacteriological analysis. On getting to the laboratory, the working benches were sterilized with 70% ethanol.

2.4.2 Handlers Samples

Blood, urine and stool were collected from each food handlers that met the inclusion criteria. A questionnaire survey was used to record the socio demographic information. All handlers were instructed on how to collect appropriate specimens.

2.4.2.1 Blood Samples Blood

Samples were collected with a sterile syringe and needle. 5ml~~s~~ of blood sample was collected from the food handlers and inserted the needle through the rubber liner of the bottle cap aseptically and dispensed into the medium bottle containing 50ml~~s~~ of the broth (Trytic soy broth) and transported to the laboratory,

2.4.2.2 Urine Specimen

Urine samples were collected using sterile universal urine bottles with tight fitting lid. All the specimens were taken to the laboratory for analysis without delay.

2.4.2.3 Stool Specimen

A total of 230 watery, semi-formed and formed stool samples were collected from subjects who met the inclusion criteria. A sterile universal stool bottle was properly labeled and given to each subject for production of stool sample. ~~–~~The samples were, packed in a cooler containing ice and transported to the laboratory for analysis.

2.5 Analysis of Specimen

Samples collected were processed and cultured using Pre-enrichment broth (peptone water), enrichment broth (Selenite F. Broth). This method allows stressed or injured *Salmonellae* to recover before exposure to selective enrichment media (*Salmonella-Shigella* agar (SSA), Blood agar (BA), Xylose –lysine desoxycholate agar (XLD) and MacConkey agar (MAC). To isolate *Salmonella* species, the methods described by Cheesebrough [21] were adopted.

2.5.1 Isolation of Bacteria

The *Salmonella* species were isolated according to the methods outlined by the WHO [22].

2.5.2 Bacteriological Examination

2.5.2.1 Ready to eat food samples (Culture using XLD, SSA and MacConkey agar. THC)

The ready to eat cooked foods were homogenously mixed with the help of a sterile spatula and labelled appropriately. Heterotrophic bacterial counts were done to estimate the viable bacteria in the food samples. It is expressed as colony forming units per milligram. Ten- folds dilution procedures were used when performing standard plate count. The viable colonies were counted and reported in cfu/ml [21]. All the agar plates used were prepared according to the manufacturer's instruction.

2.5.2.2 Urine samples (culture using Selenite F, MacConkey, XLD, SSA agar)

Using a sterile wire loop, a loop full of the urine sample was picked and inoculated into selenite F broth and sub cultured in MacConkey agar, XLD (Xylose Lysine Deoxycholate agar) and *Salmonella Shigella* agar. The plates were then incubated at 37^{O}C for 24hrs then the results were recorded accordingly.

2.5.2.3 Blood culture: (Culture using Trytic soy broth, Blood agar, SSA agar)
Using a sterile syringe and needle, 5mls of whole blood from the handlers were dispensed into 50mls of Trytic soy broth. The blood should be mixed with 10 times its volume of broth to reduce and dilute any antibiotic present in the human serum. The mixture was then incubated at 37^{o}C for 24 - 48hrs before subculture into blood agar, and *Salmonella Shigella* agar. The plates were then incubated at 37^{o}C for 24hrs. The sub culturing was done three times before conclusion that there is no growth.

2.5.2.4 Stool culture: (Culture using Selenite F, XLD agar, MacConkey and SSA)
One gram of the purulent, formed or mucoid parts of the stool samples collected were inoculated into 9mls of peptone water and incubate at 37^{O}C for 24hrs. 1ml of the inoculated peptone water was transferred into 9mls of selenite F broth medium and then incubated at 37^{0}C overnight for 18 - 24hrs, loopful of the overnight Selenite F. broth culture indicated by turbidity in the medium was streaked on Xylose lysine deoxycholates Agar (XLD), *Salmonella Shigella* Agar (SSA) and MacConkey agar, and the plates were incubated overnight at 37^{o}C for 24 hours. Typical suspected *Salmonella* colonies on XLD appeared pink-red with a black centre, on *SS*A appeared red with a black center and the production of hydrogen sulphide, on Macconkey agar, produces non-lactose fermenting pale-coloured colonies on Blood agar, colonies are moist and 2 - 3mm in diameter. The Suspected *Salmonella* colonies on the agar plates were purified by sub culturing on nutrient agar and incubated at 37^{o}C for 24 hours for isolation of pure culture and subsequent biochemical confirmation and Genomic analysis.

