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ANTIBIOTIC SUSCEPTIBILITY OF *LISTERIA* SPECIES IN SOME FROZEN, SMOKED AND CANNED FISH SOLD IN KADUNA NORTH L.G.A., NIGERIA.

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Abstract

This study was carried out to investigate theantibiotic susceptibility of *Listeria* species in frozen, smoked and canned fish in Kaduna North Local Government Area of Nigeria. A total of One hundred and eighty (180) fish samples comprising of 60 frozen, 60 smoked and 60 canned were purchased from the fish mongers at the three major markets in Kaduna North L.G.A (Kawo, Abubakar Gumi and UnguwarRimi markets). Sampling was done once a weekly. *Listeria* species were isolated using pre-enrichment selective medium and were identified by conventional biochemical tests. The antibiotic susceptibility was determined by Kirby-Bauer disk diffusion method on Muller Hilton Agar (Oxoid, UK) Nine antibiotics tested were Amoxicillin (25ug) Tetracycline (ug), Erythromycin (15ug), Gentamycin (30ug) Chloramphenicol (30ug) Ciprofloxacin (5ug) Streptomycin (10ug) Trimethoprin (5ug) and Oxacilin (1ug). Results showed that from the 180 samples, 9 of the positive isolates were *L. grayi*,while 15 were *L. ivanovii*found to be contaminated with *Listeria* species.Antibiotic susceptibility testing showed that*Listeria* sppisolates were susceptible to gentamicin (the first choice antibiotic) used in the treatment of listeriosis. The sensitivity regimens were 24(100%) for Gentamicin as the most effective, followed by 22(92%) for Chloramphenicol, 21(88.0%) for Amoxicillin and 19(79%) for Oxacillin. Antimicrobial resistance pattern Erythromycin (ER) occurred in 5 of the 24 *Listeria* species isolates. The antimicrobial resistance pattern showed by *Listeria* species in this study showed that there is a potential threat to health and safety of the public. Therefore it is recommended that the usage of antibiotics in both human and veterinary medicine should be monitored appropriately.

Key words: Antibiotics, susceptibility, Resistance, Fish, *Listeria* species.

INTRODUCTION

Listeria species are short, gram positive rods, catalase positive, oxidase negative and ferment carbohydrates (Halter *et al.*, 2012). Members of the genus are facultative anaerobes, non-sporing, non-acid fast bacteria and do not possess a capsule (Zhu *et al.*, 2005). *Listeria* species are motile by peritrichous flagella when grown at 20- 25°C and display a characteristic "tumbling" motility. The optimum growth temperature (but not for

motility) is 30-37°C (Rocourt, 1999).The bacterial genus *Listeria* currently comprises 10 species; *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri*, *Listeria grayi*, *Listeria marthii*, *Listeria rocourtiae*, *Listeria fleischannii* and *Listeria welhenstephanensis* (Zhang *et al.,* 2007; Halter *et al.,* 2012). *Listeria* speciesoccurs naturally in fresh and salt water, as well as in terrestrial environment such as livestock manure, decaying plant materials and in many raw foods associated with these environments (Gram, 2001; Zhu *et al.*, 2005; Ierene*t al.,* 2013).*Listeria* species has been isolated from river water and river sediment (Svanevik *et.al*. 2015), channels, ponds and lakes (Dijkstra, 1982;Svanevik *et.al*., 2015), as well as from sea water and water shorelines (Colburn *et al*., 1990). Because of that, it can be expected that *Listeria* speciescan be present in water organisms (Karunasagar and Karunasagar, 2000). The disease listeriosis usually occurs in high-risk groups, including pregnant women, neonates and immune compromised adults, but may occasionally occur in persons who have no predisposing underlying condition. Listeriosis is one of the most severe food borne infections, with low morbidity but high mortality of up to 30% (Rocourt *et al.,* 2001). Furthermore, there are seafood products that are eaten raw, without any listericidal step, such as cold-smoked and cold-salted fish.

Antibiotic resistance is a global public health issue. Although, the extent of the problem in the developing nations is certainly much more widespread and most of the data about the resistant bacterial strains are almost unknown (Richet*et al*., 2001). Antimicrobial resistance is a zoonotic health resulted threat. As in humans, the use of antimicrobial agents in aquatic organisms resulted in the emergence and spread of resistant bacteria. Resistant bacteria of aquatic organismsmay be passed to humans via the food chain, and may result in resistant infections. Antibiotic use whether for treatment or prophylaxis, or as performance enhancers will result in antibiotic resistant micro-organisms, not only among pathogens, but also among bacteria of the endogenous microflora of animals(Liu, 2006). However, different antibiotic resistance patternshave been reported in environmental, vegetable, fish and clinical sources (Ierene*t al.,* 2013; Chukwu *et al*., 2006 and Hansen, *et al*., 2005).

