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Prevalence and Antimicrobial Susceptibility Pattern of *Salmonella* among Food and Food Vendors in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Salmonella contamination in ready-to-eat food is seen as a health risk, and improper food processing puts customers at risk. This study therefore was carried out to investigate the prevalence and antimicrobial susceptibility pattern of *Salmonella* isolated from food vendor's hands and work bench in Port Harcourt, Rivers State. Ninety (90) samples were collected for a period of three months from three different location in Port Harcourt with five sampling points in each locations. Samples were analyzed for the presence of *Salmonella* organism using standard microbiological procedure for enumeration and identification. *Salmonella-Shigella* (SSC) counts ranged from $1.9\pm4.9x10^3$cfu/g to $3.8\pm1.4x10^3$cfu/g for choba and Aggrey road respectively in cooked rice, $0.2\pm0.3x10^3$cfu/g to $0.9\pm0.4x10^3$cfu/g for Mile 3 and Aggrey road respectively in Moi Moi, $0.6\pm0.5x10^3$cfu/g to $1.5\pm1.5x10^3$cfu/g for Aggrey road and Mile 3 in Salad, and

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$1.6\pm0.7x10^3$cfu/ml to $2.6\pm1.7x10^3$cfu/ml for Choba and Aggrey road in Egusi soup. Six (6) *Salmonella spp* were isolated in Salad and workbench from Mile 3 and Choba respectively. *Salmonella* showed a decreasing trend of resistance in the order: Ceporex, Gentamycin and Augmentin (100%) > Nalidixic acid (83.3%) > Septrin and Streptomycin (66.7%) > Tarivid, Pefloxacin and Ampicilin (33.3%). The molecular identification of the Six (6) isolates using polymerase chain reaction confirmed 100% *Salmonella spp* isolates. The six (6) *Salmonella* isolates were identified with blaTEM and blaSHV gene 6(100%). Out of the Six (6) isolates, 100% had multidrug resistance index ≥ 0.2 while 0.00% had <0.2 as their Multidrug Resistance Index. Conclusively, this study revealed prevalence *of Salmonella spp* in food and food vendors in Rivers State, posing a serious threat to consumers. Indiscriminate use of antibiotics should be discouraged to reduce the prevalence of resistant strains of *Salmonella*.

Keywords: Prevalence; food vendors; Salmonella spp; antimicrobial susceptibility.

1. INTRODUCTION

According to the World Health Organization (WHO) [1], "foodborne diseases (FBD) are diseases transmitted through contaminated food consumption. Foodborne illnesses include those caused by a microbial pathogen, parasite, chemical contaminant, or bio toxins" [2-9]. "The severity of these diseases varies from asymptomatic and mild to life-threatening, in which case life-long treatments are required. In industrialized countries, it is estimated that more than 10% of the population could suffer from a disease associated with contaminated food consumption" [10-15]. "One of the agents triggering foodborne disease is *Salmonella* spp., which causes salmonellosis disease with a high morbidity and mortality rate in industrialized and developing countries. *Salmonella species* are one of the most common causes of food and water-borne gastroenteritis in humans, which remains an important health problem Worldwide" [16-23]. According to World Health Organization (WHO) estimates, "there are about 16 million new cases and 600,000 deaths from typhoid fever each year worldwide" [1].

"Food poisoning due to pathogens is a major issue of public health concern worldwide with countries expending much resources to overcome it. Bacterial food infections are a source of worry for developed and developing countries" [24]. "In Europe, *Salmonella* and *Campylobacter* are the most important causes of foodborne illness. The European Centre for Disease Prevention and Control, asserts that aside from campylobacteriosis which had 246,571 reported cases, *Salmonella* is responsible for the highest number of human infections causing illnesses in 91,857 people in the EU in 2018. A foodborne outbreak is defined as an "incident during which at least two people contract the same illness from the same contaminated food or drink" [25-33]. There were 5146 reported foodborne outbreaks in 2018 from the EU Member States resulting in illnesses to 48,365 people. *Salmonella* alone accounted for 33% of these outbreaks" [34].