2.5.3 Characterization and Identification of *Salmonella* Species
The conventional characterization and identification of isolates were done using: colonial appearance, morphological characteristics (Gram staining and Motility) and biochemical reactions. Gram staining was done to examine the smears for the isolates Gram reaction and shape of the cells.

2.5.3.1 Gram Staining and Microscopy
Gram staining and microscopy were carried out as described by Cheesbrough, [23].

2.5.3.2 Growth on Salmonella Chromogenic Medium (SCM)
All isolated suspected to be *Salmonella* were furthered sub-culturing on *Salmonella* Chromogenic medium (OXOID, UK). The medium was prepared following the manufacturer's instructions. In brief, the bacterial cultures were streaked onto freshly prepare sterile plates containing SCM. The organisms were incubated in an incubator at 37 °C for 24 h. After incubation, colonies that showed magenta colouration were confirmed as *Salmonella* species. However, further biochemical tests were carried out for further identification.

2.5.3.3 Biochemical Screening Test of Salmonella Species
The following biochemical tests were carried out as recommended for the biochemical screening of *Salmonella* species; Kligler iron agar (KIA), Urease, Indole, Citrate, Catalase, Voges Proskauer, and methyl red (IMVIC) [4,24]. After identifying *Salmonella* strains biochemically, pure cultures were streaked on nutrient Agar slant and stored at 4^{O}C until needed for antibiotic susceptibility and molecular studies [24].

3.5.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on all the isolates of *Salmonellasp* using the modified Kirby Bauer disk diffusion technique as described in the 2016 by Clinical and Laboratory Standards Institute (CLSI) [25] guideline and interpretative criteria. Bacterial suspensions of the various isolates were prepared in 2mls of normal saline and the turbidities of each adjusted to correspond to 0.5 McFarland's standard. Within 5 - 10minns, with the aid of sterile swab sticks lawns were made from the suspensions on a Mueller Hinton agar (Oxoid, Cambridge UK) plate, allow to stayed for 5mins. Thereafter, antibiotic disks were placed using sterile forceps, equidistant from each other with a maximum of six discs per 90mm plate and each isolate had 2 plates of Muller- Hinton agar. The following antibiotics (Oxoid Cambridge; UK) were tested: Levofloxacillin 30ug, ampicillin 10ug, gentamicin 10–µg, ciprofloxacin 5–µg, ofloxacin 5µg, amoxycillin-clavulanic acid 30–µg, ceftazidime 30-µg, sulfamethoxazole/trimethoprim 25-µg, meropenem 10-µg, cefotaxime 30 ug, ceftriaxone 30–µg, Tetracycline 25–µg and erythromycin 15–µg. Incubation parameters included; ambient air for 16-18hours at 35 – 37°C.Thereafter, the zones of inhibition were measured to the nearest whole millimeter, using a ruler and compared with the zone interpretation chart [26]. The interpretation of the results was based on Clinical and Laboratory Standard Institute CLSI guidelines [25], and interpreted as resistant, intermediate or susceptible.