Because of its relatively low prices, a large sector of people in Kaduna North depend on fish as a source of protein which are imported from different geographical regions around the world.Generally, there is limited information to the best of our knowledge about the extent of *Listeria* species contamination in frozen, smoked and canned fish supplied in the study area. Therefore, the objective of this study was to determine the antibiotic susceptibility of *Listeria* species in some frozen, smoked and canned fish sold in Kaduna North L.G.A., Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Kaduna North Local Government Area of Kaduna state. It is located at Latitude 10º20'N and Longitude 7º 45' East. The city is located in the North West Geo-political zone of Nigeria.Kaduna North has an area of 72 km Sq. and a population of 364,575 according to 2006 census (KDSG, 2008).

Kaduna North falls within the Sudan savannah region; it's characterized by rainy and dry seasons with a little period of harmattan. Its headquarters is located at MagajinGari in the heart of Kaduna town. It has (3) districts; Doka, Kawo and Gabasawa respectively (KDSG, 2008).The inhabitants are mostly traders in various businesses and civil servants. In addition, it have relatively more livestock, poultry, and fish farms with fish marketing outlets which will enable field based research (FAO, 2007).

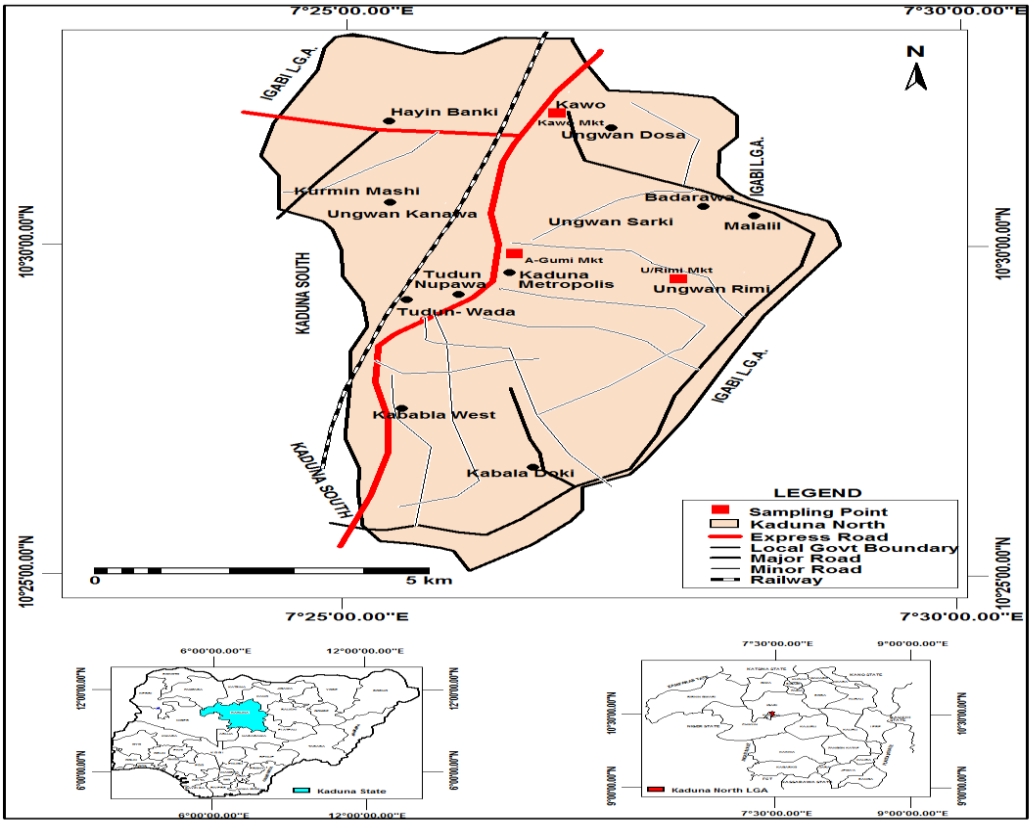


Figure 1: The Map of Kaduna North L.G.A, Nigeria showing the sampling areas.
Source: nigeriazipcodes.com Retrieved October 10 2015. 12:36 GMT

