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

[1]Musa, M.A., [2] Bello, M., [2]Kwaga, J. K. P., [1]Idi-Ogede, A.M., [3]Onimisi, H.U.,[1]Nda, A.A., [1]Yakubu, S.O. [1]Masaya, A.H. and Dagona, A.G.

[1]Department of Fisheries and Aquaculture, Federal University, Gashua.

[2]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria.

[3]Department of Fisheries and Livestock, National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria.

[4]Department of Biological Sciences' Federal University, Zaria

*Corresponding Email:musamusa25@yahoo.com Phone Number: **0803 427 2242**

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 10°20'N and Longitude 7° 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).

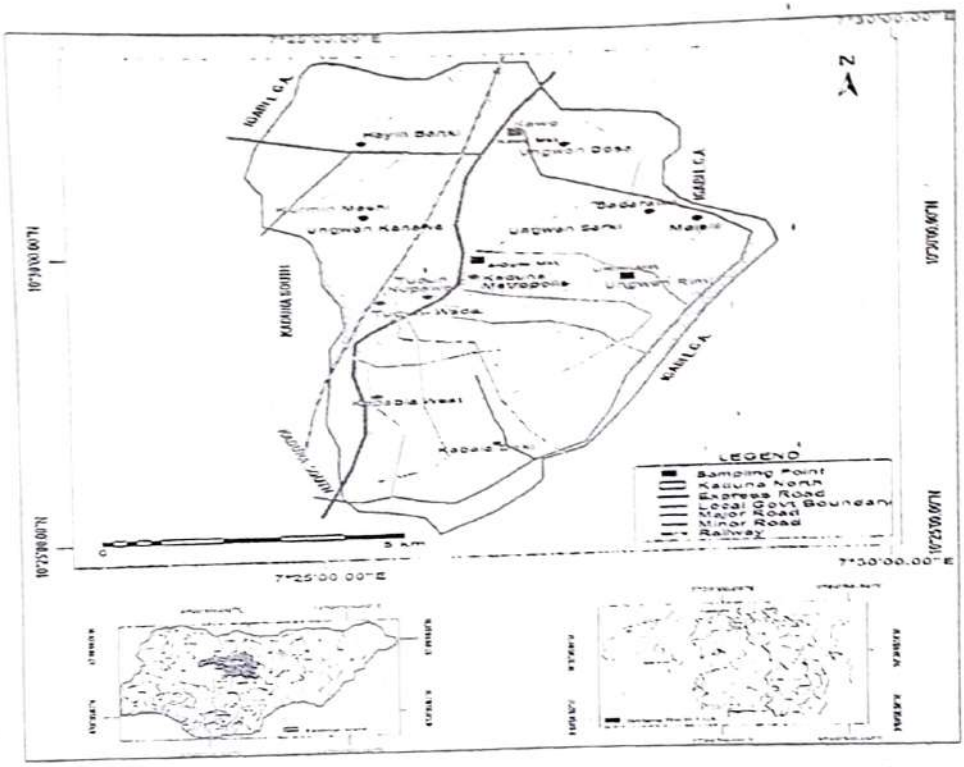

Figure 1: The Map of Kaduna North L.G.A, Nigeria showing the sampling areas.
Source: nigeriazipcodes.com Retrieved October 10 2015. 12:36 GMT

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).

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

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

$$= \quad 3.8416 \times 0.08735 / \quad 0.0025$$

$$n \quad = \quad 134.22$$

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).

