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Genomic Characterization and Plasmid Profile of *Listeria* and *Salmonella* Species Isolated from *Oreochromis niloticus* Sold in Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNO designed the study, while author VD performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript managed the analyses of the study and literature searches under the strict supervision of authors NNO and DNO. All authors read and approved the final manuscript.

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ABSTRACT

Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues. Consumption of fish may also cause diseases due to infection or intoxication, Hence, the aim of this study is to characterize using genomic analysis and plasmid profile of *Listeria* and *Salmonella* species isolated from *Oreochromis niloticus* sold in Port Harcourt. A total of one hundred and eighty samples (180) were collected from three different markets namely; Creek road, Mile one and Rumuokoro markets over a period of six months. The samples were labelled and transported in an ice packed coolers to the laboratory for analyses. Standard analytical protocols were employed to determine the

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bacteriological characteristics of the various parts such as Intestine, Gill, flesh of the sample. Statistical analyses were carried out using ANOVA and All pairs tukey-kramer. Results obtained from the study showed that the total heterotrophic bacteria count ranged from 5.1 to 5.9 x10 $x10^6$cfu/g across the markets for the flesh part, 6.0 to 7.7 $x10^6$ cfu/g (Gill) and 7.1 to 7.6 $x10^6$ cfu/g for the intestinal samples. Total coliform count ranged from 4.2 to 5.4 $x10^4$cfu/g (flesh), 5.2 to 5.4 $x10^4$ cfu/g ((Gill) and 6.1 to 8.0 $x10^4$ cfu/g (Intestine). *Listeria* count range from 2.7 to 2.9 $x10^4$ cfu/g (Fresh), 3.3 to 3.7 cfu/g (Gill)), and 3.8 to 4.3 cfu/g (Intestine), and *Salmonella* count ranged from 1.0 $x10^3$ - 1 .1 $x10^3$ cfu/g (Flesh) 1.0 to 1.6 $x10^3$ cfu/g (Gill) and 1.2 to 2.0 $x10^3$ cfu/g (intestine). This results shows that the intestines harbours more bacterial load than the gill and flesh. Mean values for all the microbial counts were significantly different ($P<0.05$) in the three samples across the sampled markets The result of the conventional and genomics identification confirms the following species of *Listeria and Salmonella: L. grayi* VD-Sfg with accession number MW020239 having a closest gene bank match with *Listeria grayi* CIP 100% identified, *L. monocytogenes* VD-Sfg with accession number MW020240 closest to *L. monocytogenes* NCTC 10357 with 99.73% identified, *L. seeligeri* VD-SFF accession number MW020241 closet to *L. seeligeri* ATCC 35967 with 98.95%, *L. welshimeri* VD-SF MW020242 closest to *L. welshimeri* ATCC 35897 with 99.9%, *L. monocytogenes* VD-Fg MW020243 closet to *L. monocytogenes* NCTC 10357 eith 99.73% identity, *S. bongori* VD-SwfiA MW020245 closest to *S. bongori* NCTC 12419 and *S. enterica* VD-SwfiD MW20244 closest with *S. entrica* LT2 both with 100% identity. Gel electrophoresis of the plasmid DNA showed that all the isolates possess plasmid. This finding is of public health concern as these organisms are the known causes of food-borne diseases and also serve as reservoirs for resistance plasmids that may be transferred to otherwise susceptible bacteria making them resistant, thus increasing the occurrence of antibiotic resistance among microorganisms.

Keywords: Genomic; characterization; plasmid profile; Listeria sp. and Salmonella sp.