"Salmonellosis is linked to the consumption of *Salmonella*-contaminated food products mostly from poultry, pork and egg products. Poor hand washing and contact with infected pets are some of the contamination routes. When infective doses are ingested, the pathogen causes sickness by colonizing the intestinal tract. The *Salmonella* outbreak in Slovakia, Spain and Poland that resulted in 1581 cases was directly linked to infected eggs. It is increasingly becoming a major concern with the global push towards ready-to-eat food products. This group of products is of greater concern because of the minimal heating they are subjected to. The fact that they can be consumed without high heat treatment further increases the risk" [35]. The aim of the current study was to determine the prevalence and antimicrobial susceptibility pattern of *Salmonella species* among food and food vendors in Port Harcourt, Rivers state.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Port Harcourt in Rivers state. Three sampling location were chosen from the study area; Mile3 in Port Harcourt Local Government Area (4°47'3"N6^{o}58'59.5"E), Choba in Obio/Akpor Local Government Area (4°54'17.4"N6°54'14.1"E) and Aggrey Road in Port Harcourt local government area of Rivers State (4°45'42.3"N7°01'19.3E), with five (5) sampling points in each location. These three (3) locations were selected based on the dense population of people living in these areas.

2.2 Sample Size Determination

The sample size was calculated by using single population proportion formula.

$$N=\frac{Z^2P\ (1-P)}{d^2}$$

Where N is the sample size; Z is the reliability coefficient (confidence level) which is 95% =1.96, p is the anticipated population proportion (Expected prevalence), d is the precision (in proportion of one of 5%, d=0.05

By using the anticipated proportion of 5.9% from a study in South Africa.

$$N=\frac{(1.96)^2 \times 0.059(1-0.059)}{(0.05)2}$$
$$=\frac{3.84 \times 0.059 \times 0.941}{0.0025}=85.28$$

Therefore the final sample size for this study was 85.28

2.3 Sampling Technique

Simple random sampling technique was used to select the 90 samples from food vendors. From each food vendors, four food samples, one vendors' hands and workbench were selected by simple random sampling technique.

2.4 Sample Collection, and Processing

A total of 90 samples (Food, Food vendor's hands and vendors workbench samples) from food vendors were collected from the three different sampling locations (Mile3, Choba and Aggrey Road) and Five (5) sampling points from each location in Rivers state, Nigeria. The food samples were put in sterile sample bottle and labelled properly. Sterile cotton swabs soaked in sterile normal saline were used to collect samples from the vendor's hands and workbenches. All samples collected were transported aseptically to the Laboratory of Department of Microbiology, Rivers State University laboratory for bacteriological analysis within 2hours of collection.

2.5 Bacteriological Analysis of Samples

Preparation of the samples was done by weighing 10g each of the food (Cooked rice, Moi moi, Salad and Egusi soup) sample and homogenizing in 90ml of the diluent (Selenite F broth used as an enrichment medium for the isolation of *Salmonella species* from food) to give 10^{-1} dilution. Further, serial tenfold dilution was done using the food samples up to 10^{-4} dilutions. Aliquot (0.1ml) of appropriate dilutions were spread plated in duplicates onto *Salmonella-Shigella* Agar medium contained in petri plates. Inoculated plates were incubated at 37^{o}C for 24 hours and used for enumeration of bacteria. Representative discrete colonies were purified by sub-culturing on freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hours to obtain pure cultures.

2.6 Isolation and Identification of *Samonella spp*

Growth of suspected colonies of *Salmonella species* were detected by their characteristic appearance on *Salmonella-Shigella* agar (SSA) showing black-centered colonies.

For further confirmation, typical and suspected colonies of *Salmonella species* were selected and streaked onto the surface of nutrient agar. Pure cultures were picked from the streaked nutrient agar plates and inoculated into Triple Sugar Iron (TSI) agar, citrate agar, hydrogen sulfde (H2S) production, indole production and motility in Sulfide–Indole–Motility (SIM) medium and incubated for 24 h at 37 °C. Culture producing alkaline slant (red color) with acid butt (yellow color) on TSI and H_2S, positive for lysine (purple color), positive for citrate utilization, positive for motility, and negative for tryptophan utilization (indole test) as well as gram negative rod were taken to be *Salmonella species.*

2.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was determined by using disc diffusion method. Identified *Salmonella* colonies from the overnight culture were suspended in nutrient broth and incubated for 4 h at 37 °C. Turbidity of broth culture was checked against 0.5 McFarland standards. A swab is dipped into the bacterial suspension and streaked over the surface of the agar plates and the procedure was repeated several times, rotating the agar plate 60° C each time to ensure even distribution of the inoculum. The plates were left to dry for 3–5 min. The antimicrobial agents used for the test were Tarivid (10µg), Nalidixic acid (30µg), Pefloxacin (10µg),

Gentamycin (10µg), Augmentin (30µg), Ciprofloxacin (5µg), Septrin (30µg), Streptomycin (30µg), Ampicilin (10µg) and Ceporex (10µg). Discs of the antibiotics were aseptically placed onto the surface of the inoculated agar plates using sterile forceps. Each disk was pressed down to ensure full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 ^{o}C to 35°C in an inverted position. After incubation, zones of inhibition were read and the diameters of growth inhibition around the discs were measured. Results were interpreted as sensitive, intermediate or resistant according to CLSI, (2017) guideline.