2.6 Statistical Analysis

The data collected was analyzed with the Statistical Package for Social Sciences (SPSS, V25, IBM, USA). The prevalence and distribution of *Salmonella sp* and antibiotic resistance patterns were presented in frequencies and percentages. All analysis was done at a 95% confidence interval and p-values less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Table 1: Different types of food handlers' samples and the isolated *Salmonella* sp

Sample	No examined	No of Positives
Blood	226 (98.3)	4 (1.7)
Stool	224 (97.4)	6 (2.6)
Urine	229 (98.3)	1 (0.4)

Number in parenthesis = percentages

Table 2: Distribution of *Salmonella* sp. isolated from food samples

Item	*Salmonella* growth (n=28)	Chi-square (p-value)
White rice/stew	5 (17.9)	
Jollof rice	3 (10.7)	

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Rice/beans stew	3 (10.7)	9.56 (0.0852)**
Porridge beans	2 (7.1)	
Beans/stew	4 (14.3)	
Moi Moi	4 (14.3)	
Africa salad	6 (21.4)	
Roasted plantain	1 (3.6)	
Total	**28 (100)**	

***distribution is not statistically significant (p > 0.05; Values in parenthesis = percentages*

Table 3: Distribution of S*almonella* sp in food handlers by demographic information

Parameter	**Total**	***Salmonella* growth**	**Chi-square (p-value)**
Age-groups			
– <40 years	156 (71.2)	8 (72.7)	0.03 (0.8600)
– ≥40 years	74 (33.8)	3 (27.3)	
Gender			
– Female	153 (66.5)	7 (63.6)	0.03 (0.844)
– Male	77 (33.5)	4 (36.4)	
Marital Status			
– Single/Separated/Widowed	100 (43.5)	6 (54.5)	2.48 (0.1147)
– Married	11 130(5 130 (56.5)	5 (44.5)	
Educational Background			
– At least Secondary	193 (83.9)	6 (54.5)	28.76 (<0.0001)*
– At most primary	37 (16.1)	5 (45.5)	

**distribution is statistically significant*

Table 4: Prevalence of *Salmonella* sp in vended food and among food handlers

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Distribution of *Salmonella* sp	Food n = 350 (%)	Food handlers n = 230(%)	Chi-square (p-value)
Yes	28 (8.2)	11 (4.8)	1.11 (0.2900)**
No	322 (92.0)	219 (95.2)	

***difference is not statistically significant (p > 0.05)*

Table 5: Total Heterotrophic Count (THC) of the Street Vended Foods

Item	Average THC x 10^4 (CFU/g)
White rice/stew	3.3 ± 2.1
Jollof rice	3.7 ± 0.9
Rice/beans stew	1.5 ± 0.7
Porridge beans	4.3 ± 1.9
White beans/stew	1.3 ± 1.1
Moi Moi	2.7 ± 1.1
Africa salad	1.8 ± 0.9
Roasted plantain.	2.9 ± 1.9
ANOVA	**0.0001**

All values are presented in Mean ±SD; *ANOVA: Analysis of variance;*
**difference is statistically significant (p < 0.05)*

Table 6: Antibiotic susceptibility pattern of *Salmonella* sp isolated from food handlers

Antibiotics	Sensitive n, (%)	Resistant n, (%)
Ofloxacin (5 µg)	2 (18.2)	9 (81.8)
Amoxiclav (30 µg)	0 (0.0)	11 (100.0)
Ampicillin (10 µg)	1 (9.1)	10 (90.9)
Ceftazidine (30 µg)	1 (9.1)	10 (90.9)
Ciprofloxacin(5 µg)	7 (63.6)	4 (36.4)
Cefotaxime(30 µg)	0 (0.0)	11 (100.0)

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Erythromycin(15 µg)	6 (54.5)	5 (45.5)
Gentamicin(10 µg)	5 (45.5)	6 (54.5)
Meropenem(10 µg)	7 (63.6)	4 (36.4)
Levofloxacin(30 µg)	2 (18.2)	9 (81.8)
Sulfamethroxazole/Trimethoprim(25 µg)	8 (72.7)	3 (27.3)
Tetracycline(25 µg)	2 (18.2)	9 (81.8)
Ceftriaxone(30 µg)	0 (0.0)	11 (100.0)

Numbers in parenthesis show the percentage susceptibility

Table 7: Antibiotic Susceptibility pattern of *Salmonella* sp isolated from Food Samples

