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Prevalence and Antibiotic Susceptibility of *Listeria monocytogenes* in Retailed Meats in Port Harcourt Metropolis, Nigeria

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Abstract *Listeria monocytogenes* is the causative agent of Listeriosis, a disease of humans and animals owing to food and environmental contamination as well as zoonotic infections. Globally, this has become an emerging and zoonotic bacterial disease having low incidence with however high case fatality rate. The essence of this study was to isolate *L. monocytogenes* from retail beef and pork samples purchased from five markets within Port-Harcourt metropolis and to detect their antimicrobial profile. One hundred samples of retail beef and pork were purchased from different vendors across the five markets using cross-sectional study design from November 2013 to March 2014. The samples were examined for the occurrence of *Listeria monocytogenes* and other *Listeria* species using standard microbiological methods. From the study, of 100 samples (50 each for beef and pork) analyzed, 19(19.0%) confirmed the presence of *Listeria* species. Of these nineteen isolates, 7(36.8%) were *Listeria monocytogenes* while 12(63.2%) were other *Listeria* species [*L. Ivanovic* 2(10.5%), *L. innocuous* 7(36.8%), *L. grayi* 2(10.5%), and *L. seeligeri* 1(5.3%)] of the total samples. Although there was insignificant difference ($p>0.05$) in the contamination levels of beef and pork meat, there was however a substantial variation ($p<0.05$) in the contamination level of the different locations with Trans-Amadi and Creek road markets having the highest incidence of 7/19(36.8%) each, Mile-1 Market did not have any positive sample for *Listeria*. The antibiotics profile of the *Listeria monocytogenes* strains was done by using the standard disc diffusion method (Muller Hinton Agar) against 14 antibiotics. All isolates were (100.0%) susceptible to Chloramphenicol, Gentamicin, Ampiclox, Clotrimoxazole and Streptomycin. It also showed that they were all (100.0%) resistant to Augmentin, Erythromycin, Tetracycline, Rifampicin, and Cloxacillin. The isolates also showed varying degree of resistance to Norfloxacin (4 resistant, 57.2%), Levofloxacin (5 resistant, 71.4%), and Ciprofloxacin (5 resistant, 71.4%). This study further confirms the presence of *Listeria monocytogenes* and other *Listeria* species in beef and pork meat samples in Port Harcourt metropolis, Nigeria which is a cause for public health concerns because of the danger this poses especially through cross-contamination and inadequate cooking of meat products. There is a need for relevant public health agencies in Nigeria to create awareness to vendors and consumers about *Listeria* in food and its potential as a food pathogen of interest.

Keywords Listeria monocytogenes, Listeria species, Antimicrobial drugs, Public Health

1. Introduction

Contaminated raw meats are one of the chief causes of foodborne infection [1, 2]. *Campylobacter* spp., *Listeria monocytogenes* and *Escherichia coli* O157: H7 were the major pathogens associated with meat and meat products. These organisms have been linked to a number of cases of human illnesses [3, 4].

L. monocytogenes is the causal agent of listeriosis, which is one of the most virulent foodborne diseases that controlling and monitoring agencies across the globe have been trying to contained [5].

It is well established that listeriosis causes a range of manifestations including flu-like symptoms such as fever, fatigue, nausea, vomiting, and diarrhoea, and severe symptoms such as septicemia and meningitis [6]. Listeriosis has an approximately 30% case-fatality rate that increases to seventy-five percent in groups at high-risk which include pregnant women, fetuses, neonates, persons above 60years and immunocompromised adults [7, 8]. The obvious upsurge of contamination in food industry particularly poultry and meat products by pathogens has brought great public health concern. Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, however, *L. monocytogenes* is solely and consistently related to human illness [9].

Listeria species has been isolated from poultry and red meat products in several countries such as Yugoslavia,

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Belgium, New Zealand, Australia and Japan [10]. The existence of *Listeria* species in meat calls for public health concerns in relations to the safety of consumers, as these *Listeria* species have the capability of developing on both cooked and raw meat during refrigeration [10]. *Listeria* species particularly *L. monocytogenes* has been related to an extensive diversity of food sources mainly chicken and red meat [11].

Genus *Listeria* contains ubiquitous bacteria that are extensively disseminated in normal environments. The ubiquitous trait of these pathogens unavoidably results in contamination of many foods and meat products [10]. *Listeria* species are resistant to harsh environments such as high salt environment, low temperatures and low pH [12, 13]. Consequently, they are present in a diversity of environments which include foods, water, soil, effluents, silage and sewage. Members of *Listeria* genera are extensive in the surroundings and spreading to animals. The results of their occurrences are advanced in the environment via milk, blood and faeces. In these conditions, food substances of the animal source are opened to contamination in a substantial degree that could happen in the course of transportation, processing and storage [14, 15]. Although milk and its products remained categorized to be mainly accountable in circumstances of listeriosis owing to food consumptions, researchers have revealed that *L. monocytogenes* can contaminate meat [16].

With globalization and intensified consumption of ready-to-eat foods globally, it is barely astonishing that *L. monocytogenes* has emerged as a vital foodborne pathogen of public health implication [17]. This study evaluated the occurrence of *L. monocytogenes* in retail meat and pork sold in different markets within Port Harcourt metropolis. It is necessary because of the significance of *L. monocytogenes* as an emerging pathogen of public health concern and considering the role and nutritional benefits of meat and pork as an essential part of human diet especially with the growing habit of eating half cooked meat (sushi meals) that is gradually being take in this part of the world.

