Original Research Article

Extended Spectrum Beta-Lactamase (ESBL) Resistant genes in *Salmonella spp* isolated from ready-to-eat foods and food handlers in Port Harcourt Metropolis, Nigeria

ABSTRACT

Aim: To assess the Extended Spectrum Beta-Lactamase (ESBL) resistant genes in *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 and molecular methods. *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 the isolate. Antibiotic susceptibility patterns of the *Salmonella* isolates were determined using Kirby Bauer disk diffusion method. The presence of Extended Spectrum Beta Lactamase genes (CTX-M, SHV, and TEM) in the *Salmonella* isolates were screened using double disc synergy method and also investigated using Polymerase chain reaction (PCR) technique.

Results: Results showed that of the 11 *Salmonella* species isolated from the food handlers, 7(63.6%) harboured extended spectrum beta-lactamase (ESBL) genes while 18 (64.3%) out of 28 *Salmonella* isolated from street vended food sample were positive for ESBL genes. However, there was no significant difference in the proportion of ESBL producers observed in the street vended food and those in food handlers' sample (P=0.4764). PCR result revealed the presence of genes encoding for TEM, SHV and CTX-M.

Conclusion: Street vended foods such as white rice/stew, jollof rice, rice/beans stew, porridge beans, beans/stew, moi moi, abacha, and roasted plantain in Port Harcourt harbour *Salmonella* species that possess the following Extended Spectrum Beta Lactamase (ESBL) genes: CTX-M, SHV and TEM. This should receive particular attention, as the presence of extended spectrum beta-lactamase genes isolated, indicate public health hazard and gives a warning signal for the possible occurrence of food borne diseases and multi-drug resistance.

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Keywords: Extended Spectrum Beta-Lactamase (ESBL), Resistant genes, Salmonella spp, ready-to-eat foods, food handlers, Port Harcourt, Nigeria

1. INTRODUCTION

Antimicrobial resistance is the ability of a microbe to resist the effects of medication that once could successfully treat the microbe [1]. Resistant microbes are more difficult to treat, requiring alternative medications or higher doses of antimicrobials. Microbes resistant to multiple antimicrobials are called multidrug resistant (MDR). Those considered extensively

drug resistant (XDR) or totally drug-resistant (TDR) are sometimes called "superbugs" [1,2,3]. Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from. All classes of microbes can develop resistance. Fungi develop antifungal resistance. Viruses develop antiviral resistance. Protozoa develop antiprotozoal resistance, and bacteria develop antibiotic resistance. Resistance can appear spontaneously because of random mutations. However, extended use of antimicrobials appears to encourage selection for mutations which can render antimicrobials ineffective.

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Bacteria with resistance to antibiotics predate medical use of antibiotics by humans [4]. However, widespread antibiotic use has made more bacteria resistant through the process of evolutionary pressure [5]. Reasons for the widespread use of antibiotics in human medicine include; increasing global availability over time since the 1950s, uncontrolled sale in many low- or middle-income countries, where they can be obtained over the counter without a prescription, potentially resulting in antibiotics being used when not indicated [6]. Other causes include: Antibiotic use in livestock feed at low doses for growth promotion is an accepted practice in many industrialized countries and is known to lead to increased levels of resistance. Releasing large quantities of antibiotics into the environment during pharmaceutical manufacturing through inadequate wastewater treatment increases the risk that antibiotic-resistant strains will develop and spread [7].

Rising drug resistance is caused mainly by use of antimicrobials in humans and other animals, and spread of resistant strains between the two [8]. 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 [9]. Most cases of salmonellosis in human's samples are the consequence of consuming contaminated food mostly prepared by street food vendors [10]. Contaminated street foods are among the important sources for food-borne infection or outbreak than from any other animal or food products [11,12,13]. Prompt and accurate detection of *Salmonella* spp.in contaminated food provides an opportunity to prevent the contaminated food from entering the food chain [14]. For this reason, sensitive and specific laboratory methods for the isolation, identification and serotyping of *Salmonella* for *Salmonella* infections are essential elements for monitoring and control programs [14]. There is also growing resistance of bacteria to antibiotics which has been linked to greater access to antibiotics drug in the developing countries.