2.8 Determination of Multiple Antibiotic Resistance (MAR) Index

"Multiple antibiotic resistance is the resistance of *Salmonella* isolate to three or more antibiotics. Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where ''a'' represent the number of antibiotics to which the test isolate depicted resistance and ''b'' represent the total number of antibiotics to which the test isolate has been evaluated for susceptibility" [36].

2.9 Data Processing and Analysis

"Statistical Package for Social Sciences (SPSS) version 25 was used to statistically analyse the data obtained from counts and the measurement of the zones of inhibition. Descriptive statistics was used to summarize all data obtained. Analysis of variance (ANOVA) was carried out to test for significant difference ($p \leq 0.05$) in the bacterial counts from the various locations. Duncan multiple range test was used to separate the means where difference existed" [36].

3. RESULTS AND DISCUSSION

Results of *Salmonella-Shigella* population of cooked rice from various locations sampled are presented in Table 1.

Result of the Total *Salmonella-Shigella* count ranged from $1.9 \pm 4.94 \times 10^3$cfu/g to $3.8+1.4 \times 10^3$cfu/g. The results revealed that Aggrey road had the highest bacterial contamination while Choba had the least bacterial contamination. There was no significant difference ($p>0.05$) in the total *Salmonella-Shigella* count between the sampling locations.

Results of *Salmonella-Shigella* population of Moi Moi from various locations sampled are presented in Table 1. Result of the *Salmonella-Shigella* count ranged from $0.2 \pm 0.28 \times 10^3$cfu/g to $0.9+0.4 \times 10^3$cfu/g. The results of this analysis revealed Aggrey road had the highest bacterial contamination while Mile 3 had the least bacterial contamination. There was no significant difference ($p<0.05$) in the total *Salmonella-Shigella* count between the sampling locations.

Results of *Salmonella-Shigella* population of Salad from various locations sampled are presented in Table 1. Result of the *Salmonella-Shigella* count ranged from $0.6 \pm 0.51 \times 10^3$cfu/g to $1.5+1.5 \times 10^3$cfu/g. The results of this analysis revealed Mile 3 had the highest bacterial contamination while Aggrey road had the least bacterial contamination. There was no significant difference ($p \leq 0.05$) in the total *Salmonella-Shigella* counts between the sampling locations.

Results of *Salmonella-Shigella* population of Egusi soup from various locations sampled are presented in Table 1. Result of the Total *Salmonella-Shigella* count ranged from $1.6 \pm 0.7 \times 10^3$cfu/g to $2.6 \pm 1.7 \times 10^3$cfu/ml. The results of this analysis revealed Aggrey road had the highest bacterial contamination while choba had the least bacterial contamination. There was no significant difference ($p \leq 0.05$) in the total *Salmonella-Shigella* counts between the sampling locations.

Improper washing of hands during food processing can help in transmitting bacterial in ready to eat food.

Table 1. Distribution of *Salmonella-Shigella* population of food from various locations sampled

Location	Cook Rice cfu/g/ml	Moi Moi cfu/g/ml	Salad cfu/g/ml	Egusi soup cfu/g/ml
	SSC $x10^3$	SSC $x10^3$	SSC $x10^3$	SSC $x10^3$
Mile 3	2.82 ± 2.2^a	0.2 ± 0.3^a	1.5 ± 1.5^a	1.8 ± 1.4^a
Choba	1.9 ± 4.9^a	0.3 ± 0.4^a	0.8 ± 0.8^a	1.6 ± 0.7^a
Aggrey road	3.8 ± 1.4^a	0.9 ± 0.4^a	0.6 ± 0.5^a	2.6 ± 1.7^a

*Key: SSC (Salmonella-Shigella count). *Mean with the same superscript along the rows is not significantly different (p≤0.05)**