Hence, the need to study the prevalence and antibiotic resistance of *L. monocytogenes* isolated from beef and pork which are commonly consumed in a developing country like Nigeria. Thus, the aim of this study was to assess the prevalence of *Listeria monocytogenes* in beef and pork meat from selected markets in Port Harcourt metropolis, as well as the drug sensitivity pattern of *L. monocytogenes,* isolates from the sample area.

2. Material and Methods

Study area

This study was conducted with beef and pork samples purchased at five markets viz Trans-Amadi Market, Rumuokoro Market, Creek Road Market, Mile 1 Market, and Mile 3 Market all within Port Harcourt metropolis. Samples were collected from five markets within Port Harcourt metropolis, they include Rumuokoro market (RM), Trans-Amadi slaughter market (TA), Mile 1 market (MM), Mile 3 market (MT) and Creek Road market (CR). The sampling was conducted between November 2013 and March 2014 during which samples were collected from the different markets periodically for analysis.

Sample Collection and Preparation

Fifty beef and fifty pork meat samples were examined for the presence of *Listeria monocytogenes*. They were subjected to microbiological analysis to determine their microbial quality. Samples were collected from the five markets used as a study area and aseptically transported to the Laboratory in cold-boxes. Samples were kept at 4°C and analyzed within 2 hours. The samples were given specific labels based on the location and type of meat (beef or pork). Trans-Amadi Market Meat (TMM), Trans-Amadi Market Pork (TMP), Rumuokoro Market Meat (RMM), Rumuokoro Market Pork (RMP), Mile-1 Market Meat (MMM), Mile-1 Market Pork (MMP), Mile-3 Market Meat (MTM), Mile-3 Market Pork (MTP), Creek Road Market Meat (CRM) and Creek Road Market Pork (CRP). All media were prepared and sterilized at 121°C for 15 min in line with the manufacturer's stipulation. Twenty-five grams of each sample with 225mls sterile 0.1% peptone water was placed in a stomacher bag for two minutes homogenization. A ten-fold serial dilution in sterile peptone water was prepared.

Enumeration, Isolation and Identification of *Listeria Monocytogenes* and *Listeria* Species

These were done according to the International Standards Organization (ISO 11290-1) methods of 1996 and 2004 [18, 19]. Briefly, Fraser broth (Oxoid, CM0895) was prepared and sterilized at 121°C (15 lbs. psi) for 15 min in line with the manufacturer's stipulation. Spread plate method was used in which 0.1ml of 10^{-4}-10^{-6} was spread plated in duplicates on the different media. The plates were incubated at 37°C for 24h. Selective plating and characterization of *Listeria* colonies were carried out using Polymixin Acriflavine Lithium Chloride Ceftazidime Aesculine Mannitol (PALCAM) agar. The main discriminating enhancement step involves using a selective broth with reducing concentrations of Fraser broth. Also, 25g of meat samples were aseptically dispensed into a sterile stomacher bag enclosing 225ml of half Fraser broth (Oxoid CM0895, Basingstoke, United Kingdom) to attain a 1: 10 dilution factor [20, 21]. The mixture was vigorously mixed for 2min in the stomacher circulator (Unit 400, Seward, United Kingdom) and was incubated at 30°C for 24h. The second selective enhancement medium used is Fraser Broth (Oxoid, CM0895). After the incubation period, 0.1ml of pre-enriched cultures from half-Fraser broth culture was decanted into 10ml of Fraser broth for further enrichment. This was incubated at 37°C for 48h [20, 21]. A loopful of positive Fraser broth enriched culture was speckled onto PALCAM agar plates (Oxoid, CM0856) prepared and supplemented as directed. The plates were incubated at 37°C for 24 to 48h and thereafter observed for distinct colonies of *Listeria*. Proof of

identity of *Listeria* colonies on PALCAM agar centred on their ability to hydrolyzed aesculin (blackening of the medium) and fermentation of mannitol (colour change from grey or red to yellow) owing to the production of acids [22] (Molla *et al*., 2004).

Confirmation of *Listeria monocytogenes* and *Listeria* species

Distinct colonies of *Listeria* were transferred onto pre-dried Tryptone Soya Agar (Oxoid, M290) plates complemented by 0.6 percent of Yeast Extract Powder ([TSYEA], Oxoid, LP0021) and incubated at 37°C for 24 hours after which further biochemical characterizations were carried out. Confirmation of *Listeria* isolates was based on motility, Gram reaction, catalase, and oxidase and further confirmed by their haemolytic ability on blood agar and their ability to ferment rhamnose mannitol and xylose sugar [23, 24]. Confirmation was also based on Christe Atkins Munch Peterson (CAMP) test according to ISO 11290-1 techniques [18, 19] (ISO, 1996, 2004).

Antibiotic Susceptibility Testing

Antibiotic sensitivity and resistance profile of *L. monocytogenes* isolate on Mueller Hinton Agar (MHA, Oxoid) were carried out using the disk diffusion technique. An inoculum from a cell suspension of approximately 10^6cells/ml was used for the test. The cell suspension was prepared by inoculating sterile normal saline with a pure culture of the test organism and incubating for 4hrs. Following this, the cell suspension turbidity was attuned to equal 0.5 McFarland Standard (0.5ml of 1% $BaCl_2$ + 99.5ml of 1% H_2SO_4). To confirm the matching, the turbidities were also read through a spectrophotometer at 625nm prior to inoculation. The inoculum was applied onto Mueller-Hinton Agar (MHA, Oxoid) plates. These were left to dry at room temperature prior to aseptically application of the antibiotic discs and incubated at 37°C for 24h. A Clear zone of inhibition diameter was measured (in mm) and interpreted agreeing to the British Society for Antimicrobial Chemotherapy guidelines [25]. The activities of 14 antibiotics used in this study and the interpretations of the diameters of zone of inhibitions of the isolates were categorized as resistant, intermediate or susceptible in line with the recommendations by BSAC [25] standards. The abbreviations and concentration of the antibiotic discs (Oxoid, Australia) used were Ampiclox (APX) 20µg; Ciprofloxacin (CPX) 10µg; Chloramphenicol (CHL), 30µg; Rifampicin (RF) 5µg; Erythromycin (ERY) 5µg; Tetracycline (TET) 10µg; Gentamicin (GEN) 10µg; Amoxil (AML) 20µg; Augmentin (AUG) 30µg; Norfloxacin (NFX) 10µg; Levofloxacin (LEV) 20µg; Streptomycin (STR) 30µg; Clotrimoxazole (COT) 25µg; and Cloxacillin (CXC) 5µg.