1. INTRODUCTION

According to Omorodion et al. [1], genus *Listeria* is a gram-positive, non- spore forming, rod-shaped bacteria of 0.4-0.5 x 0.5- 2 *µm* in size with rounded ends and can also be coccoid at times, occurring singly or in short chains and not encapsulated. *Listeria monocytogenes* is reported to be pathogenic in humans and animals and is the causative agent of Listeriosis. It is also an agent of several food-borne disease outbreaks [2] and causes serious infection in the elderly, neonates, pregnant women and immune-compromised persons. According to Nakamura et al. [3], Listeriosis has a high fatality (20 to 30%) rate and infected persons may show signs of meningitis and septicaemia. Bryan et al. [4] reported that *Listeria monocytogenes* is resistant to stress and is widely distributed in the environment by virtue of its occurrence in water, soil and plant material. The species *L. monocytogenes* is widely studied in the developed world and is known as an entero-invasive gastrointestinal pathogen [5]. Humans can become infected when contaminated food is ingested because the acidic stomach environment and its surface proteins can help the organism to attach to the gut and multiply in the host's cell cytosol [6], Pizarro-Cerdá et al. [7]. Attachment of *Listeria* on stainless steel at temperatures below 25°C is aided by flagella [8]. The organism has recently been suggested as a model for understanding how an environmental bacterium switches to life within human cells [5]. ListerIosis may be caused by unsanitary handling of food products in commercial food processing plants or in homes. To detect the organism and study strains associated with outbreaks, serological and genomic techniques are usually employed. *Listeria* can be subdivided into 15 serovars [9] and various sub-typing studies have shown that the serovars of *Listeria* can be divided into four evolutionary lineages [10,11]. Some strains have had their whole genome sequenced [12,13].

Microorganisms are varied in its susceptibility to antimicrobial drugs and influenced by antimicrobial use to humans and animals, as well as the geographical differences [14,15]. *Salmonella* is rod-shaped, gram-negative enterobacterium that has an ability to colonize a wide range of hosts. Disease outcome in the host is dependent on the bacteria serovar, inoculation dosage, and host immune status [15]. The genus *Salmonella* is divided into two main species, *S. bongori and S. enterica.* These species are then further classified into several subspecies, with more than 2500 serovars identified (Foley et al., 2013). In poultry, salmonellosis can be classified

more simply into two main types of infection, with either poultry-adapted strains or nonhost specific strains [16]. *Salmonella gallinarum* and *Salmonella pullorum* are host-adapted strains that cause fowl typhoid and Pullorum disease respectively, with high morbidity and mortality in avian species [17,16]. Because these strains are host-specific, they pose a minimal threat to human health and have been mostly eradicated from the commercial poultry industry in developed countries [17]. However, the persistence of broad-host range strains like *Salmonella enteritidis* (SE) in chickens is a major concern for food safety and public health due to their ability to cause disease in humans. Poultry serve as a main reservoir of infection for humans, and consumption of contaminated egg and poultry products has resulted in outbreaks of foodborne illness worldwide [18]. Silent infections in chickens, broad-host range colonization, and high zoonotic potential are the major characteristics associated with SE that make eradication of the pathogen highly challenging.

According to Kalpana and Harish (2015) plasmid is a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium Plasmids are also known as extra chromosomal materials which carries genes that code the production of β-lactam resistance encoding genes [19]. Plasmids are often implicated in increasing drug resistance as they are able to transfer the genes both within species and between different species [12], Plasmid profiling has proved to be relevant in the epidemiologic study of drug resistance as this explains the pattern, occurrence and likely future picture of the resistance when linked with some parameters (Wilberforce et al. 2013) Plasmid profiling also aids in surveillance in relating strains with outbreaks [20], Plasmids are often implicated in increasing drug resistance as they are able to transfer the genes both within species and between different species [15].

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as good source of protein and other elements for maintenance of healthy body [21]. Rivers, homes to diverse fishes, are often exposed to waste from agricultural and industrial processes and products of human, and animal origins. Pollution of aquatic environments with organic waste of animal and human origin may lead to transfer of pathogens to the inhabitant fishes thereby making them carriers of the pathogens such as *Vibrio* spp., *Listeria monocytogenes*, *Yersinia* spp., *Salmonella* and *Clostridium botulinum* [22], therefore, this study will provide specific information as regards the microbial load of fishes, with the aim to characterize using genomic studies and plasmid profile of *Listeria* and *Salmonella* species isolated from *Oreochromis niloticus* sold in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Description of Study Area

Port Harcourt is located in the Niger Delta region; Southern Nigeria. The city is situated between latitudes 3°37' and 3°56' N. and longitude 11°10' and 11°45' E, approximately 50 km from the Atlantic coast. Precipitation averages 3,030 mm annual and a temperature average of 23°C. Port Harcourt is located in the Niger Delta region; Southern Nigeria. The city is situated between latitudes 3°37' and 3°56' N. and longitude 11°10' and 11°45' E, approximately 50km from the Atlantic coast. Precipitation averages 3,030 mm annual and a temperature average of 23°C. One of Major occupations known with residents/inhabitants of Port Harcourt is fishing around the marine communities in spite of the various exploitation of oil by multinational companies. Fig. 1 shows the map of Port Harcourt indicating the sampled markets.