Study Design

A cross sectional study was carried out. Frozen, smoked and canned fish were purchased from three major markets in the study area for a period of 10 weeks (October, 2015-January, 2016).The required sample size was determined according to the formula as described by Campbell (1997) at 95% confidence interval using 9.67% (Shinkafi and Ukwaja, 2010).

$$n = t^2 \, x \, p \, (1 - p)/m^2$$

$$n = 1.96^2 \, x \, 0.0967 \frac{(1 - 0.0967)}{0.0025}$$

= 3.8416×0.08735/ 0.0025

n = 134.22

Therefore, n = 135; p was derived from the formula, and p-value of 9.67% (Shinkafi and Ukwaja, 2010), but for precision 45 samples were added and (n) was adjusted to 180.

Description

n = required sample size. t = confidence interval = 95 % (1.96). p = prevalence of the disease,
m = allowable error = 5 % (0.05)

Sample Collection and Transportation

A total of one hundred and eighty (180) samples which comprised of 60 frozen (20 samples from each market), 60 smoked (20 samples from each market) and 60 canned fish (20 samples from each market) were purchased from fish vendors and retail outlets of the major markets in three (3) districts of Kaduna North L.G.A. namely; Kawo Market, Abubakar Gumi market and UnguwarRimi market, based on convenience. Sampling was done once weekly for a period of 10 weeks (October, 2015- January, 2016), so as to ensure collection of new batches of fish samples.

Each sample was wrapped in sterile aluminum foil, packed and labeled appropriately in sterile polythene bags. Frozen fish were transported in a Coleman box containing ice packs to the Bacterial Zoonoses Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria for microbiological analysis and Multi-user Post Graduate Research Laboratory in the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria for heavy metal analysis. Information on the container/labels was recorded to include National Agency for Food and Drug Administration and Control (NAFDAC) number, manufacture and expiry dates, batch number and Manufacturer's address where available. Canned containers were examined for evidence of defects before purchase; those with defects were excluded from sampling.

4. Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined by Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Oxoid, Basingstoke, UK). The antibiotics that were tested included: Amoxicillin (25µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Erythromycin (15µg), Gentamicin (30µg), Streptomycin (10µg), Tetracycline (30µg), Trimethoprim (5µg) and Oxacillin (1µg).Three to five well isolated colonies of *Listeria* were transferred into 5ml on Mueller-Hinton broth (Oxoid) and incubated at 37°C for 16 to 18 hrs. The overnight culture broth was diluted using sterile distilled water to a turbidity equivalent to 0.5 McFarland standard (Harakeh *et al.,* 2005), and swabbed onto the entire surface of the dried Mueller-Hinton Agar (MHA, Oxoid) using sterile swab stick. The inoculated MHA plates were allowed to dry at room temperature before placing the antibiotic discs followed by incubation at 37°C for 24hrs. The zone of inhibition were measured in millimeter and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines (CLSI, 2011). The multiple antibiotic resistances (MAR) index was determined for each of the isolates. MAR index was calculated by dividing the number of antibiotics to which each isolate was resistant (a) by the total number of antibiotics to which the isolate was tested (b) (Singh *et al.,* 2010)

MAR index = a/b

MAR index = Multiple- antibiotic resistance indices above the bench score of 0.20

a = number of antibiotics to which each isolate was resistant to

b = total number of antibiotics to which the isolate was tested.

Data Analyses

Data was subjected to descriptive statistics using Statistical Package for Social Science (SPSS) version 20.0 (Ierene*t al.*, 2013). Probability less than or equal to($p \leq 0.05$) was considered statistically significant.

RESULTS

1. Antibiotic resistance of *Listeria* isolates from Fish obtained in Kaduna North L.G.A, Nigeria.

Figure 2 showed the susceptibilities of the 24 isolates tested against 9 antibiotics. From the percentage distribution of antibiotics susceptibilities of the 24 *Listeria* isolates to 9 antimicrobial agents, all the 24 *Listeria* isolates were susceptible (100%) to gentamicin. The isolates showed susceptibilities to the following antimicrobial agents at various percentages; amoxicillin(87.5%),chloramphenicol(91.7 %), ciprofloxacin(58.3%), erythromycin(37.5%), streptomycin(66.7%),tetracycline(66.7%), trimethoprim(70.8%), oxacillin(79.2%) and chloramphenicol(91.7%). The isolates showed least resistance to oxacillin(20.8%), amoxicillin(12.5%) andchloramphenicol(8.3%), (Figure 2).