Antibiotics	**Sensitive n, (%)**	**Resistant n, (%)**
Ofloxacin (5 µg)	7 (25.0)	21 (75.0)
Amoxiclav (30 µg)	0 (0.0)	28 (100.0)
Ampicillin (10 µg)	3 (10.7)	25 (89.3)
Ceftazidine (30 µg)	2 (7.1)	26 (92.9)
Ciprofloxacin (5 µg)	16 (57.1)	12 (42.9)
Cefotaxime (30 µg)	7 (25.0)	21 (75.0)
Erythromycin (15 µg)	16 (57.1)	12 (42.9)
Gentamicin (10 µg)	16 (57.1)	12 (42.9)
Meropenem (10 µg)	23 (82.1)	5 (17.9)
Levofloxacin (30 µg)	7 (25.0)	21 (75.0)
Sulfamethroxazole/Trimethoprim (25µg)	20 (71.4)	8 (28.6)
Tetracycline (25 µg)	8 (28.6)	20 (71.4)
Ceftriaxone (30 µg)	0 (0.0)	28 (100.0)

Numbers in parenthesis shows the percentage of ssusceptibility

This study showed that the proportion of positive culture of food handlers' samples based on the type of sample were 6 (2.6%) for positive stool sample (Table 1). Moreover, 4 (1.7%)

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were positive blood samples, only 1 (0.4%) urine sample was positive, the stool samples had the highest occurrence of positive *Salmonella* species compared to blood and urine samples. Table 2 shows the distribution of *Salmonella* sp isolated from the different food samples that were collected in this study. Most of the *Salmonella* sp isolated were found in the African salad (21.4%), followed by white rice/stew (17.9 %), white beans/stew (14.3 %) and the least *Salmonella* sp isolated was in the roasted plantain (3.6 %). Chi-square statistic shows no significant difference in the distribution of the *Salmonella* sp isolated from the different food samples ($p = 0.0852$). Table 3 also shows the distribution of *Salmonella* sp growth by demographic information among the food handlers. It was observed that 8 (72.7%) of *Salmonella* sp were isolated in persons <40 years, 7 (63.6%) of the *Salmonella* sp growth was observed in female food handlers, 6 (54.5%) *Salmonella* spwere observed in unmarried persons and 6(54.5%) of the *Salmonella* sp isolated were observed in persons with at least secondary education. The distribution of *Salmonella* sp by demographic data was not statistically significant ($p>0.05$) except in the distribution by educational background, where the distribution of *Salmonella* sp was significantly higher among persons with at least secondary education ($p <0.0001$).

Different studies have reported the prevalence of *Salmonella* in these foods. However, few have evaluated the prevalence of beta-lactamase genes in the isolated *Salmonella* species. Adu-Gyamfi & Nketsia-Tabiri [27] isolated *Salmonella paratyphi* B from street-vended Jollof rice. Similarly, Ossai, [28] evaluated the bacteriological quality and safety of street vended foods and reported the presence of *Salmonella* sp. in the street vended Jollof rice, beans, white rice and stew in Delta State, Nigeria. Therefore, the report of this study on the contamination of street vended Jollof rice with *Salmonella* sp. is consistent with other reports.

The result obtained from the study, revealed that there was 4.8% and 8.2% prevalence of *Salmonella* among food handlers and food specimens analyzed, respectively (Table 4). This result is in agreement with a similar work done by Naik et al. [29], which showed the prevalence of 9% and 7% in food samples and food handlers, respectively. However, this is not consistent with the reports of similar studies that indicated a 10 to 15–% prevalence *Salmonella* sp. in food specimens in tropical region [30]. In addition, prevalence of *Salmonella* sp. in street vended food has been reported among food handlers in similar studies [28,31–32].