Studies have shown Extended Spectrum Beta-Lactamase (ESBL) production by *Salmonella spp* to be plasmid mediated and readily transmitted amongst members of the Enterobacteriaceae family as a result [15,16]. This potential exacerbates the spread of antimicrobial resistance amongst the other members of the Enterobacteriaceae family against beta-lactam antibiotics and other commonly used antibiotics [17]. Studies have also shown that ESBLs occur because of mutations in the *blaTEM-1*, *blaTEM-2* or *blaSHV-1* genes, which are commonly found in the Enterobacteriaceae family. Others enzymes, notably from the cefotaxime resistance family (CTX-M), represent plasmid acquisition of broad-spectrum beta-lactamases originally determined by chromosomal genes [18]. These mutations gave rise to the alterations in their amino acid configuration and conferred on these enzymes the ability to hydrolyze a broader spectrum of antibiotics [18].

The β-lactamase enzymes are classified based on their molecular structure and/or functional characteristics. Structurally they are placed into four main categories (A, B, C, or D). There are three functional groupings based on the substrate specificity: the cephalosporinases, the serine β-lactamases, and the metallo (zinc-dependent) β-lactamases. These enzymes may also be commonly known by their enzyme family; for example: the TEM (named after the first patient) family, the SHV (sulphydryl variable) family, and the CTX (preferentially hydrolyze cefotaxime) family. Gram negative bacteria may produce β-lactamases from all

four structural groups. The β-lactamases found in gram positive bacteria are mainly from group A, with some from group B [19,20]. These enzymes may be innately found on the bacterial chromosome or may be acquired via a plasmid. Many members of the Enterobacteriaceae family of gram-negative bacteria possess chromosomal β-lactamase genes. Other gram-negative bacteria that possess these include *Aeromonas spp., Acinetobacter spp*., and *Pseudomonas spp*. Plasmid-carried β-lactamase genes are most commonly found in the Enterobacteriaceae, but may also be found in some species of gram-positive bacteria such as *Staphylococcus aureus, Enterococcus faecalis*, and *Enterococcus faecium* [19,21]. The aim of this study was to assess the Extended Spectrum Beta-Lactamase (ESBL) resistant genes in *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 [22]. The prevalence rates of 33.5% [22], and 17.2% (Oghenevo *et al* [23] for the two sample types respectively, were used to determine the sample size using the formular below:

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

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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.

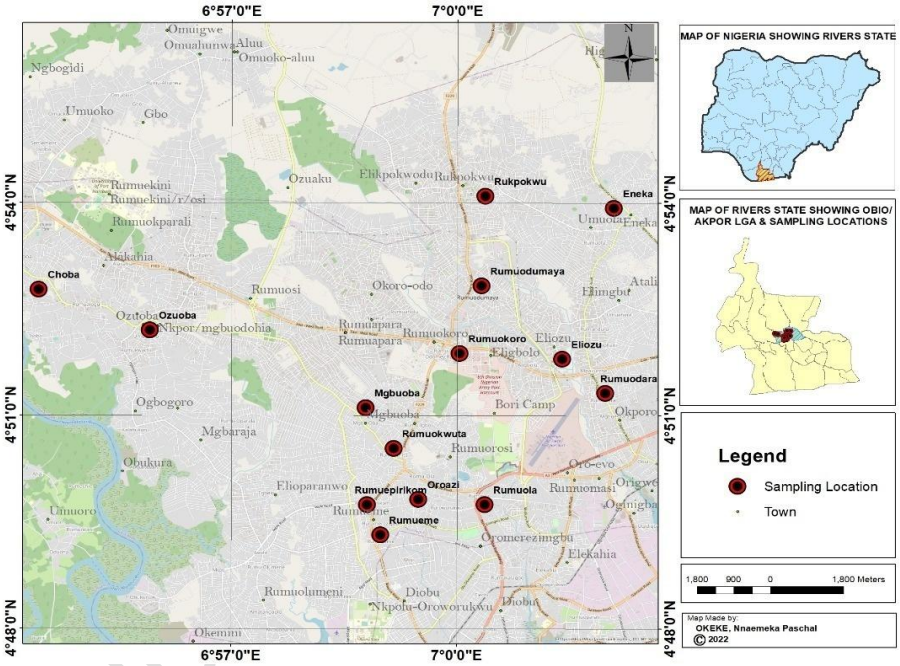

Figure 1: Map of Rivers State Showing Sample Locations.