3. Results

CAMP test

The Christie-Atkins-Munch-Peterson test was used for confirmation of *Listeria*. All *Listeria* species were small colonies, positive for catalase and motile Gram-positive rods. They hydrolyze esculin and ferments dextrose sugar. Some of the species ferments rhamnose, mannitol and xylose with the release of acid. *L. grayi* ferments mannitol with the release of acid. *L. monocytogenes* and *L. seeligeri* induce hemolytic activities which were enriched in the zone predisposed by the *Staphylococcus aureus* streaked on sheep blood agar and were consequently CAMP test positive. However, *L. monocytogenes* does not utilize xylose and was positive for rhamnose utilization (Table 1).

Table 1. CAMP hemolytic activities of *Listeria* species

***Listeria* Species**	**CAMP reaction with *Staphylococcus aureus***
L. grayi	-
L. ivanovii	-
L. welshimeri	-
L. innocua	-
L. monocytogenes	+
L. seeligeri	+

Table 2. Cultural, morphological and biochemical characteristics of *Listeria* species isolated from fresh beef samples in Port Harcourt metropolis, Nigeria

Isolate code	Cell morphology	Gram rxn	Catalase	Oxidase	Motility	Haemolysis	Glucose	Mannitol	Xylose	Rhamnose	H_2S	CAMP test	Identified *Listeria* species
CRM3	Rod	+	+	-	+	-	+	-	-	-	-	-	*L. innocua*
RMM3	Rod	+	+	-	+	+	+	-	+	-	-	+	*L. seeligeri*
MTM4	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
TAM4	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
CRM5	Rod	+	+	-	+	+	+	-	+	-	-	-	*L. ivanovii*
CRM10	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
TAM10	Rod	+	+	-	+	-	+	+	-	-	-	-	*L. grayi*
TAM1	Rod	+	+	-	+	+	+	-	-	-	-	-	*L. innocua*

Key: CRM= Creek Road Market Meat, TAM= Trans-Amadi Market Meat, MTM= Mile 3 Market Meat, RMM= Rumuokoro Market Meat, + = Positive, - = Negative

Table 3. Cultural, morphological and biochemical characteristics of *Listeria* species isolated from fresh Pork samples in Port Harcourt metropolis, Nigeria

Isolate code	Cell morphology	Gram rxn	Catalase	Oxidase	Motility	Haemolysis	Glucose	Mannitol	Xylose	Rhamnose	H_2S	CAMP test	Identified *Listeria* species
CRP1	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
MTP1	Rod	+	+	-	+	-	+	-	-	-	-	-	*L. innocua*
CRP2	Rod	+	+	-	+	-	+	-	-	-	-	-	*L.innocua*
RMP4	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
TAP4	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
RMP5	Rod	+	+	-	+	+	+	-	+	-	-	-	*L. ivanovii*
CRP5	Rod	+	+	-	+	-	+	-	-	-	-	-	*L.innocua*
TAP5	Rod	+	+	-	+	-	+	+	-	-	-	-	*L. grayi*
CRP6	Rod	+	+	-	+	+	+	-	-	+	-	+	*L. monocytogenes*
TAP6	Rod	+	+	-	+	-	+	-	-	-	-	-	*L. innocua*
TAP8	Rod	+	+	-	+	-	+	-	-	-	-	-	*L. innocua*

Key: CRP= Creek Road Market Pork, MTP= Mile 3 Market Pork, RMP= Rumuokoro Market Pork, TAP= Trans-Amadi Market Pork, MMP= Mile 1 Market Pork, + = Positive, - = Negative

Overall Prevalence of *Listeria* species in beef and Pork meat samples in Port Harcourt metropolis, Nigeria

Of the total 100 meat samples analyzed, 19(19.0%) confirmed the presence of *Listeria* species. Of these 19 isolates, 7(36.8%) were *Listeria monocytogenes* while 12(63.2%) were other *Listeria* species [*L. ivanovii* 2(10.5%), *L. innocua* 7(36.8%), *L. grayi* 2(10.5%), and *L. seeligeri* 1(5.3%)]. Of the 7 *Listeria monocytogenes* isolated, 3(42.8%) were found in beef and 4(57.1%) in pork (Table 4).