The contamination of food samples has been generally linked to poor hygiene among food handlers and residents of a particular area [33]. In Rivers State, a study done by Omorodion et al. [34] found out that 70 – 80% of bacterial food poisoning cases are due to *Salmonella* that originates in poultry, eggs, beef and pork. It has also been reported that the majority of human *Salmonella* infections are caused by strains of only a few serotypes such as *Salmonella typhi* and *Salmonella enteritidis*. Therefore, serotype determination is an important aspect of epidemiological surveillance and disease assessment [35]. Changes in the prevalence of specific serotypes can result from the movements of people, animals, and [35]. In comparison to other bacteria isolated using conventional method, there was a statistically significant difference in the prevalence of *Salmonella* sp. in food handlers and food specimen.

A wide range of pathogens play vital roles in foodborne disease. Most of these have a zoonotic origin and thus can be carrier in healthy food animals from which they spread to an increasing variety of foods of animal origin and are considered as major vehicles of foodborne infections [32]. Among the pathogens, *Salmonella* is considered the most prevalent foodborne pathogen worldwide and has long been recognized as an important zoonotic microorganism of economic significance in animals and humans, predominantly in

the developing countries [33]. Consumption of raw or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors to outbreak of salmonellosis in humans [36].

Determination of antimicrobial susceptibility pattern of *Salmonella* species isolated from ready-to-eat foods and food handlers as shown in Table 5. Isolates from food handlers were analysed for susceptibility to 13 antibiotics and these showed 100% resistance to amoxyclave, cefotaxime and ceftriaxone, 90.9% resistance to ampicillin and ceftaxidime, respectively. In addition, the isolates showed 81.8% resistance to ofloxacin, levofloxacin and tetracycline (Table 6). Similarly, the susceptibility pattern of *Salmonella* isolated from food samples showed 100% resistance to amoxiclav and ceftriaxone, 92.9% to ceftazidine, 89.3% to ampicillin, 75.0% to ofloxacin, cefotaxime and levofloxacin, respectively. There was also 71.4% resistance to tetracycline (Table 7). Antimicrobial susceptibility pattern of *Salmonella* sp. isolated from this study to commonly used antibiotics show different level of susceptibility and resistance. From the study, it was observed that at least 1 in every 2 *Salmonella* isolated were resistant to some of the first- and second-generation antibiotics. The susceptibility pattern of the *Salmonella* sp. isolated from the food handlers and street vended foods showed that 50% of all the *Salmonella* isolates were resistant to amoxiclav, cefotaxime, ceftriaxone with percentage resistance of 100%. The study also revealed that the isolates were 90.0% resistant to ampicillin, ceftazidine, and 81.8% resistant to ofloxacin, levofloxacin and tetracycline, respectively. The study showed that more than 50% of the *Salmonella* sp isolated from food samples were resistant to amoxiclav, ceftriaxone, ceftazidine, ofloxacin, ampicillin, ceftazidine and levofloxacin. The *Salmonella* isolates were commonly resistant to the antibiotics amoxiclav, ceftriaxone and ofloxacin; the frequency of resistance to this antibiotic observed in the current study is similar to findings of other studies carried out in different parts of Nigeria [37].

The isolates resistant to four or more separate classes of antimicrobials were defined as multi-drug resistant. The incidence of resistance (i.e., resistance to two drugs) and multidrug-resistance (i.e., resistance to four or more drugs) of all *Salmonella* strains was observed in the current study. However, Kohinur, et al. [38] reported the isolation of *Salmonella sp* from Vended foods that were sensitive to ciprofloxacin (100%) and ofloxacin (93.33%). The sensitivities of the other antibiotics were as follows: gentamicin (66.67%) and -nitrofuratoin (50%) while cefuroxime, amoxicillin/clavulanic acid, ampicillin, and ceftazidime were resistant to the *Salmonella* species isolated. Quinolone resistance of *Salmonella* sp. is usually associated with point mutations in the quinolone resistance-determining regions (QRDR). The mutations conferring resistance cause amino acid substitutions in the target enzymes of these antibiotics, i.e. gyrase and topoisomerase IV (gyrA, gyrB, parC, parE). Antimicrobial resistance is a significant problem for food safety. Trade globalization and the international movement of food products result the spread of resistant bacteria to consumers all around the world. In addition, horizontal gene transfer can enhance the dissemination of resistant bacteria, which increases the risk that new mechanism of resistance may be transferred via the food chain to the consumer.