2.2 Collection of Samples

Total one hundred and eighty samples (180), samples were obtained from the three different markets namely; Creek road, Mile one and Rumuokoro markets over a period of six months (February to July). Sterile containers were used to store the frozen fishes after purchase and then transported to the Laboratory for analyses within 2 hours of collection in a thermos box containing ice pack.The samples were collected using sterile bags properly labeled according to standard microbiological procedures [1].

2.3 Bacteriological Analyses of Fish Samples

2.3.1 Preparation of the samples in the laboratory

The fish samples were prepared for bacteriological analysis as described by Danba et al. [23] with required modification; the fish

samples were rinsed with water to remove surface dirt, and then the body surface were swabbed with ethanol to remove external microorganisms. The skin, gills, and intestine were dissected with the aid of sterile knife and forceps, and separately macerated aseptically.

2.3.2 Serial dilution

One gram each of the respective samples was separately added to 9 ml of 0.1% peptone water diluents. After thorough shaking, further tenfold (v/v) serial dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluents to a range of 10^{-6} dilutions [24].

2.3.3 Inoculation and Incubation

Aliquots (0.1 ml) of various dilutions were inoculated to surface dried Plates Count Agar TM Media product, (PCA) in triplicates for enumeration of total heterotrophic bacterial population, McConkey agar) (TM Media product),for total coliform population, *Salmonella-Shigella* agar (SSA TM Media product) for *Salmonella* and *Shigella* populations, and *Listeria* selective agar base supplemented with *Listeria* Selective Supplement II (Oxoid product, FD063 or FD063I) for *Listeria* population in triplicates and spread evenly with flamed bent glass spreader. The plates were incubated at 37°C for 24 hours.

2.3.4 Enumeration and isolation of pure culture

2.3.4.1 Total heterotrophic bacteria

Total Heterotrophic Bacteria was enumerated as described by Prescott et al. [24]. Bacterial Colonies that appeared on plate count agar plates (PCA) were counted and the mean expressed as cfu/g for the respective samples (Odokuma and Nrior 2015). The colony forming unit per gram of the sample was calculated using the formula below;

$$\text{CFU/G} = \frac{\text{number of colonies}}{\text{Dilution x volume plated}}$$

The discrete colonies were sub cultured on fresh Nutrient agar plates in order to isolate pure cultures.