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 4hr in a cooler with ice pack for bacteriological analysis.

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.

2.4.2.1 Blood Samples Blood
Samples were collected with a sterile syringe and needle. 5mls 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 50mls 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 [24] were adopted.

2.5.1 Isolation of Bacteria

The *Salmonella* species were isolated according to the methods outlined by the WHO [14].

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 [24]. 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^{0}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^{0}C for 24 hours. Typical suspected *Salmonella* colonies on XLD appeared pink-red with a black centre, on SSA 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^{0}C for 24 hours for isolation of pure culture and subsequent biochemical confirmation and Genomic analysis.

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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, [25].

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.

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) [26] guideline and interpretative criteria.

2.5.5 Determination of Beta Lactamases production in isolate of *Salmonella sp.*

Salmonella isolates that showed resistance against third generation cephalosporin by disc diffusion method were selected for further detection of ESBL production by double disc synergy test (DDST) [27]. A disc of amoxillin-clavulanic acid (30µg) was placed on the centre of the Muller-Hinton agar (Hardy diagnostics) plate, which was previously inoculated with resistant strain. Each cephalosporin disc of ceftriaxone-30µg, cefotaxime-30µg and ceftazidime-30µg was placed around the amoxillin-clavulanic acid disc 20mm apart and incubated for 18-24 hours at 37^{O}C. A clear extension of the edge of the inhibition zone of any of the antibiotics towards the central disc (amoxillinclavulanic acid) disc was interpreted as positive for Beta Lactamase production [28]. *Klebsiella pneumoniae* and *E. coli* were used for the control strain of positive and negative ESBLs production.

2.5.6 Molecular Analysis for Resistance Genes

The pure colonies obtained were inoculated into Luria Bertani medium for 10 hours. During this period, the isolates formed turbidity, then 0.5ml (500 microlitre) of the turbid broth containing the organism was poured into 1.5ml capacity Eppendoff tube and centrifuged at 12000rpm for 4 minutes. The supernatant was discarded and another 1ml of normal saline added to the deposit in each tube, vortexed and centrifuged again. The deposits contain

microbial cells. This process was done two times and is called washing of cells. The supernatant was discarded and 500ul (0.5ml) of elution buffer was added in each tube. The tubes were vortexed and placed on heating block for 20 minutes. The temperature of heating block was set at 95^{O}C. After 20 minutes, tubes were removed from heating block and allowed to stand for 10 minutes in freezer. This is to allow the cell to bust (heat stocking), the tubes were centrifuged for 4 minutes at 2000rpm.
After the centrifugation, another set of Eppendorf tube were arranged and labeled accordingly. Then from each tube containing cytoplasmic DNA and elution buffer, 200ul (0.2ml) of supernatant was collected with micropipette (200ul capacity) and transferred into second corresponding Eppendorf tube.

2.5.6.1 DNA quantification
The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestral, the upper pedestral was brought down to contact the extracted DNA on the lower pedestral. The DNA concentration was measured by clicking on the “measure” button.

2.5.6.2 Amplification of SHV genes
SHV genes from the isolates were amplified using the SHV F:5' CGCCTGTGTATTATCTCCCT-3' (20 bases, bar code: 52A07) and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' (20 bases, Barcodes: S2A07) primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95^{O}C for 5 minutes; denaturation, 95^{O}C for 30 seconds; annealing, 56^{O}C for 40 seconds; extension, 72^{O}C for 30 seconds for 35 cycles and final extention, 72^{O}C for 5 minutes. The product was resolved on a 1% agarose gel at 120Voltage for 25 minutes and visualized on a UV transilluminator for a 400bp product size [29].

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2.5.6.*3 Amplification of TEM genes*
TEM genes from the isolates were amplified using the TEMF: 5’-ATGAGTATTCAACATTTCCGTG-3’ and TEMR: 5’-TTACCAATGCTTAATCAGTGAG-3’ primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of theextracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95^{O}C for 5 minutes; denaturation, 95^{O}C for 30 seconds; annealing, 58^{O}C for 30 seconds; extension, 72^{O}C for 30 seconds for 35 cycles and final extention, 72^{O}C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 400bp product size.