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4. CONCLUSION

Salmonella isolates were identified from white rice/stew, jollof rice, rice/beans stew, porridge beans, beans/stew, moi moi, abacha, and roasted plantain) and their handlers. Antibiotics susceptibility pattern of the *Salmonella* isolates showed that they were susceptible to ciprofloxacin, erythromycin, gentamicin, meropenem, sulfamethroxazole/Trimethoprim with more isolates being sensitive to meropenem. However, they were resistant to routinely used prophylactic and chemotherapeutic antibiotics such as amoxiclav, ceftazidine, cefotaxime, levofloxacin and ceftriaxone.

Comment [PM2]: Must be improve and developp

CONSENT

Participants were duly enlightened about the study and structured questionnaire was administered to each participant. Written informed consent were also obtained from all subjects before specimen was collected.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCES

Comment [PM3]: Add DOI for all references

Comment [PM4]:

1. Rodriguez-Rivera, L. D., Moreno Switt, A. I., Degoricija, L., Fang, R., Cummings, C. A., Furtado, M. R., Wiedmann, M., & Den Bakker, H. C. Genomic characterization of *Salmonella Cerro* ST367, an emerging *Salmonella* subtype in cattle in the United States. *British Medical College of Genomics*, 2014; 15(1): 32-9.
2. Zhang, W. H., Lin, X. Y., Xu, L., Gu, X. X., Yang, L., Li, W., Ren, S. Q., Liu, Y. H., Zeng, Z. L., & Jiang, H. X. CTX-M-27 producing *Salmonella enterica* serotypes *typhimurium* and Indiana are prevalent among food-producing animals in China. *Frontiers in Microbiology,* 2016; 7(3): 219 - 26.
3. Zhao, X., Ye, C., Chang, W., & Sun, S. Serotype distribution, antimicrobial resistance, and class 1 integrons profiles of *Salmonella* from animals in slaughterhouses in Shandong Province, China. *Frontiers in Microbiology*, 2017; 8(7): 1049 – 53.
4. Kaniz, F., Mahfuzur, R., Suvomoy, D. & Mehadi, H. M. Comparative analysis of multi-drug resistance pattern of *Salmonella* spp. isolated from chicken faeces and poultry meat in Dhaka city of Banghladash. *Journal of Pharmacy and Biological Sciences,* 2014; 9: 147-9.
5. Akinyemi, K. O., Iwalokun, B. A., Oyefolu, A. O. B. & Fakorede, C. O. Occurrence of extended-spectrum and AmpC β-lactamases in multiple drug resistant salmonella isolates from clinical samples in Lagos, Nigeria. *Infection and Drug Resistance*, 2017; 10: 19–25.
6. Ellaine, S., Robert, M. H., Frederick, J. A., Robert, V.T., Marc-Alain, W. & Sharon, L.R. *Emerging Infectious Disease,* 2011; 17 (1): 1-5.
7. Omer, M. K., Álvarez-Ordoñez, A., Prieto, M., Skjerve, E., Asehun, T., & Alvseike, O. A. A Systematic Review of Bacterial Foodborne Outbreaks Related to Red Meat and Meat Products. *Foodborne Pathogens and Disease,* 2018; 15(10): 598–611.
8. CDC. Center for disease control and prevention. food borne outbreak online database (FOOD). http:// wwwn.cdc.gov/foodborne outbreaks/Default.aspx. 2018.
9. Manyi-Loh, C., Mamphweli, S., Meyer, E. & Okoh, A. Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Blood*, 2018; 23(4): 213 – 9.