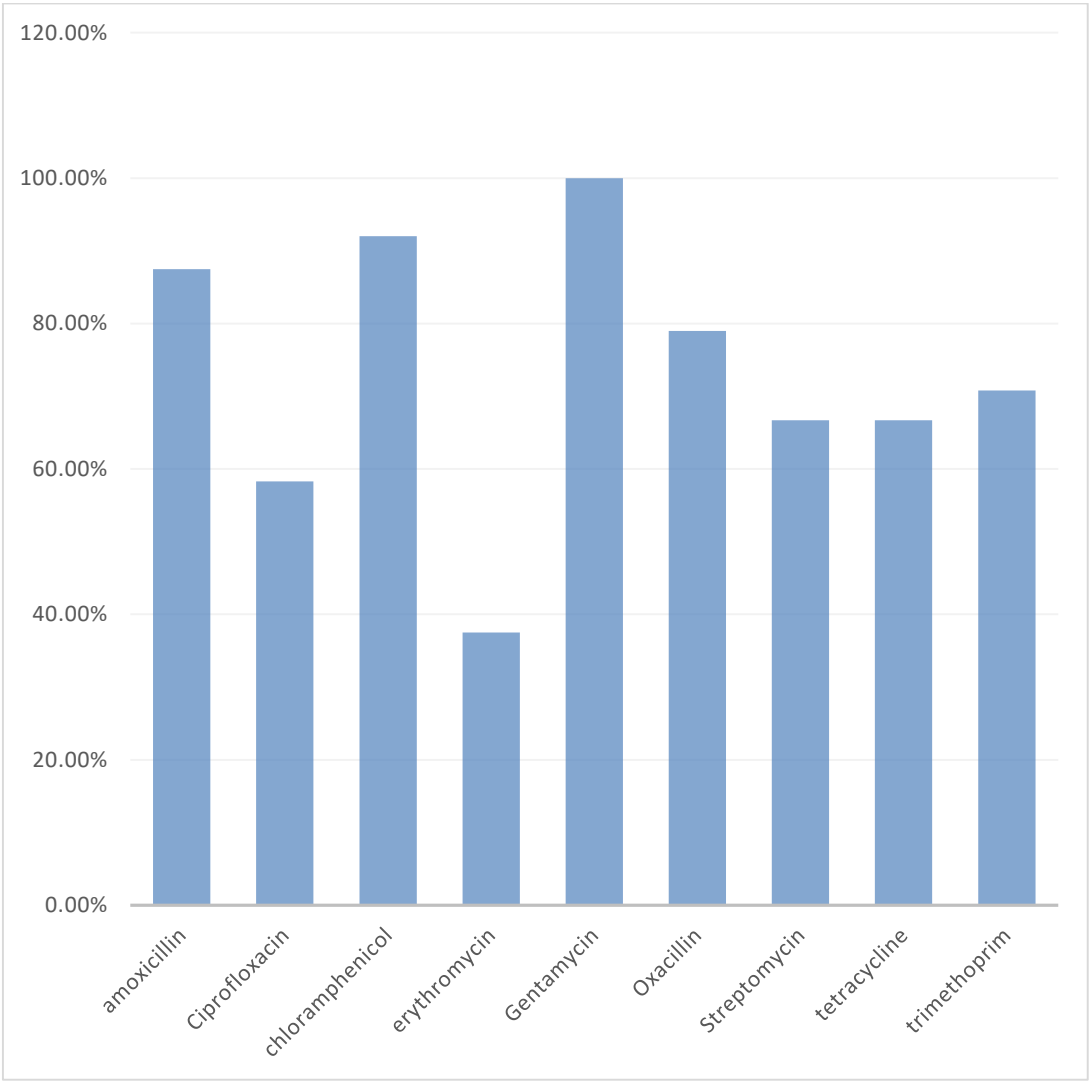


Figure 2: Susceptibilities of 24 *Listeria* Isolates from Fish to 9 antimicrobial agents obtained in Kaduna North L.G.A, Nigeria.

5: Multiple Antibiotic Resistance (MAR) index of the 24 *Listeria* species isolates obtained from Fish in Kaduna North L.G.A, Nigeria.

The mean MAR index ranged from 0.22 to 0.67. Fourteen (14) isolates out of the 24 isolates had multiple- antibiotic resistance indices above the bench score of 0.20 (Figure 3).

Two *Listeria* isolates showed resistance to six (6), five (5) and four (4) antimicrobial agents respectively. Three *Listeria* isolates showed resistance to 3 antimicrobial agents, while 5 isolates showed resistance to only 2 antimicrobial agents respectively (Figure 3).