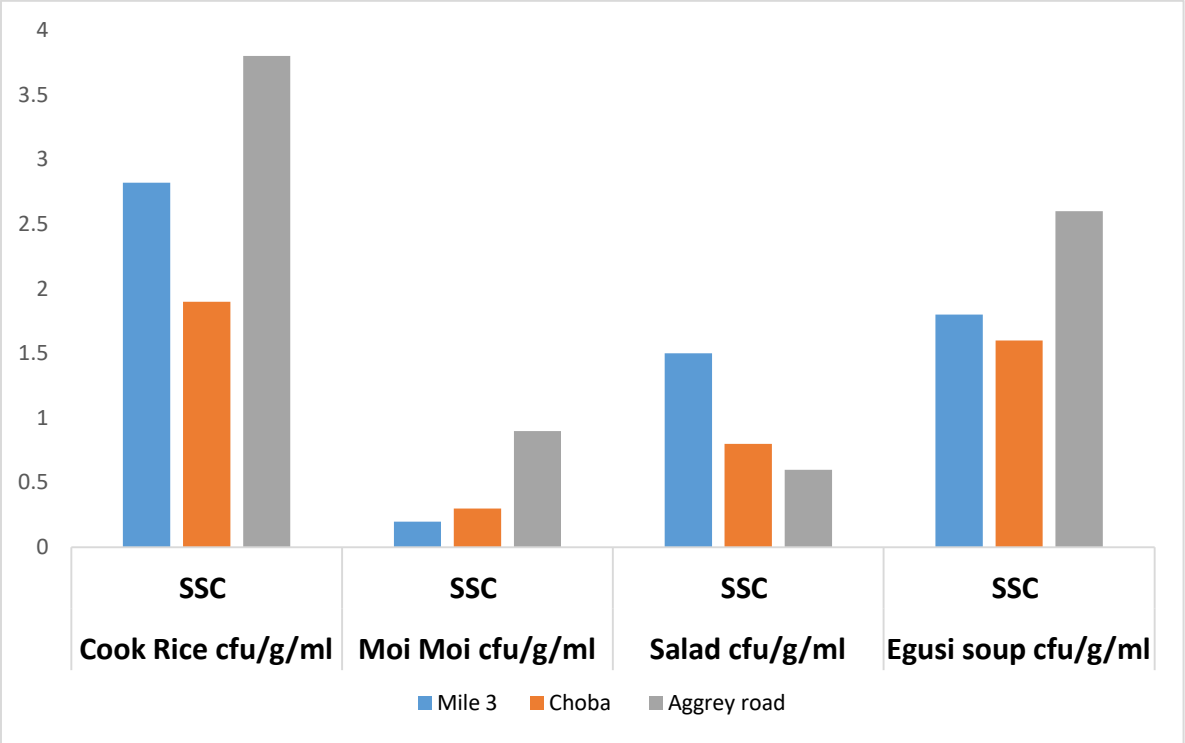

Fig. 1. Distribution of *Salmonella-Shigella* population of food from various locations sampled

The presence of *Salmonella spp* in food samples indicated poor hygiene, poor handling of food and poor sanitary conditions [37] Food poisoning caused by microorganisms is a significant global public health hazard, with countries investing significant resources to combat it. Ready-to-eat meals have a significant potential of contamination and cross-contamination, which can result in disease. The results obtained in the current study on the prevalence and antimicrobial susceptibility pattern of *Salmonella* isolated from food and vendors (vendors hands and work benches) gives us ideas on the gene that confers resistance in *Salmonella spp,* drug of choice for an outbreak of food-borne *Salmonella* infections when food contaminated with this organism is consumed as well as better handling and processing methods of ready to eat foods.

Contamination of Ready to eat food by *Salmonella spp* may be as a result of contaminated water used for food preparation, person to person contact, improper washing of vending utensils [38]. The total *Salmonella-Shigella* count of Aggrey Road were the highest followed by Mile 3 and Choba. Afreen et al., [39] recorded similar values in his research on ready to eat food ($(3.8+1.3x10^3$cfu/g). There was no significant difference ($p>0.05$) in the total *Salmonella-Shigella* count from the three locations sampled. Three species isolated were *Salmonella enterica, Salmonella typhimurium and Salmonella bongori.*

The prevalence of *Salmonella spp* was high in both Mile 3 and Choba samples (33.3%) occurrence, Hence Mile 3 and Choba had the highest and the same percentage occurrence could be due to the environmental condition of the area and the used of contaminated water in the preparation of food and there was no presence of *Salmonella spp* in the samples obtained from Aggrey road. The high percentage of occurrence is could also due to poor sanitary conditions, poor hand hygiene and cross contamination [37].