10. Liljebjelke, K. A., Hofacre, C. L., White, D. G., Ayers, S., Lee, M. D., & Maurer, J. J. Diversity of Antimicrobial Resistance Phenotypes in *Salmonella* Isolated from Commercial Poultry Farms. *Frontiers in Veterinary Science*, 2017; 4(6): 23 - 43.
11. Sapkota, T. B., Jat, M. L., Aryal, J. P., Jat, R. K., & Khatri-Chhetri, A. Climate change adaptation, greenhouse gas mitigation and economic profitability of conservation agriculture: Some examples from cereal systems of Indo-Gangetic Plains. *Journal of Integrative Agriculture,* 2015; 14(8): 1524–33.
12. de Moraes, M. H., Soto, E. B., González, I. S., Desai, P., Chu, W., Porwollik, S., McClelland, M., & Teplitski, M. Genome-wide comparative functional analyses reveal adaptations of *Salmonella newport* to a plant colonization lifestyle. *Frontiers in Microbiology*, 2018; 9(10): 1–10.
13. Brown, A. D. Identities in Organization Studies. *Organization Studies,* 2019; 40(1): 7–21.
14. Anyanwu, A. L., Fasina, F. O., Ajayi, O. T., Rapu, I. & Fasina, M. M. Antibiotic resistant *Salmonella* and *Escherichia coli* isolated from day-old chicks, Vom, Nigeria. *African Journal of Clinical and Experimental Microbiology,* 2010; 11(1): 129-36.
15. Akond, M. A., Shirin, M., Alan, S., Hassan, S. M. R., Mahbubur Rahman, M.D. & Hoq, M. Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh. *Stamford Journal of Microbiology* 2012; 81: 2074-5346.
16. Braden, C. R. *Salmonella enterica* serotype Enteritidis and eggs: A National Epidemic in the United States. *Clinical Infectious Disease,* 2006; 43: 512-7.
17. Kabir, S. M. L. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns*. International Journal of Environmental Research Public Health,* 2010; 7: 89-114.
18. Habtamu, T. M., Rathore, R., Dhama, K. & Agarwal, R. K. Isolation, identification and polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin*, International Journal of Microbiological Resources,* 2011; *2*: 135-42.
19. Okafor, U.C., Okafor, S. C. & Ogugua, A. J. Occurrence of multidrug-resistant *Salmonella* in cattle carcass and contact surfaces in Kwata slaughterhouse, Awka, Anambra State, Nigeria. *International Journal of Public Health,* 2020; 6(1): 49-55.
20. Oghenevo O, Bassey B, Yhiler N, Francis U, Angela O. Antibiotic Resistance in Extended Spectrum Beta-Lactamases (Esbls) *Salmonella* Species Isolated from Patients with Diarrhoea in Calabar, Nigeria. *Journal of Clinical Infectious Diseases,* 2016; 1: 107 – 16.
21. Cheesbrough, M. District Laboratory Practice in Tropical Countries. In District Laboratory Practice in Tropical Countries. Cambridge University Press. 2010.
22. WHO, Laboratory protocol isolation of *Salmonella* spp. from food and animal faeces.5th ed. WHO global food borne infection network, 2010; 8: 16-30.
23. Cheesbrough, M. District Laboratory Practice in Tropical Countries Part 2 Second Edition.Cambridge university press. 2006.
24. WHO *Salmonella* health topic. Available at: http://www.who.int/topics/*Salmonella*/ en/ index.html. 2015.
25. Clinical and Laboratory Standards institute (CLSI): *Performance standards for antimicrobial susceptibility testing*, Wayne, Pennsylvania, USA, 2006.
26. Muragkar, H.V., Rahman, H, Ashok, K.& Bhattacharyya, D. Isolation, phage typing and antibiogram of Salmonella from man and animals in north Eastern India. *Indian Journal of Medical Research,* 2004; 122: 237-42.
27. Adu-Gyamfi, A. and Nketsia-Tabiri, J. Elimination of bacterial isolates from Jollofrice meal by irradiation and chilled storage. *Journal of Food Safety*, 2008; 28(2): 210–9.
28. Ossai, O. S. Bacteriological quality and safety of street vended foods in Delta State, Nigeria. *Journal of Biology, Agriculture and Healthcare*, 2012; 2(5): 114-9.