Table 4. Frequency of occurrence of *Listeria* species obtained from beef and Pork samples in Port Harcourt metropolis, Nigeria

Listeria Species	No. Positive (%)	Beef samples (%)	Pork samples (%)
L. monocytogenes	7(36.8)	3(42.8)	4(57.1)
L. ivanovii	2(10.5)	1(50.0)	1(50.0)
L. innocua	7(36.8)	2(28.5)	5(71.4)
L. welshimeri	0(0.0)	0(0.0)	0(0.0)
L. seeligeri	1(5.3)	1(100.0)	0(0.0)
L. grayi	2(10.5)	1(50.0)	1(50.0)
Total	**19(100.0)**	**8(42.1)**	**11(57.9)**

Prevalence of *Listeria Listeria* species in beef samples in Port Harcourt metropolis, Nigeria

Of the 50 beef samples analyzed, 8(16.0%) had *Listeria* species. Of these 8 isolates, 3(37.5%) were *Listeria monocytogenes* while 5(62.5%) were other *Listeria* species [*L. ivanovii* 1(12.5%), *L. innocua* 2(25.0%), *L. grayi* 2(12.5%), and *L. seeligeri* 1(12.5%)]. Trans-Amadi market and Creek road market had the highest number of positive samples for *Listeria* spp. with 3(37.5%) each, Rumuokoro market had 1(12.5%), Mile 3 market 1(12.5%) of the total samples while Mile 1 market had no positive sample (Table 5). Of the three *Listeria monocytogenes* isolated from beef meat, one beef meat sample each from Trans-Amadi (33.3%), Creek road (33.3%), and Mile-3 (33.3%) markets were positive for *L. monocytogenes*. Other *Listeria* species were also isolated from Trans-Amadi Market (*L. innocua* and *L. grayi*), only *L. seeligeri* was isolated from Beef meat from Rumuokoro Market and only *L. ivanovii* and *L. innocua* were obtained from Creek Road market. Mile-1 Market had no positive sample (Table 5). Only *Listeria monocytogenes* was isolated from beef meat collected from the Mile-3 market (Table 5).

Prevalence of *Listeria* species in Pork in Port Harcourt metropolis, Nigeria

Of the 50 Pork meat samples analyzed, a total of 11(22.0%) of the pork samples were positive for *Listeria* spp., of which 4(36.4%) were *L. monocytogenes*, *L. ivanovii* 1(9.1%), *L. innocua* 5(45.4%), and *L. grayi* 1(9.1%). Trans-Amadi Market (36.4%) and Creek road Market (36.4%) had the highest number of positive samples (n=4) each of the total pork samples analyzed. Rumuokoro Market had 2(18.2%), Mile 3 Market had 1(9.1%) while Mile 1 market had no positive sample (Table 6). Of the 4(36.4%) *Listeria monocytogenes* isolated from pork meat, Trans-Amadi (12.5%) and Rumuokoro (12.5%) Markets had one sample each that was positive for *L. monocytogenes* while Creek Road Market had two (50.0%) positive samples. Trans-Amadi Market also had three samples positive for other *Listeria* species; Creek Road Market had two while Rumuokoro and Mile 3 Markets had one each. Mile 1 Market had no positive sample (Table 6).

The Antimicrobial profile of the *Listeria monocytogenes* strains

The antimicrobial profile of the *Listeria monocytogenes* strains was also assessed by using the standard disc diffusion method (Muller Hinton Agar) and it was tested against 14 antimicrobial drugs. The sensitivity pattern of *L. monocytogenes* strains to 14 antibiotics are presented in Table 7. It showed 57.1% (8/14) overall percentage resistance and 35.7% (5/14) susceptibility. All isolates (100.0%) were susceptible to Gentamicin, Chloramphenicol, Ampiclox and Clotrimoxazole and Streptomycin and all isolates (100.0%) were resistant to Erythromycin, Tetracycline, Cloxacillin, Augmentin, and Rifampicin, with varying degree of susceptibility (0.0% to 71.4%) to others (Table 7).

Table 5. Frequency of occurrence of *Listeria* species obtained from beef samples collected from different markets in Port Harcourt metropolis, Nigeria

Market/*Listeria* species	No. (%)	Number of Samples Positive for *Listeria* species				
		Trans-Amadi	Rumuokoro	Creek Road	Mile 1	Mile 3
L. monocytogenes	3(37.5)	1(33.3)	0(0.0)	1(33.3%)	0(0.0)	1(33.3%)
L. ivanovii	1(12.5)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)
L. innocua	2(25.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)
L. grayi	1(12.5)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
L. seeligeri	1(12.5)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)
Total	**8(16.0)**	**3(37.5)**	**1(12.5)**	**3(37.5)**	**0(0.0)**	**1(12.5)**

Table 6. Frequency of occurrence of *Listeria* species obtained from Pork sold in Port Harcourt Metropolis, Nigeria

Market/*Listeria* Species	No. (%)	Number of Samples Positive for *Listeria* species				
		Trans-Amadi	Rumuokoro	Creek Road	Mile 1	Mile 3
L. monocytogenes	4(36.4)	1(12.5)	1(12.5)	2(50.0)	0(0.0)	0(0.0)
L. ivanovii	1(9.1)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)
L. innocua	5(45.4)	2(40.0)	0(0.0)	2(40.0)	0(0.0)	2(20.0)
L. grayi	1(9.1)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
L. seeligeri	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	**11(22.0)**	**4(36.4)**	**2(18.2)**	**4(36.4)**	**0(0.0)**	**1(9.1)**

Table 7. Activity of antimicrobial agents tested against strains of *L. monocytogenes* isolated from beef and pork samples in Port Harcourt Metropolis, Nigeria