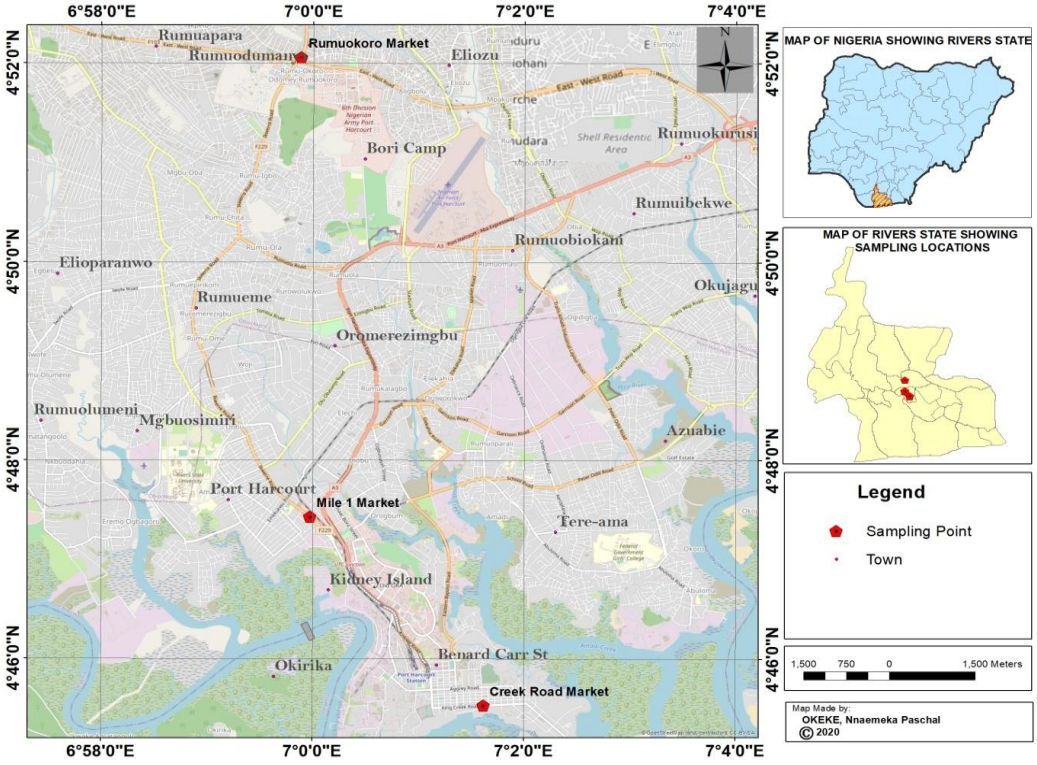

Fig. 1. Map showing study area of the various sampling markets

2.3.4.2 Total coliform counts

Total Coliform Counts was enumerated as described by Prescott et al. [24]. Bacterial Colonies that appeared on the MacConkey agar plates were counted and the mean expressed as cfu/g for the samples (Nrior and Odokuma, 2015). The discrete colonies were sub cultured on fresh Nutrient agar plate in order to isolate pure culture cultures.

2.3.4.3 Isolation and resuscitation of Salmonella species

For isolation and resuscitation of *Salmonella* species, Ten gram (10 g) of the fish sample was agitated in 90 ml sterile peptone broth incubated at 37°C for 48 h., for enrichment, then 10 ml of the incubated peptone containing the sample were transferred into Selenite F broth at 37°C for 24 hr., for further enrichment, 0.1 ml aliquot was inoculated on *Salmonella-Shigella* Agar (SSA), incubate at 37°C for 24 hours.

2.3.4.4 Isolation and Resuscitation of Listeria specie

For *Listeria* species resuscitation and isolation, ten grams (10) of respective samples was transferred into 90 ml of Half Fraser broth and incubated at 37°C for 24 h. Ten mililiter of Half Fraser were transferred into full Fraser broth and incubate at 37°C for 24hour, after which 0.1 ml of Full Fraser was inoculated on PALCAM media and incubated at 37°C for 24 hours.

2.3.4.5 Maintenance of pure culture

Discrete bacterial colonies that grew on the respective media were sub cultured using streak plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure culture. The pure bacteria cultures were then maintained according the method described by Amadi et al. [25] using ten percent (v/v) glycerol suspension at -4°C

2.4 Morphological and Biochemical Characterization of the Bacterial Isolates

Pure bacterial culture from the respective parts of frozen tilapia fish was obtained by streaking on Plate count agar and incubated for 24 hours, The pure isolates were characterized and identified using their morphological and biochemical tests [26-28]. Further identification of the pure isolates were carried out using genomic analysis.

2.5 Molecular Identification

2.5.1 DNA extraction (Boiling method)

Five millilitres of an overnight broth culture of the bacterial isolate in Luria Bertani broth (LB) was spun at 14000 rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20°C for further analyses [12].

2.5.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer [12].

2.5.3 16S rRNA amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 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 the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; anealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extention, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 20 minutes and visualized on a blue light transilluminator [29].

2.5.4 Sequencing

Sequencing was done using the BigDye Terminator kit on on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25 ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min [29].

2.5.5 Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [29]

2.5.6 Plasmid extraction

Plasmid was extracted using the Zyppy plasmid mini-prep extraction kit manufactured by Zymo Research Inc, USA. was used according to the manufacturer's procedure.