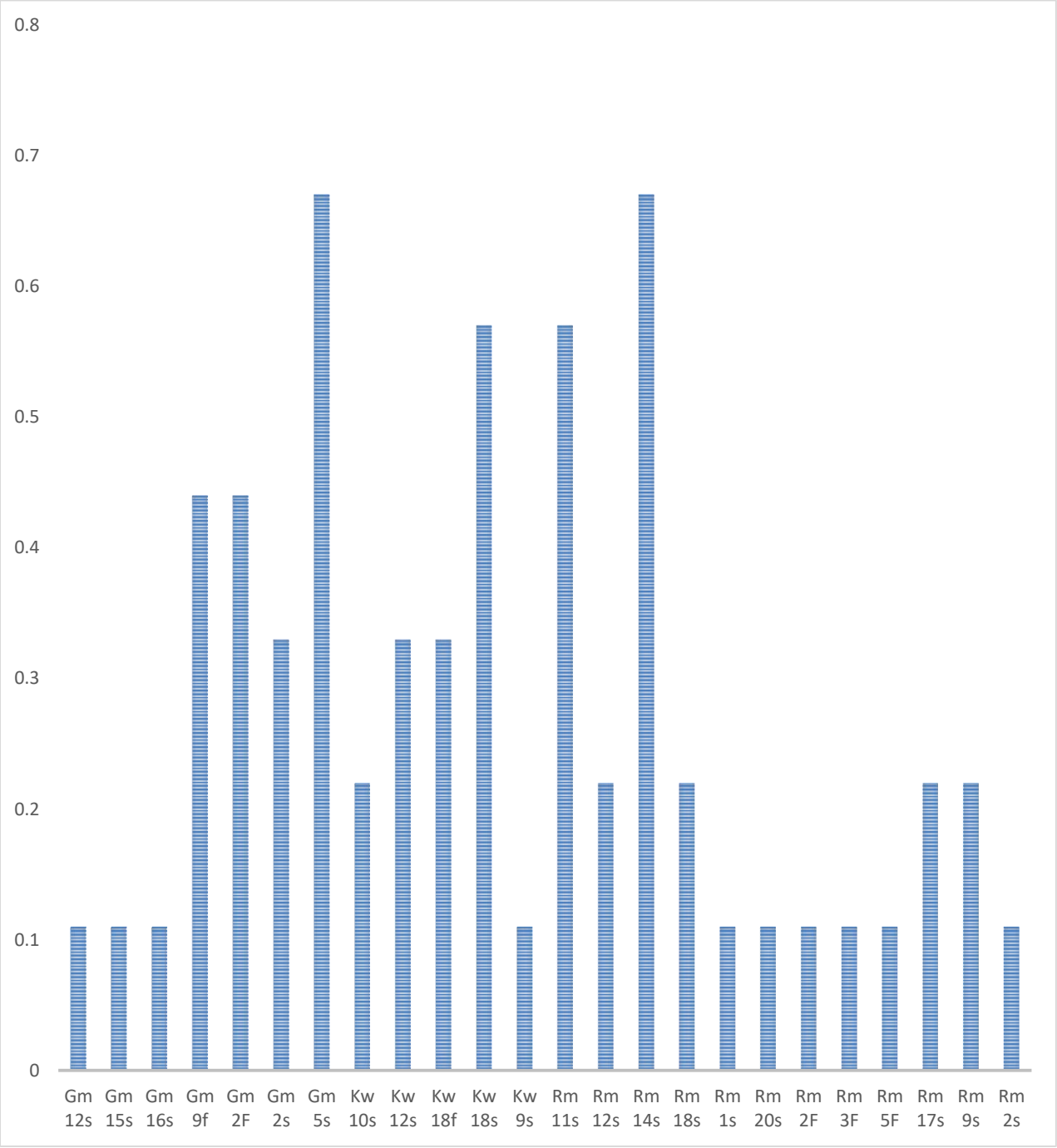


Figure 3. Multiple Antibiotic Resistance (MAR) index of the 14*Listeria* species isolates obtainedfrom Fish in Kaduna North L.G.A, Nigeria.

DISCUSSION

The findings of this study on drug susceptibility showed that *Listeria* speciesisolates were 100% susceptible to the first drug of choice (Gentamicin) used in the treatment of listeriosis, followed by Chloramphenicol (91.7%), Amoxicillin (87.5%) and then Oxacillin (79.2%).