29. Naik,V.K., Shakya,S .,& Bhoomika. Isolation and molecular characterization of *Salmonella spp* from street vended food and food handlers collected from different districts of chhattisgarh india. *Journal of animal science,* 2012; 82(6): 557-60.
30. Zhang, C.Z., Ding, X.M., Lin, X.L., Sun, R.Y., Lu, Y.W., Cai, R.M., Webber, M. A., Ding, H.Z., & Jiang, H.X. The Emergence of Chromosomally Located blaCTX-M-55 in *Salmonella* From Foodborne Animals in China. *Frontiers in Microbiology,* 2019; 2(6): 1268 - 73.
31. Arnott, A., Wang, Q., Bachmann, N., Sadsad, R., Biswas, C., Sotomayor, C., Howard, P., Rockett, R., Wiklendt, A., Iredell, J. R. & Sintchenko, V. Multidrug-resistant *Salmonella enterica* sequence type 34, New South Wales, Australia, 2016-2017. *Emerging Infectious Diseases*, 2018; 24(4): 751–3.
32. Cao, Y., Shen, Y., Cheng, L., Zhang, X., Wang, C., Wang, Y., Zhou, X., Chao, G. & Wu, Y. Combination of multilocus sequence typing and pulsed-field gel electrophoresis reveals an association of molecular clonality with the emergence of extensive-drug resistance (XDR) in *Salmonella*. *Microbiological Research*, 2018; 207: 170–6.
33. Leon, I. M., Lawhon, S. D., Norman, K. N., Threadgill, D. S., Ohta, N., Vinasco, J., & Scott, H. M. Serotype diversity and antimicrobial resistance among *Salmonella enterica* isolates from patients at an equine referral hospital. *Applied and Environmental Microbiology*, 2018; 84(13): 221 - 7.
34. Omorodion, Nnenna & Adesewa, Ogunekun. Microbial and physic chemical screening of Ready to eat street food. *Asian Journal of Research in Basic Science*, 2022; 4(1): 24-35.
35. Han, J., David, D.E., Deck, J., Lynne, A.M., Kaldhone, P., Nayak, R., Stefanova, R., & Foley, S. L. Comparison of *Salmonellaenterica* serovar Heidelberg isolates from human patients with those from animal and food sources. *Journal of Clinical Microbiology,* 2011; 49: 1130-3.
36. Dmowski, M., Golębiewski, M., & Kern-Zdanowicza, I. Characteristics of the conjugative transfer system of the IncM plasmid pCTX-M3 and identification of its putative regulators. *Journal of Bacteriology*, 2018; 200(18): 128 – 36.
37. Walther, B., Klein, K. S., Barton, A. K., Semmler, T., Huber, C., Wolf, S. A., Tedin, K., Merle, R., Mitrach, F., Guenther, S., Lübke-Becker, A., & Gehlen, H. Extended-spectrum beta-lactamase (ESBL) producing *Escherichiacoli* and *Acinetobacterbaumannii* among horses entering a veterinary teaching hospital: The contemporary "Trojan Horse." *Procedure Library of ScienceOne*, 2018; 13(1): 321 – 9.
38. Kohinur, B., Tanvir, A. R., Margia, H., Akil, H., Kabirul Hassan, F. M., Shaik, N. H., Nargis, A., Aliza, A., & Uptal, B. Isolation, identification and antibiotic resistance pattern of *Salmonella* spp. from chicken eggs, intestines and environmental samples. *Bangladesh Pharmaceutical Journal,* 2010; 13 (1): 4601-06.
39. Colavecchio, A.; Cadieux, B., Lo, A., Goodridge, L. D. Bacteriophages Contribute to the Spread of Antibiotic Resistance Genes among Foodborne Pathogens of the Enterobacteriaceae Family – A Review. *Frontiers in Microbiology,* 2017; 8(2): 1108–09.