2.5.7 Gel electrophoresis

Plasmids were separated by electrophoresis in 1% agarose (Sigma Aldrich, USA) at a voltage of 4.5 V/cm; buffer used was TAE (Tris-Acetate EDTA) for 3 hours. Following electrophoresis, the gels were stained for 15 minute with ethidium bromide solution (1.0 µg/ml EtBr in 0.5 x TrisAcetate-EDTA (TAE)), and then observed under UV light. The image was registered and analyzed using Quantity One software, version 4.1 [29].

3. RESULTS AND DISCUSSION

The result of bacteriological evaluation of different parts (Intestine, Gill and Flesh) of Frozen fish obtained from three markets namely Rumuookoro, Mile 1 and Creek Road is presented in Table 1.

The result revealed that there were significant difference in bacteriological counts of the various samples parts across three sampled markets at $P<0.05$, except for *Salmonella* count that showed no significant difference and total heterotrophic and *Listeria* count from the flesh and intestine respectively, while across the various fish parts there were significant difference at $P<0.05$ in the bacteriological counts except for the *Salmonella* count from the set of samples obtained from Rumuokro market.

Total heterotrophic bacteria count ranged from 5.1 to 5.9 x10 x10^6cfu/g across the market for the flesh part, 6.0 to 7.7 x10^6 cfu/g (Gill) and 7.1 to 7.6 x10^6 cfu/g for the intestine. Total coliform count ranged from 4.2 to 5.4 x10^4cfu/g (flesh), 5.2 to 5.4 x10^4 cfu/g (Gill), and 6.1 to 8.0 x10^4 cfu/g (Intestine). *Listeria* count range from 2.7 2.9 x10^4 cfu/g (Fresh), 3.3 to 3.7 cfu/g (Gill), and 3.8 to 4.3 cfu/g (Intestine), and *Salmonella* count ranged from 1.0 x10^3 to 1.1 x10^3 cfu/g (Flesh) 1.0 x10^3 to 1.6 x10^3 cfu/g (Gills) and 1.2 to 2.0 x10^3 cfu/g (Intestine), (Table 1). This results show that the intestines harbours more bacterial load than the gills and flesh respectively. The results obtained in this study is in agreement with that report by Adebayo-Tayo et al. [21] on microbial quality of frozen fish, in their research, high microbial load was also obtained from the intestine of various fishes they worked on. Arannilewa et al. [8] also found that the total coliform count range in fish was between 3.0 x 10^3 - 7.5 x 10^6 cfu/g with increasing values, as the duration increases. From the result of this study, it can be seen that frozen fish sold in the market has high contamination and may be as a result of certain factors like temperature which favours some organisms and the character of fish handler, by not maintaining personal hygiene.

The result of the conventional and genomics identification confirms the following species of *Listeria and Salmonella: L. grayi* VD-Sfg with accession number MW020239 having a homologous with gene bank match with *Listeria grayi* CIP 100% identified, *L. monocytogenes* VD-Sfg with accession number MW020240 closet to *L. monocytogenes* NCTC 10357 was with 99.73% identified, *L. seeligeri* VD-SFF accession number MW020241 homologous with to *L. seeligeri* ATCC 35967 with 98.95%, *L. welshimeri* VD-SF MW020242 closest to *L. welshimeri* ATCC 35897 with 99.9%, *L. monocytogenes* VD-Fg MW020243 closest to *L. monocytogenes* NCTC 10357 eith 99.73% identity, *S. bongori* VD-SwfiA MW020245 homologous with to *S. bongori* NCTC 12419 and *S. enterica* VD-SwfiD MW20244 clost with *S. entrica* LT2 both with 100% identity. Species of *Listeria* were phylogenetically related having common ancestral relationship with *L. welshimeri* and *L. seeligeri* being the closest in the phylogenetic setup. Table 2, Shows the accession number of the isolates and homologous with Genbank match of the identified *Listeria* and *Salmonella* species while Fig. 2 shows the Phylogenetic tree showing the evolutionary distance between the bacteria.