Although the incidence of antibiotic resistance was high in this study, the range of antibiotics to which resistance has been acquired is wide. It is of concern that this expanding range now includes a number of antibiotics used in the treatment of listeriosis, e.g tetracycline and streptomycin(Liu, 2006).However, the isolation of resistant strains of *Listeria* specieswas high, even though evidence of the emergence of resistant strains from various sources has been reported (Liu, 2006).

Amoxicillin or penicillin and gentamicin remain the drugs of choice for most manifestations of listeriosis. Trimethoprim is considered to be a second choice therapy; but erythromycin is also used respectively to treat bacteremia and pregnant women diagnosed with listeriosis (Charpentier and Courvalin, 1999). The majority of *Listeria* species isolated in this study were susceptible to the antibiotics commonly used in veterinary and human listeriosis, even though more than one isolate were resistant to Amoxicillin, Erythromycin and Trimethoprim. A similar pattern of resistance has also been found by other authors (Aurelli*et al*., 2003) suggesting the worldwide increase in antibiotic resistance.

Most of the *Listeria* speciesisolates in this study showed high frequency of resistance to Erythromycin and Ciprofloxacin.This suggests the extent of distribution of resistance of *Listeria* species to commonly used antimicrobial agents in the study area.The widespread use of antibiotics in human and veterinary therapy, alongside the length of time over which it has been available in Nigeria and other countries of the world could account for this trend. *Listeria* speciescan either acquire or transfer antibiotic resistances' genes from plasmids and transposons of other bacterial species including *Enterococcus* spp. either *in vivo* or *in vitro* in the intestinal tract (Pourshaban *et al*., 2002).

However, (Akano *et al.*, 2009) reported that the rate of abuse of antibiotics could promote the acquisition of resistance genes by bacteria in developing nations like Nigeria. This finding is also at variance with findings in previous studies conducted where high resistance levels were observed among food bacterial isolates (Ebigwei and Olukoya, 1991; Olasupo *et al.*, 2002a). The high resistance level among food bacterial isolates have been partly attributed to possible transfer of resistance trait from indigenous micro flora associated with the sources of the raw materials used in the preparation and processing of foods (Olasupo*et al.*, 2002b). Since water is one of such materials used, contamination from environmental isolates is quite a possibility(Olasupo *et al.*, 2002b).

The resistance shown to erythromycin (62.7%) in this study indicates that, this widely used drug may be ineffective in the treatment of listeriosis. The resistance to antibiotics can be due to selective antibiotic pressure (Hanchung*et al*., 2004) or intergons and other insertion elements (Didier *et al.*, 2000).The emergence of antibiotic resistant isolates from fish samples is of medical and public health importance (Hanchun *et al.*, 2004), which will defy almost all the antibiotic

chemotherapy.Therefore it is recommended that the usage of antibiotics in both human and veterinary medicine should be monitored appropriately.

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4: Antibiotic Resistance patterns of *Listeria* isolates from Fish obtained in Kaduna North L.G.A., Nigeria.

Table 1:Antimicrobial resistance patterns of 24 *Listeria* species isolates obtained from Fish in Kaduna North L.G.A., Nigeria.

S/No.	Antibiotic resistance Pattern.	Frequency (Antibiogram)	*Listeria* species
1.	CIP, CL, TE, TM, OX, ER	1	*L. grayi*
2.	CIP, TE, TM, S, OX, ER	1	*L. ivanovii*
3.	CIP, TE, OX, AML, ER	1	*L. ivanovii*
4.	CIP, TE, TM, S, ER	1	*L. ivanovii*
5.	CIP, TM, OX, ER	1	*L. ivanovii*
6.	CIP, CL, S, ER	1	*L. grayi*
7.	CIP, TE, TM	1	*L. ivanovii*
8.	S, AML, ER	1	*L. ivanovii*
9.	TE, TM, ER	1	*L. ivanovii*
10.	CIP, ER	2	*L. grayi*,*L. ivanovii*
11.	CIP, TE	1	*L. grayi*
12.	OX, ER	1	*L. grayi*
13.	S, AML	1	*L. grayi*
14.	ER	5	*L. ivanovii*(3), *L. grayi*(2).
15.	TE	2	*L. ivanovii*(2).
16.	S	3	*L. grayi*,*L. ivanovii*(2).

Key: AML, Amoxicillin; CL, Chloramphenicol; CIP, Ciprofloxacin; ER, Erythromycin; TM, Trimethoprim; S, Streptomycin; TE, Tetracycline; OX, Oxacillin.