Antimicrobial agent	ug/disc	Resistant (%)	Intermediate (%)	Susceptible (%)
Ciprofloxacin	10	5(71.4)	2(28.6)	0(0.0)
Norfloxacin	10	4(57.2)	3(42.8)	0(0.0)
Gentamicin	10	0(0.0)	0(0.0)	7(100.0)
Amoxil	20	0(0.0)	0(0.0)	0(0.0)
Streptomycin	30	0(0.0)	0(0.0)	7(100.0)
Rifampicin	20	7(100.0)	0(0.0)	0(0.0)
Erythromycin	30	7(100.0)	0(0.0)	0(0.0)
Chloramphenicol	30	0(0.0)	0(0.0)	7(100.0)
Ampiclox	20	0(0.0)	0(0.0)	7(100.0)
Levofloxain	20	5(71.4)	2(28.6)	0(0.0)
Clotrimoxazole	25	0(0.0)	0(0.0)	7(100.0)
Cloxacillin	5	7(100.0)	0(0.0)	0(0.0)
Augmentin	30	7(100.0)	0(0.0)	0(0.0)
Tetracycline	10	7(100.0)	0(0.0)	0(0.0)
Total percentage		**8/14(57.1)**	**3/14(21.4)**	**5/14(35.7)**

4. Discussion

In the present study, a total of hundred meat samples, fifty each of beef and pork across five different locations within Port-Harcourt metropolis were tested. Of the total 100 meat samples analyzed, 19(19.0%) had *Listeria* species, of which 7(36.8%) were *Listeria monocytogenes* while 12(63.2%) were other *Listeria* species [*L. ivanovii* 2(10.5%), *L. innocua* 7(36.8%), *L. grayi* 2(10.5%), and *L. seeligeri* 1(5.3%)]. The presence of *L. monocytogenes* in beef and pork meat samples is line with findings in previous studies and it has been reported in many countries, thus, underlining that contaminations may have occurred in the course of processing meat to the end and ready-to-eat products [26].

Of the 7(36.8%) *Listeria monocytogenes* isolated in this study, 3(42.8%) were found in beef meats and 4(57.1%) in pork meats samples. This result is in discordance with other studies regards to the presence of *L. monocytogenes* in foods. Eruteya *et al*. [27] recorded a rate of 1.29% of *L. monocytogenes* in raw cow and goat meat; Salihu *et al*. [28] reported 4.7% prevalence in beef. A number of authors reported a 4.65% and 6.4% (Bulgaria), 5.1% (Ethiopia), 6.66% (India), 17.7% (Portugal), 20.0% (Greek), 31.0% (Denmark) and 35.0% (Spain) prevalence of *L. monocytogenes* from raw beef. However, the high prevalence reported for *L. monocytogenes* in this study, 42.8% in beef meats and 57.1% in pork meats are in agreement with previous findings. Other authors had significantly higher percentage prevalence. Yusuf and Abdul-Hamid [29] recorded an incidence rate of 60.0% for *L. monocytogenes* in Kilishi, a meat product. Previous studies reported the prevalence of Listeria to be in the range of 0.0%-68.0% in pork meats [30-34].

The prevalence reported for *L. monocytogenes* in this study is higher than what was reported by some authors. Adetunji and Olaoye [35] also reported a rate of 20.0% in milk; while Nwachukwu *et al*. [36] recorded an even higher level in water samples; Gamboa *et al*. [37] reported 33.9% and Akya *et al*. [38] reported 27.2% in meat products. However, Okonko *et al.* [39] reported no occurrence of *L. monocytogenes* in kilishi. Okonkwo *et al.* [40] reported no occurrence of *L. monocytogenes* in raw vegetables and meat. Other previous studies in Nigeria, India, Serbia and Bangkok also reported the inability to isolate *L. monocytogenes* in beef and pork meat making it obvious that the occurrence rates of *L. monocytogenes* may vary from one place to the other depending on the methods used and also the predisposing conditions.

The overall findings in this study coincide with that of previous studies which specified 1.0%-70.0% *Listeria* species prevalence in beef samples [22, 32, 34]. The custom of eating raw or undercooked meat (e.g. sushi) aggravates the public health risk related to *L. monocytogenes* [22]. The prevalence of *L. monocytogenes* in this study was higher in the pork meat samples with a prevalence rate of 8.0% than in beef meat samples (6.0%). It is also in disagreement with the work carried out by Molla *et al.* [22] in which an incidence rate of 7.5% was recorded in Pork meat and which was higher than all other meat samples. Lanciotti *et al*. [41] also observed the high occurrence of *L. monocytogenes* (17.6%) in pork in their study.

The occurrence of these pathogens in swine is linked to the detail that swine are vectors of *L. monocytogenes*. For instance, it has been exposed that pork lodges *L. monocytogenes* in intestine and tonsils [42-44]. Owing to the facts, if there is rupture of the organ in the course of evisceration, the carcass can be contaminated with this pathogen. Even though it is recurrent for processing plants, slaughters and carcasses to be contaminated with *L. monocytogenes*, a subsequent daily wash of slaughtering slabs and sterilizing techniques ought to remove contamination. Meat products that are free of pathogens are contingent upon the efficiency of these sterilizing procedures [42].

In this study, *L. monocytogenes* and *L. innocua* were most predominant with a prevalence rate of 7(36.8%) each, as compared with other species of Listeria. Thus, of all the *Listeria* species isolated, *L. monocytogenes* and *L. innocua* had an occurrence rate of 36.8% each, *L. ivanovii* and *L. grayi*, 10.5 % each while *L. seeligeri* had 5.3% occurrence of all the *Listeria* species. This is in agreement with other studies, Skovgaard and Morgen [45] showed that the occurrence of *L. innocua* as the dominating species in beef and poultry products. Also, Barros *et al*. [26] reported a higher prevalence of *L. innocua* in meat than all other *Listeria* species. Former studies showed *L. innocua* as the most predominant *Listeria* species observed in foods [22, 41].