2.5.6.4 Amplification of CTX-M genes
CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR:5'-ACCGCGATATCGTTGGT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95^{O}C for 5 minutes; denaturation, 95^{O}C for 30 seconds; annealing, 52^{O}C for 30 seconds; extension, 72^{O}C for 30 seconds for 35 cycles and final extention, 72^{O}C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 500bp product size.

2.6 Statistical Analysis

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

3. RESULTS AND DISCUSSION

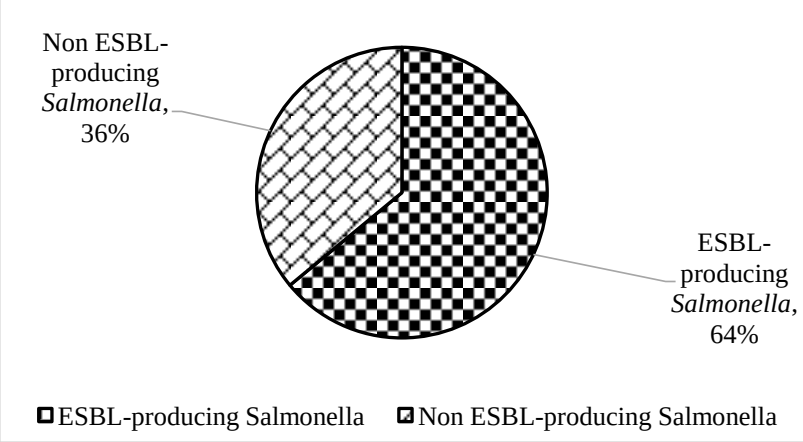

Figure 2: Occurrence of ESBL- and Non ESBL-Producing *Salmonella* sp. in food handlers

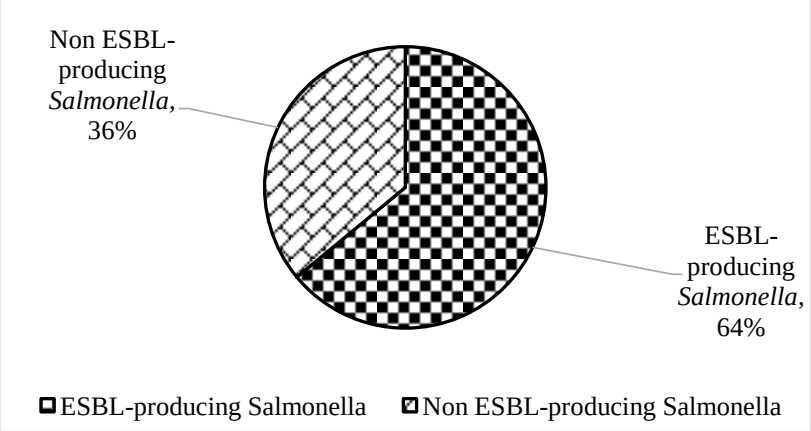

Figure 3: Occurrence of ESBL- and Non ESBL-Producing *Salmonella* sp. in food samples

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How many replica was conducted to achieve these results?