Table 1. Mean Bacteriological counts for samples collected from the three markets

Sample	Bacteriological counts				
THB	**Creek road**	**Mile one**	**Rumuokoro**	**P value**	**Significant difference**
Frozen Fish Flesh	$5.9 \times 10^6 \pm 0.99^{ab}$	$5.7 \times 106 \pm 1.06^{abcd}$	$5.1 \times 10^6 \pm 0.53^{abc}$	0.364	No
Frozen Fish Gill	$7.5 \times 10^6 \pm 0.8^{ab(x)}$	$7.7 \times 10^6 \pm 0.98^{ab(x)}$	$6.0 \times 10^6 \pm 0.63^{ab(y)}$	**0.014***	**Yes**
Frozen Fish Intestine	$7.6 \times 10^6 \pm 0.96^{ab}$	$7.1 \times 10^6 \pm 0.68^{abc}$	$7.3 \times 10^6 \pm 0.84^{a}$	0.555	No
P value	**0.0006***	**<0.0001***	**0.0003***		
Significant Difference	**Yes**	**Yes**	**Yes**		
Total Coliform					
Frozen Fish Flesh	$5.4 \times 10^4 \pm 0.69^{bcd(x)}$	$4.5 \times 10^4 \pm 0.39^{bc(xy)}$	$4.2 \times 10^4 \pm 0.42^{bc(y)}$	**0.011***	**Yes**
Frozen Fish Gill	$6.6 \times 10^4 \pm 0.58^{ab(x)}$	$5.7 \times 10^4 \pm 0.55^{ab(y)}$	$5.2 \times 10^4 \pm 0.27^{ab(y)}$	**0.002***	**Yes**
Frozen Fish Intestine	$8.0 \times 10^4 \pm 0.44^{a(x}$	$6.4 \times 10^4 \pm 0.65^{a(y)}$	$6.1 \times 10^4 \pm 0.44^{a(y)}$	**0.002***	**Yes**
P value	**<0.0001**	**<0.0001**	**<0.0001**		
Significant Difference	**Yes**	**Yes**	**Yes**		
***Listeria* Count**					
Frozen Fish Flesh	$2.9 \times 10^4 \pm 0.7^{b}$	$2.7 \times 10^4 \pm 0.68^{b}$	$2.9 \times 10^4 \pm 0.23^{b}$	0.758	**No**
Frozen Fish Gill	$3.7 \times 10^4 \pm 0.35^{ab(x)}$	$3.3 \times 10^4 \pm 0.15^{ab(y)}$	$3.3 \times 10^4 \pm 0.27^{ab(y)}$	**0.045***	**Yes**
Frozen Fish Intestine	$4.3 \times 10^4 \pm 0.57^{a}$	$3.8 \times 10^4 \pm 0.44^{a}$	$3.9 \times 10^4 \pm 0.34^{a}$	0.193	**No**
P value	**<0.0001**	**<0.0001**	**<0.0001**		
Significant Difference	**Yes**	**Yes**	**Yes**		
***Salmonella* Count**					
Frozen Fish Flesh	$1.2 \times 10^3 \pm 0^{b}$	$1.0 \times 10^3 \pm 0.58^{b}$	$1.0 \times 10^3 \pm 0$	0.833	No
Frozen Fish Gill	$1.6 \times 10^3 \pm 0.58^{b}$	$1.3 \times 10^3 \pm 0^{b}$	$1.0 \times 10^3 \pm 0$	0.218	No
Frozen Fish Intestine	$2.0 \times 10^3 \pm 1.41^{b}$	$1.5 \times 10^3 \pm 1.0^{b}$	$1.2 \times 10^3 \pm 0.5$	0.586	No
P value	**<0.0001***	**0.0006***	0.646		
Significant Difference	**Yes**	**Yes**	No		

Mean with different superscript (^{abcde}) shows Significant Difference along columns
Mean with different superscript (^{xyz}) Significant Difference across rows