Antibiotic susceptibility testing of the strains of *L. monocytogenes* isolated from beef and pork meats showed that an overall percentage resistance to be 57.1% and susceptibility to being 35.7%. All isolates (100.0%) were susceptible to chloramphenicol, gentamicin, ampiclox, cotrimoxazole and streptomycin. It also showed that they were all isolates (100.0%) resistant to augmentin, erythromycin, tetracycline, rifampicin, and cloxacillin. The isolates also showed varying degree of resistance to norfloxacin (4 resistant, 57.2%), levofloxacin (5 resistant, 71.4%), and ciprofloxacin (5 resistant, 71.4%). The high resistance of *L. monocytogenes* to these antibiotics could be owing to recent proliferation in the application of antibiotics as growth promoters [46]. Sometimes, antibiotics were added to animal feed for increased bulk of the animals.

The 100.0% susceptibility of *L. monocytogenes* to gentamicin and ciprofloxacin as observed in our study was previously reported by Rahimi *et al*. [47] and Yakubu *et al*. [46]. They observed that more than 80.0% of *L. monocytogenes* were sensitive to these antibiotics.

The 100.0% resistance of *L. monocytogenes* to erythromycin in the present study is in conflict with what was reported in Botswana where Morobe *et al*. [48] reported that all isolates in their study were sensitive to erythromycin. It is also in agreement with the study by Adetunji and Olaoye [35] in which 100.0% resistance was observed for augmentin

and cloxacillin. High resistance observed for augmentin and cloxacillin (100.0%) shows that these widely used antibiotics will be unsuccessful in the treatment of Listeriosis. This was similarly observed in Ado-Ekiti where David and Odeyemi [49] reported that environmental isolates of *L. monocytogenes* were resistant to augmentin and cloxacillin. The observed resistance to antibiotics could be owing to selective antibiotic pressure [50].

The multi-drug resistance (MDR) observed in the present study has been previously observed by Lotfollahi *et al*. [51] and Yakubu *et al*. [46]. Yakubu *et al*. [46] in their study observed that 20.0% of isolates obtained from dairy foods showed resistance to two or more antibiotics. Yakubu *et al*. [46] also stated that none of the isolates in their study was resistant to less than one antibiotic. These observations are similar to what we observed in the present study with all isolates (100.0%) been resistant to greater than two antibiotics. This observation was also supported by Lotfollahi *et al*. [51] who found multi-drugs resistances to antibiotics in *L. monocytogenes* strains obtained from humans.

5. Conclusions

This present study has further confirmed the existence of *L. monocytogenes* in beef and pork meat samples in Port Harcourt metropolis, Nigeria, which infers that likelihoods of acquiring listeriosis are significantly increased when undercooked meat and meat products are consumed. The presence of *Listeria monocytogenes* in meats is a cause for public health concerns because of the danger this poses especially through cross contamination and inadequate cooking of meat products. From the present finding, it can be inferred that *Listeria monocytogenes* is gradually becoming multi-drug resistant with emerging strains displaying multi-drug resistance (8 out of 14 drugs tested). There is a need for relevant public health agencies in Nigeria to create awareness to vendors and consumers about *Listeria* in food and its potential as a food pathogen of interest. In future, a sustained surveillance for emerging multi-drug resistance of this pathogen to antibiotics is highly essential. Also, this study advocates the essential for enhanced food safety by application of aseptic procedures at all stages of manufacture to consumption with specific highlights on ready-to-eat food substances which need no additional heat action.

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REFERENCES

[1] Bhandare, S.G., Paturkar, A.M., Warskar, V.S. and Zende, R.J. (2009). Bacteriological Screening of environmental sources of contamination in an abattoir and meat shops in Mumbai, India. *Asian Journal of Food* and *Agro-Industry*. 2(03), 280-290.

[2] Podpecan, B., Pengov, A., Vadnjal, S. (2007). The source of contamination of ground meat for the production of meat products with bacteria *Staphylococcus aureus*. *Slovenian Veterinary Research*, 44: 24 – 30.

[3] Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M., and Laegreid, W. W. (2000). Correlation of entero hemorrhagic *Escherichia coli* 0157 Prevalence in feces, hides and carcasses of beef cattle during processing. *Proceedings of the National Academy of Science*, 97: 2999-3003.

[4] Madden, R. H., Espie, W. E., Moran, L., McBride, J. and Scates, P. (2001). Occurrence of *E. coli* 0157:H7, *Listeria monocytogenes, Salmonella and Campylobacter spp.* on beef carcasses in Northern Ireland. *Meat Science*, 58: 343-346.

[5] Vitas, A. I., Aguado, V and Garcia-Jalon, E. I. (2004). Occurrence of *Listeria monocytogenes* in Fresh and Processed Foods in Navarra (Spain) *International Journal of Food Microbiology*. 90:349-356.

[6] Awaisheh, S. S. (2010). Incidence and Contamination Level of *Listeria monocytogenes* and other *Listeria spp.* In Ready-to-Eat Meat Products in Jordan. *Journal of Food Production*. 73(3): 535-540.

[7] Rocourt, J., BenEmbarek, P., Toyofuku, H. and Schlundt, J. (2003). Quantitative Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods: The FAO/WHO Approach. *FEMS Immunology and Medical Microbiology* 35: 263-267.

[8] Goulet, V., Hedberg, C., Monnier, A. Le. and de. Valk, H. (2008). Increasing Incidence of Listeriosis in France and Other European Countries. *Emerging Infectious Diseases*. 14:734-740.

[9] Seafood Network Information Center (2007). Compendium of Fish and Fishery Product Processes, Hazards and Controls. Chapter 15: *Listeria monocytogenes. http://seafood.ucdavis.edu/haccp/compedium/chapt15.html.* accessed 23/06/2014.