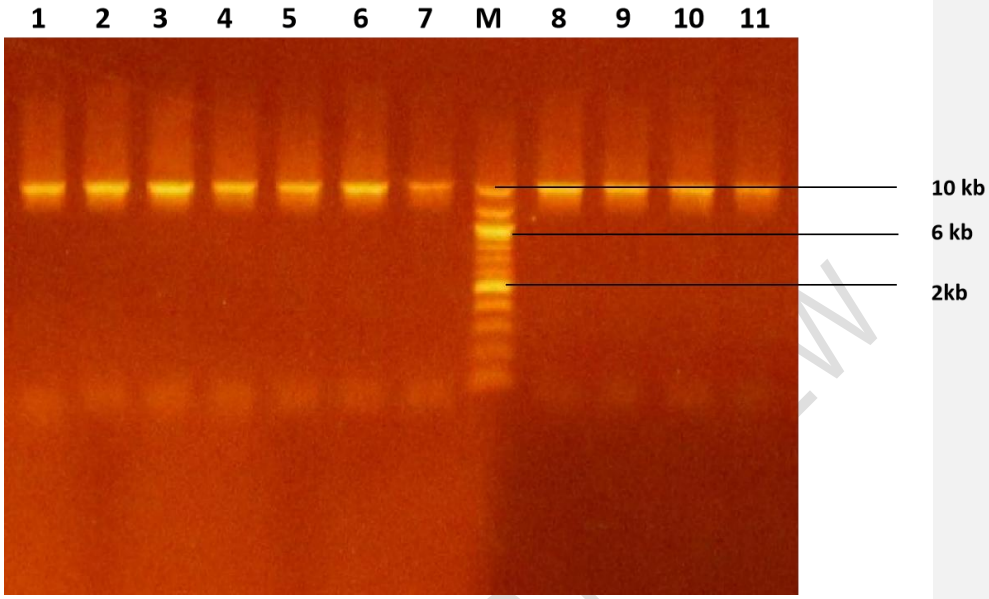


Plate 1: Agarose gel electrophoresis of extracted DNA from the *Salmonella* isolates. Lane *1 – 11 represents DNA bands (10 kb). Lane M represents the 1 kb Molecular ladder.*

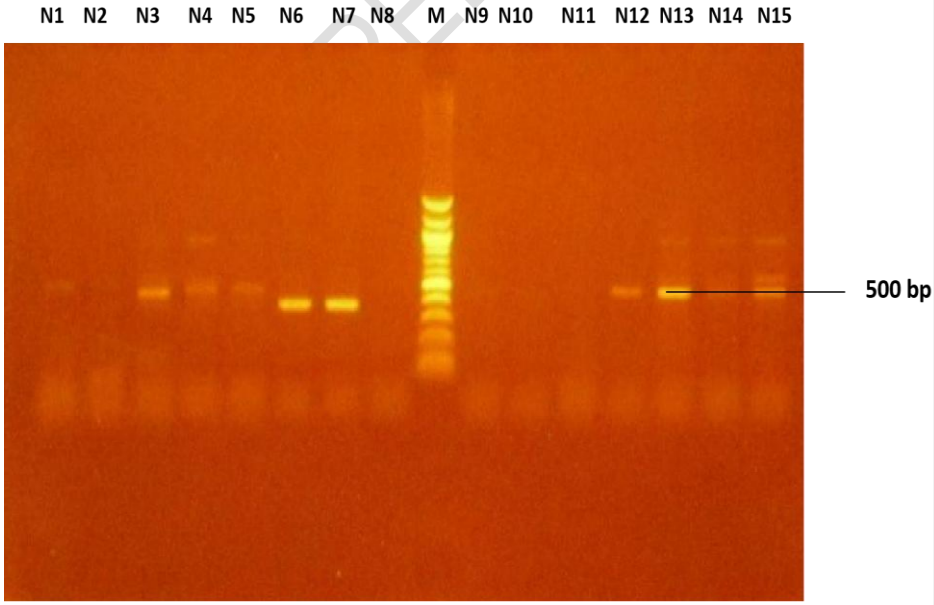


Plate 2: Agarose gel electrophoresis of CTX-M gene amplification by *Salmonella* isolates from street vended food

Lane N1, N3-N7 and N13-N15 represents the positive CTX-M gene bands (500bp). Lane M represents the 1 kb Molecular ladder.