Results of the antibiotic sensitivity (Table 2) obtained in this study revealed that high percentage of *Salmonella spp* was susceptible to Ciprofloxacin (100%) which is the most effective drug against *Salmonella spp*. This result is in agreement with the work of Jain *et al*., 2019 which showed that *Salmonella* was most sensitive to Ciprofloxacin. The drug interferes with nucleic acid synthesis during DNA replication by inhibiting either DNA gyrase or topoisomerase IV [37]. This susceptibility showed was Ciprofloxacin (100%) followed by Tarivid and Ampicilin (66.7%), then Septrin (33.3%) and Pefloxacin (16.7%). The effect of Ciprofloxacin

Table 2. Susceptibility pattern of *Salmonella spp* isolated from food sample and work bench during the study

Antibiotics	Conc. (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFX	10	2(33.3)	0(0.00)	4(66.7)
NA	30	5(83.3)	1(16.7)	0(0.00)
PEF	10	2(33.3)	3(50.0)	1(16.7)
CN	10	6(100)	0(0.00)	0(0.00)
AU	30	6(100)	0(0.00)	0(0.00)
CPX	10	0(0.00)	1(16.7)	6(100)
SXT	30	4(66.7)	0(0.00)	2(33.3)
S	30	5(83.3)	0(0.00)	0(0.00)
PN	30	2(33.3)	0(0.00)	4(66.7)
CEP	10	6(100)	0(0.00)	0(0.00)

Key: OFX (Tarivid), NA (Nalidixic acid), PEF (Pefloxaxin), CN (Gentamycin), AU (Augmentin), CPX (Ciprofloxacin), SXT (Septrin), S (Streptomycin), PN (Ampicilin), CEP (Ceporex)

and Ampicilin on *Salmonella spp* isolated in this study is not surprising because it is known to be effective against most gram-negative bacteria including *Salmonella.* This drug binds to the cell and inhibits protein synthesis and the acquisition of the aac gene by *Salmonella* (Crump *et al.,* 2008). Similar findings were reported by previous workers where *Salmonella* demonstrated susceptibility to Ciprofloxacin, Ampicilin and Septrin [40]. *Salmonella spp* showed a decreasing trend of resistance in the order: Ceporex, Gentamycin and Augmentin (100%) > Nalidixic acid (83.3%) > Septrin and Streptomycin (66.7%) > Tarivid, Pefloxacin and Ampicilin (33.3%).

This showed that a high percentage of the organism was resistant to Augmentin, Ceporex and Gentamycin (100%) as well as Nalidixic acid and Ciprofloxacin (83.3%). The organism was more resistant to Augmentin (100%). Augmentin belongs to the penicillin class of antibiotic and interferes with the penicillin binding protein (PBP) that leads to the disruption of synthesis of cell wall [41].

The Multiple Antibiotic Resistance Index of *Salmonella spp* isolated from food sample, Fingernails and work benches is shown on Table 3. Out of the Six (6) isolates, 3(50.0%) had multidrug resistance index of 0.5 and 0.7. It is crucial to remember that MAR index values larger than 0.2 signify sources of contamination with a significant risk of contamination and frequent usage of antibiotics [41,42]. MAR index greater than (>) 0.5 indicate existence of isolate from high risk contamination source with frequent use of antibiotics [40]. One Hundred percent (100%) of the *Salmonella* isolated in this study showed multiple resistance to antibiotics, probably due to indiscriminate use of antibiotics arising from infections by *Salmonella spp* acquired from this source [40].

Table 3. MAR indices of *Salmonella spp* (N=6)

MAR index	Number (%)
0.1	0(0.00)
0.2	0(0.00)
0.3	0(0.00)
0.4	0(0.00)
0.5	3(50.0)
0.6	0(0.00)
0.7	3(50.0)

Key: Multiple Antibiotic Resistance (MAR)

4. CONCLUSION AND RECOMMENDATIONS

Salmonella contamination of prepared food is seen as an unsanitary situation that may put customers at danger. Its growing resistance to the majority of antibiotics could seriously endanger public health. This study showed a high bacterial load in food sold in Port Harcourt, Rivers State with differences in microbial load of the three sampling locations with Five (5) sampling point. High prevalence of *Salmonella* observed in this study could be of public health concern when compared with recommended standards for Ready to eat Food. From the study, it can be inferred that the risk of *Salmonella* infection was higher in the samples obtained from Mile 3 than samples from Choba while samples from Aggrey road do not yield any growth of *Salmonella*. In this study, the Vendors hands samples do not yield any growth of *Salmonella spp*. *Salmonella* resistance to several groups of antibiotics which could pose a serious public health problem has been confirmed in this

study. Results showed that Ciprofloxacin, Streptomycin and Gentamicin can be used as drug of choice for treatment of *Salmonella* associated foodborne diseases arising from consumption of Ready to eat food obtained from this source. The presence of bla*TEM* and blaSHV gene in some of the isolates has been found in this study to be a possible factor that confer resistance to antibiotics in *Salmonella spp.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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