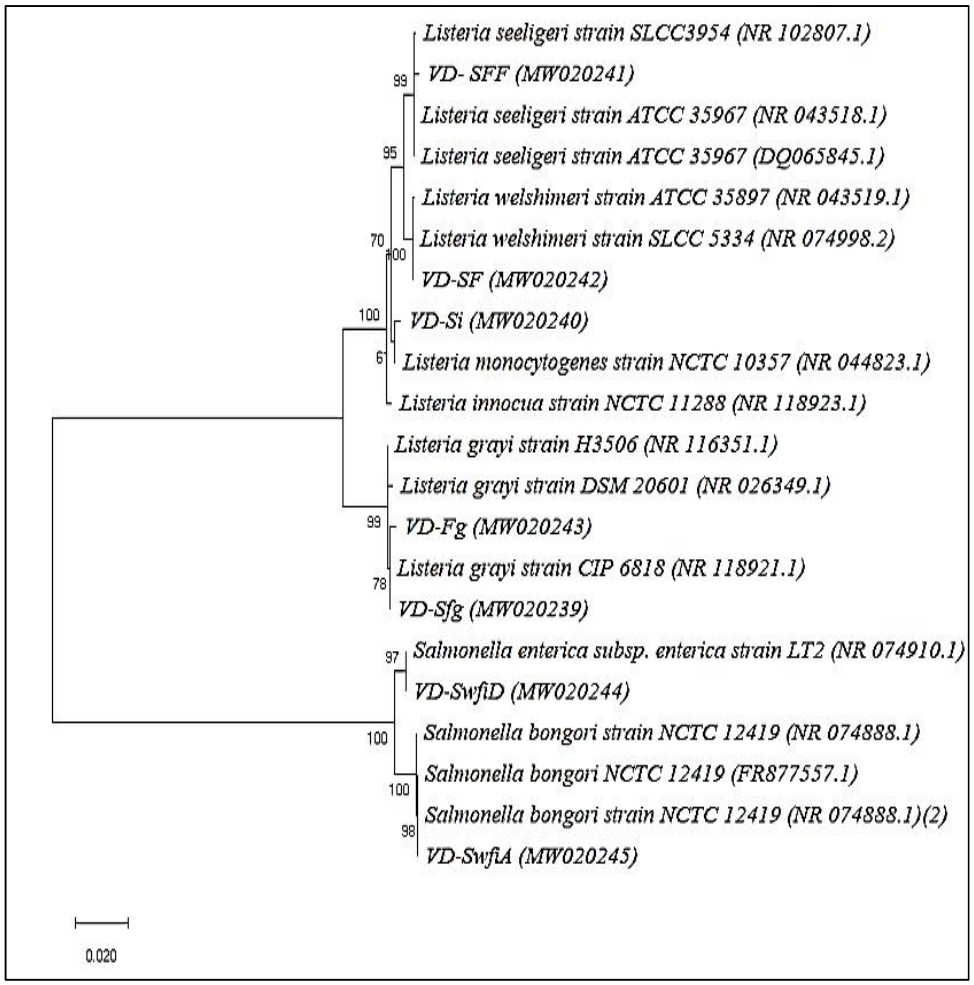

Fig. 2. Phylogenetic tree showing the evolutionary distance between the bacteria

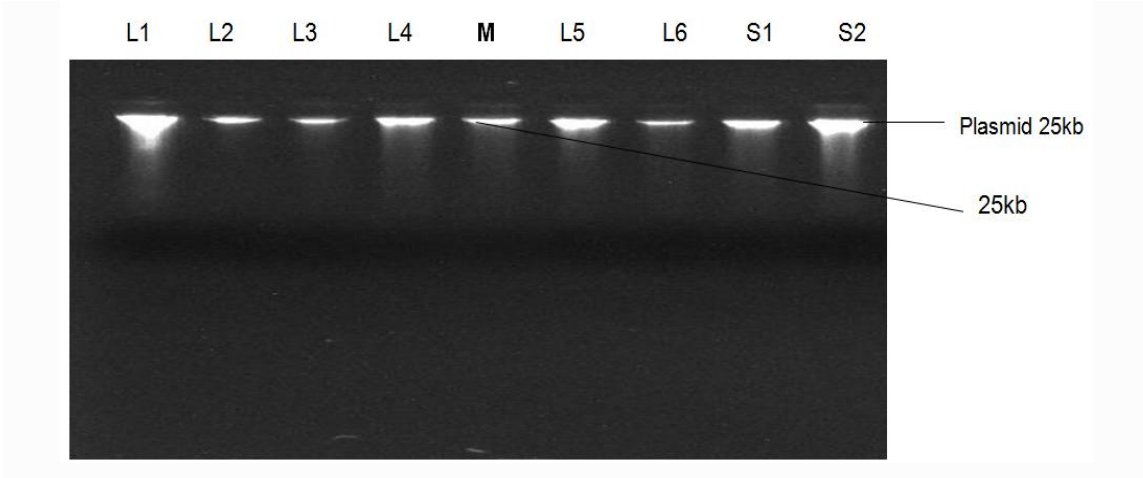

Plate 1. Agarose gel electrophoresis showing the extracted plasmid band sizes at 25kb. Lane M represents the 1kb molecular ladder

Table 2. Shows the accession number of the isolates and closest Genbank match

S/N	Name	Accession No.	Closest GenBank Match	% Identity
1	*Listeria grayi*	MW020239	*Listeria grayi* CIP	100
2	*Listeria monocytogenes*	MW020240	*Listeria monocytogenes* NCTC 10357	99.73
3	*Listeria seeligeri*	MW020241	*Listeria seeligeri* ATCC 35967	98.95
4	*Listeria welshimeri*	MW020242	*Listeria welshimeri* ATCC 35897	99.9
5	*Listeria monocytogenes*	MW020243	*Listeria monocytogenes* NCTC 10357	99.73
6	*Salmonella enterica*	MW020244	*Salmonella enterica* LT2	100
7	*Salmonella bongori*	MW020245	*Salmonella bongori* NCTC 12419	100

Other bacterial isolates were identified as *Vibrio* spp, *Bacillus* spp *Staphylococcus spp Shigella* spp *Pseudomonas* spp. *Enterobacter* spp*. E. coli Micrococcus* spp*. Acinetobacter* spp*. Klebsiella* spp. The presence of contaminating bacteria obtained in this study could be attributed to cross- contamination from environment, source, and handling by the sellers. These results are in line with reports of other studies in Nigeria by various authors [30,31,32,33]. It showed that the organism isolated in this study suggest the high level of contamination of environment where the samples were obtained, meaning that water body is not free from microorganisms. *Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease [34]. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteraemia and enteric fever [35]. The severity of *Salmonella* infections in humans varies depending on the serotype involved and the health status of the human host.