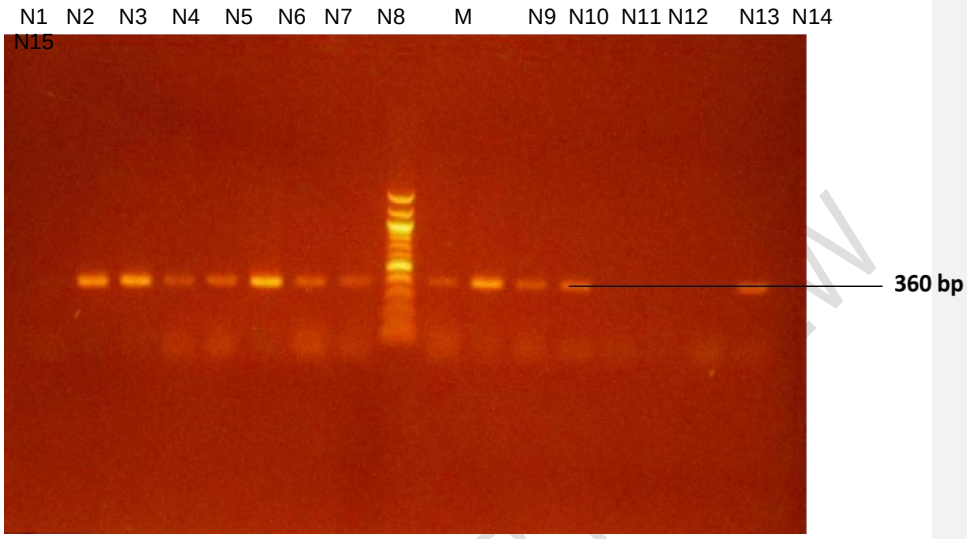
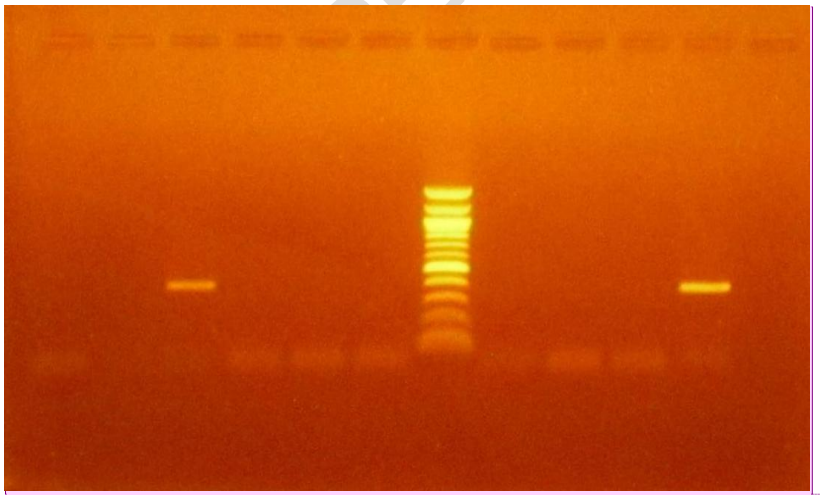


Plate 3: Agarose gel electrophoresis of TEM gene amplification by *Salmonella* isolates from street vended food

Lane N2, N3-N7 and N9-N12; N15 represents the positive TEM gene bands (360bp). Lane M represents the 1 kb Molecular ladder.



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Unless the picture is cropped.

Plate 4: Agarose gel electrophoresis of SHV gene of some selected bacteria isolates from food samples

Lanes N4 and N14 represent a SHV band (400bp). Lane M represents the 100bp Molecular ladder of 1000bp.

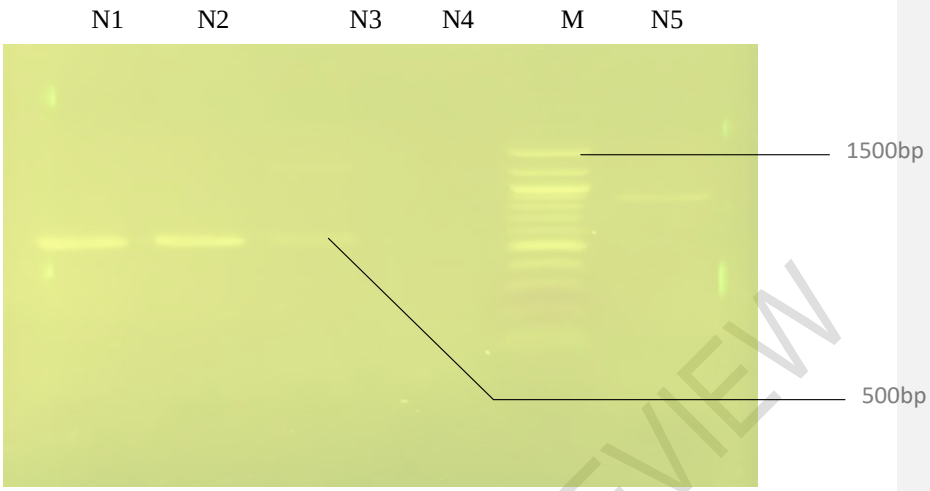

Plate 5: Agarose gel electrophoresis of CTX-M gene amplification by *Salmonella* isolates from street vended food
Lane N1-N3 represent positive CTX-M gene at 500bp. Lane M represent the 100bp molecular ladder