[10] Abd El-Malek, A. M., Hassan Ali, S. F., Hassanein, R., Abdelazeem, M., and Elscyh, K. I. (2010). Occurrence of *Listeria species* in Meat, Chicken products and Human Stools in Assiut City, Egypt with PCR use for rapid Identification of *Listeria monocytogenes. Veterinary World*. 3(8): 353-359.

[11] Endang, P., Radu, S., Ismail, A., Kgueen, C. Y., and Maurice, L. (2003). Characterization of *Listeria monocytogenes* isolated from chicken meat. Evidence of conjugal transfer of Plasmid-mediated Resistance to Antibiotic. *Journal of Animal Veterinary Advancement* 2: 237-246.

[12] Sleator, R. D., Gahan, C. G. M., and Hill, C. (2003). A Postgenomic Appraisal of Osmotolerance in *Listeria monocytogenes*. *Applied and Environmental Microbiology* 69: 1-9.

[13] Liu, D., Lawrence, M., Austin, F. W., and Ainsworth, A. J. (2005). Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *Federation of European Microbiological Societies Microbiology Letters* 243: 373-378.

[14] Schlech III. W.F. (2000). Foodborne listeriosis. *Clinical Infectious Diseases* 31:770-775.

[15] Schlech, W. F., Haldane, H., Mailman, T. L., Warhuus, M., Grouse, N., Haldane, D. J. M. (2005). Does sporadic *Listeria* gastroenteritis exist? A 2-year population based survey in Nova Scotia, Canada. *Clinical Infectious Disease* 41: 778-784.

[16] Tirziu, E., Nichita, I., Cumpanasoiu, C., Gros, R.V., and Seres, M. (2010). *Listeria monocytogenes* Monographic Study. *Animal Science and Biotechnologies.* 43(1):441-446.

[17] Jeyaletchumi, P., Tununa, R., Margaret, S. P., Son, R., Farinazleen, M. G., and Cheah, Y. K. (2010). Detection of *Listeria monocytogenes* in Foods. *International Food Research Journal* 17: 1 – 11.

[18] International Organization for Standardization 11290-1. (ISO, 1996). Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the detection and Enumeration of *Listeria monocytogenes*. –Part 1: Detection method. 1-16.

[19] International Organization for Standardization 1129-1. (ISO, 2004). Microbiology of Food and Animal feeding stuff - Horizontal Method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection Method, Geneva, Switzerland. 1 – 20.

[20] Gurler Z, Pamuk S, Yildirim Y, Ertas N. (2015). The microbiological quality of ready-to-eat salads in Turkey: A focus on *Salmonella* spp. and *Listeria monocytogenes.* International Journal of Food Microbiology, 196: 79–83.

[21] Niyonzima E, Ongol MP, Brostaux Y, Koulagenko NK, Daube G, Kimonyo A, Sindic M. (2017). Consumption patterns, bacteriological quality and risk factors for *Salmonella* contamination in meat-based meals consumed outside the home in Kigali, Rwanda. Food Control 73(Part B): 546–554.

[22] Molla, B., Yilma, R., and Alemayehu, D. (2004). *Listeria monocytogenes* and other *Listeria species* in Retail meat and milk products in Addis Ababa, Ethiopia. *Ethiopia Journal of Health Development*. 18(3): 208 – 212.

[23] Janzten, M. M., Navas, J., Corujo, A., Moreno, R., Lopez, V. and Martinez-Suarez, J. V. (2006). Review Specific Detection of *Listeria monocytogenes* in food using commercial methods: from chromogenic media to real-time PCR. *Spanish Journal of Agricultural Research*, 4(3): 235-247.

[24] Jemmi, T. and Stephen, R. (2006). *Listeria monocytogenes*: Foodborne pathogen and hygiene indicator. *Review of Science and Technology* 25: 571-580.

[25] British Society for Antimicrobial Chemotherapy (BSAC) (2013). BSAC Methods for Antimicrobial Susceptibility Testing, version 12.

[26] Barros, M. A. F., Nero, L. A., Silva, L. C., d'Ovidio, L., Monteiro, F. A., Tamanini, R., Fagnani, R., Hofer, E. and Beloti, V. (2007). *Listeria monocytogenes:* Occurrence in Beef and Identification of the Main Contamination Points in Processing Plants. *Journal of Meat Science* 76: 591-596.

[27] Eruteya, O. C., Odunfa, S. A. and Lahur, J. (2014). *Listeria spp.* in Raw Cow and Goat Meat in Port Harcourt, Nigeria. *British Biotechnology Journal* 4(2): 204-214.

[28] Salihu, M. D., Junaidu, A. U., Manga, S. B., Gulumbe, M. L., Magaji, A. A., Ahmed, A., Adamu, A. Y., Shittu, A. and Balarabe, I. (2008). Occurrence of *Listeria monocytogenes* in Smoked fish in Sokoto, Nigeria. *African Journal of Biotechnology*. 7(17): 3082-3084.

[29] Yusuf, M. A. and Abdul Hamid, T. H. A. (2013). Isolation and identification of *Listeria sp* from ready-to-eat (RTE) kilishi in retailed outlet in Bauchi, Nigeria. *International Journal of Pharmaceutical Science Invention*. 2(1):22-25.

[30] Uytteendaele, M., Troy, P. D. and Debevere, J. (1999). Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *International Journal of Food Microbiology;* 53: 75 – 80.

[31] Farber, J. M. (2000). Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. *International Journal of Food Microbiology;* 62: 247-251.

[32] Rocourt, J. and Cossart, P. (2001). *Listeria monocytogenes. In*: Doyle, M. P., L. R. Beuchat and T. J. Montville (eds)*, Food microbiology fundamentals and frontiers.* ASM Press, Washington D. C. U.S.A, pp 337-351.