According to Agbagwa et al. plasmids have been known to be extra-chromosomal elements that can replicate on their own. They are distinct from chromosomal DNA in that they can exist independent of the host. In this study the plasmid profile of multi-resistant isolates of *Listeria* and *Salmonella* species were determined. Gel electrophoresis of the plasmid DNA showed that all the isolates had at least one plasmid Plate 1.

The presence of plasmid in Bacteria especially enteric organisms has been reported by other researchers in Nigeria [36] The presence of plasmid DNA in all the isolates implies that the resistance to commonly used antibiotics by this bacteria may be plasmid-mediated and also they serve as reservoirs for resistance plasmids that may be transferred to otherwise susceptible bacteria making them resistant, thus increasing the ocurrence of antibiotic resistance among microorganism. This in agreement with the finding of Ogbonna and Azuonwu [36].

4. CONCLUSION AND RECOMMENDATIONS

Bacteriological evaluation of *Oreochromis niloticus* sold in Port Harcourt were determined in this study. The results obtained shown that *Oreochromis niloticus* are highly. The finding contaminated with potential pathogens such *Listeria* and *Salmonella* species and others. These bacteria are of public health importance as are the known causes of food-borne diseases and also the presence of plasmid in this organisms can make them serve as reservoirs for resistance plasmids that may be transferred to otherwise susceptible bacteria making them resistant. This study provides information on the occurrence of *Listeria moncytogenes* and *Salmonella* species in fishes sold in Port Harcourt. It therefore recommended that all critical control points should be followed during food processing to avoid food borne infections.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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