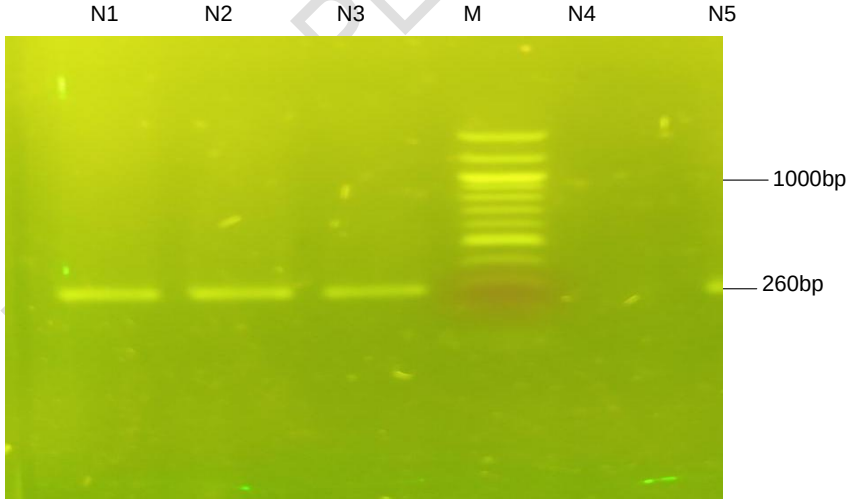

Plate 6: Agarose gel electrophoresis showing SHV gene of some selected bacteria isolates from food handlers.
Lane N1 – N3 represent positive SHV gene at 260bp. Lane M represent the 100bp Molecular ladder.

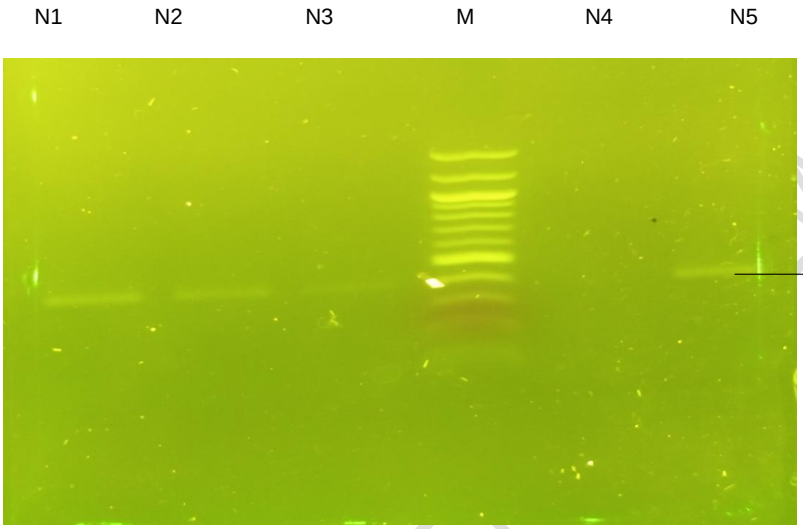

Plate 7: Agarose gel electrophoresis showing TEM gene of some selected bacteria isolates from food handlers.

Lane N1 – N3 and N5 represent positive BlaTEM gene at 300bp. Lane M represent the 1000bp Molecular ladder.

Table 1: Distribution of ESBL genes (CTX-M, SHV, TEM) from *Salmonella* isolates *from* street food and handlers isolates.

S/N	SAMPLE CODE	CTX-M	SHV	TEM
1	N1H	+	+	+
2	N2H	+	+	+
3	N3H	+	+	+
4	N4H	-	-	-
5	N5H	-	+	+
6	N1F	+	-	-
7	N2F	-	-	+
8	N3F	+	-	+
9	N4F	+	+	+
10	N5F	+	-	+
11	N6F	+	-	+
12	N7F	+	-	+
13	N8F	-	-	+
14	N9F	-	-	+
15	N10F	-	-	+
16	N11F	-	-	+
17	N12F	+	-	+

18	N13F	+	-	-
19	N14F	+	+	-
20	N15F	+	-	+

Positive (+), Negative (-); N1H-N5H = Isolates from Food Handlers; N1F - N15F = isolates from Street vended food.