[33] Malik, S. V. S., Barduddhe, S. B. and Chaudhari, S. P. (2002). Listeric Infections in Humans and Animals in Indian Subcontinent: A review. *Tropical Animal Health Production*. 34:359-381.

[34] Dhanashree, B., Otta, S. K., Karaunasagar, I., Goebel, W. and Karaunasagar, I. (2003). Incidence of *Listeria species* in clinical and food samples in Mangalore, India. *Food Microbiology;* 20: 447-453.

[35] Adetunji, V. O. and Olaoye, O. O. (2012). Incidence and Antibiotic Susceptibility pattern of *Listeria monocytogenes* isolates from milk of West African Dwarf and Red Sokoto breeds of goat from Southwestern Nigeria. *New York Science Journal* 5(11):68-73.

[36] Nwachukwu, N.C., Orji, F.A., Iheukwumere, I., Ekeleme, U.G. (2010). Antibiotic-Resistant Environmental Isolates of *Listeria monocytogenes* from Anthropogenic Lakes in Lokpa-Ukwu, Abia State of Nigeria. *Australian Journal of Basic and Applied Sciences*. 4:1571-1576.

[37] Gamboa-Marin, A., Sonia Buitrago, M., Perez-Perez, K., Mercado, M., Poutou-Pinales, R., and Carrascal-Camacho, A. (2012). Prevalence of *Listeria monocytogenes* in Pork-meat and other processed products from the Colombian Swine Industry. *Revista MVZ Cordoba*, 17(1):2827 – 2833.

[38] Akya, A., Najafi, F., Moradi, J., Mohebi, Z., and Adabagher, S. (2013). Prevalence of food Contamination with *Listeria spp*. In Kermanshah, Islamic Republic of Iran. *Eastern Mediterranean Health Journal* 19(5): 474 – 477.

[39] Okonko, I.O., Odu, N.N. and Igboh, I.E. (2013). Microbiological Analysis of Kilishi sold in Port Harcourt, Nigeria. *New York Science Journal* 6(7): 37-43.

[40] Okonkwo, L. N., Chukwuezi, F. O. and Ozuogwu, N. E. O. (2014). Isolation of *Listeria monocytogenes* from Raw vegetables and meat sold in open market in Onitsha metropolis, Anambra State, Nigeria. *Advances in Biological Research* 8(2): 79-82.

[41] Lanciotti, R., Gardini, F., Bandini, G., Vannini, F. and Guerzoni, M. E. (1999). Survey of the incidence of *Listeria species* in the food of animal origin in northern Italy. *Advance Food Science.* 21(5/6): 197-202.

[42] Hellstrom, S., Laukkanen, R., Siekkinen, K. M., Ranta, J., Maijala, R., Korkeala, H. (2010). *Listeria monocytogenes* in pork can originate from farms. *Journal of Food Protection;* 73(4): 641 – 648.

[43] Kanuganti, S. R., Wesley, I. V., Reddy, P. G., Mckean, J., Hurd, H. S. (2002). Detection of *Listeria monocytogenes* in Pigs and Pork. *Journal of Food Protection*. 65(9): 1470 – 1474.

[44] Thevenot, D., Dernburg, A., Vernozy-Rozand, C. (2006). An updated review of *Listeria monocytogenes* in the pork meat industry and its products. *Journal of Applied Microbiology;* 101: 7 – 17.

[45] Skovgaard, N. and Morgen, C. A. (1988). Detection of *Listeria spp*. in faeces from animals, in feeds, and in raw foods of animal origin. *International* Journal *of Food Microbiology*. 6:229-242.

[46] Yakubu, Y., Salihu, M. D., Faleke, O. O., Abubakar, M. B., Junaidu, A. U., Magaji, A. A., Gulumnbe, L and Aliyu, R. M (2012). Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in raw milk from cattle herds within Sokoto metropolis, Nigeria. *Sokoto Journal of Veterinary Sciences*. 10 (2): 13-17.

[47] Rahimi, E., Momtaz, H., Sharifzadeh, A., Behzadnia, A., Ashtari, M. S., Zandi Esfahani, S., Riahi, M., and Momeni, M. (2012). Prevalence and Antimicrobial Resistance of *Listeria species* Isolated from traditional dairy products in Chahar Mahal and Bakhtiyari, Iran. *Bulgarian Journal of Veterinary Medicine*. 15(2): 115 – 122.

[48] Morobe, I. C., Obi, C. L., Nyila, M. A., Gashe, B. A. and Matsheka, M. I. (2009). Prevalence, antimicrobial resistance profiles of *Listeria monocytogenes* from various foods in Gaborone, Botswana. *African Journal of Biotechnology*. 8:6383-6387.

[49] David, O. M. and Odeyemi, A. T. (2007). Antibiotic resistant pattern of environmental isolates of *Listeria monocytogenes* from Ado-Ekiti, Nigeria. *African Journal of Biotechnology* 6: 2135-2139.

[50] Hanchung, Y., Sheng, C., David, G.W., Shouhua, Z., Patrick, M.D., Robert, W. and Jianghong, M. (2004). Characterization of multiple antimicrobial resistant *Escherichia coli* Isolates from Chicken and Swine in China. *Journal of Clinical Microbiology*. 42:3484-3489.

[51] Lotfollahi, L., Nowrouzi, J., Irajian, G., Masjedian, F., Kazemi, B., Eslamian, L., Falahat, A., Ramez, M. (2011). Prevalence and antimicrobial resistance profile of *Listeria monocytogenes* in spontaneous abortions in humans. *African Journal of Microbiological Research*. 5:1990-1993.