This study employed molecular technique to investigate the prevalence of beta-lactamse (CTX-M, SHV and TEM) resistant genes in *Salmonella* isolated from street-vended foods and food handlers at fourteen different locations in Port Harcourt, Rivers State, Nigeria. In this study, the following street-vended foods: White Rice/stew, Jollof Rice, Rice/Beans stew, Porridge Beans, Beans/Stew, Moimoi, Abacha, Roasted plantain analysed for the presence of *Salmonella* isolates harbouring beta lactamase genes.

The *Salmonella* isolates were evaluated for ESBL production and the data obtained revealed that 50% of the isolates were able to produce ESBL. Out of 11 *Salmonella* isolates from food handlers tested for ESBL production 7(63.6%) were positive, similarly 64.3% (Figure 2) of the isolated *Salmonella* sp. from the vended foods were positive for ESBL production (Figure 3). These levels of ESBL production present a concern to public health and food scientists as it implies high prevalence of antibiotic resistance in the population. Studies have suggested that excessive use of antibiotics in aquaculture is increasing the prevalence of antibiotic-resistance bacteria in aquatic food products. In addition, Ndihokubwayo et al. [30] noted that inappropriate and irrational use of antibiotics are the leading factors promoting the spread of resistance. Chukwu et al. [31] reported that about a third of the general Nigerian public consume antibiotics without prescription and thus attributed high rate of antibiotic resistance in Nigeria as a result of wrong or absence of prescription before antibiotic consumption. Furthermore, Iheanacho & Eze [32] reported high antimicrobial resistance in Nigeria.

Beta-Lactams and fluoroquinolones are generally used to treat invasive *Salmonella* infections, but emergence and spread of antibiotic-resistant strains are increasingly becoming a global challenge. Detection of extended-spectrum β-lactamases (ESBLs) in *Salmonella* serotypes is particularly an emerging threat worldwide [33]. Increasing occurrence of antimicrobial resistance in both typhoidal and nontyphoidal *Salmonella*

infections is a serious public health problem. The findings of the current study is consistent with the reports of Phoba et al. [33] on the existence of resistance determinants in the isolated *Salmonella sp.* The study also revealed the detection of carb-like gene (carbenicillinase) in the isolates that exhibited resistance or decreased susceptibility to beta lactam antibiotics, suggesting that this resistance is mediated by carb-like gene which encoded beta lactamase enzyme. The study showed that expression of the blaTEM, blaSHV and blaCTX-M genes among the *Salmonella* isolates can be correlated with the occurrence of ampicillin-resistance in the same isolates [34,35].

In the present study, the isolates from street vended food and handler showed CTX-M and TEM genes but only 2 of the isolates expressed SHVgenes. Only 1 isolate harboured all three genes namely CTX-M, TEM and SHV. Data obtained from this study correlate with report from other researchers Obajuluwa et al. [36] who showed that ESBLs genes may vary from source of infection to another and from one location to another. Other studies have also implicated other bacterial isolates such as *Proteus mirabilis* as reservoirs of these resistance genes. The presence of ESBLs in *Providencia statuii* indicate that there could be spontaneous mutation or transfer of genetic material (plasmids) from one organism to another in a mixed culture.

The presence of TEM genes in a higher number of isolates in this study than other resistance genes showed that this gene may be peculiar to particular regions with different activities. The study is in disagreement with a work in Rivers State but in different locations where more SHV genes was detected [37] but though the research did not consider TEM genes.

4. CONCLUSION

Extended Spectrum Beta Lactamase (ESBL) genes: CTX-M, SHV and TEM found in *Salmonella* isolates from sampled street vended foods should receive particular attention, as the presence of extended spectrum beta-lactamase genes isolated, indicate public health hazard and gives a warning signal for the possible occurrence of food borne diseases and multi-drug resistance.

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.

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