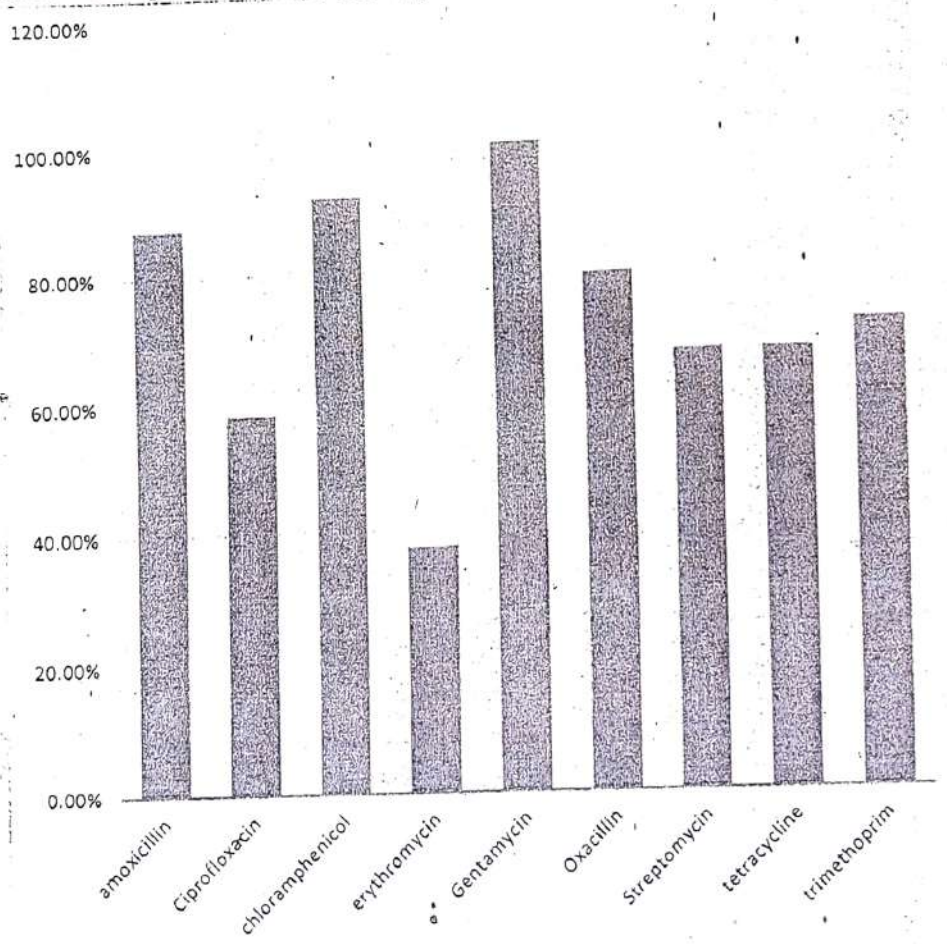

Figure 2: Susceptibilities of 24 *Listeria* Isolates from Fish to 9 antimicrobial agents obtained in Kaduna North L.G.A, Nigeria.

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).

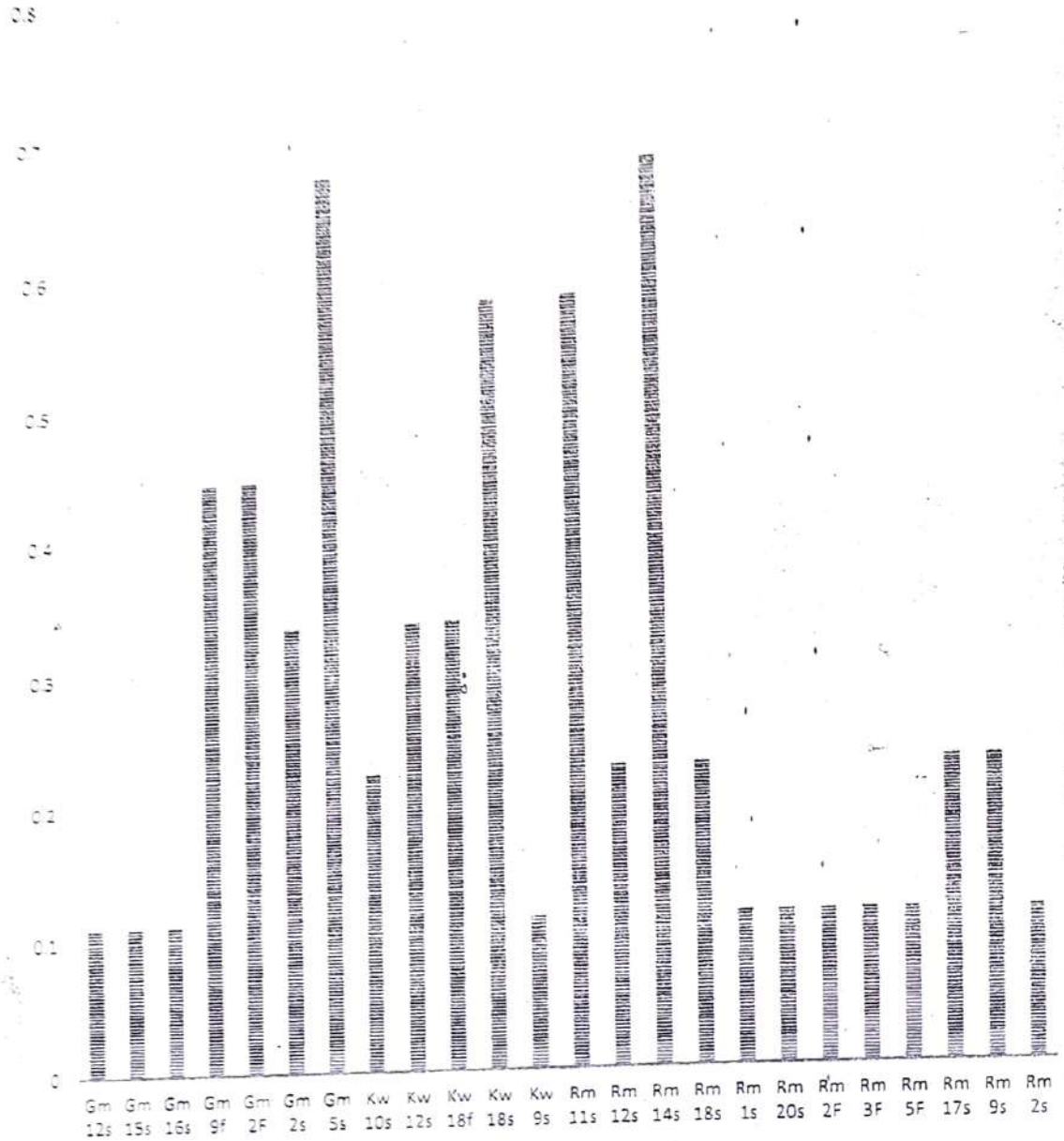

Figure 3. Multiple Antibiotic Resistance (MAR) index of the 14*Listeria* species isolates obtainedfrom Fish in Kaduna North L.G.A, Nigeria.

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

REFERENCES

Aurell, F., Ferrini, A.M., Mannoni, V., Hodzic, S., Wedel-Weergaard, C. and Oliver, B. (2003). Susceptibility of *Listeriamonocytogenes* isolated from food in Italy to antibiotics. *International Journal of Food Microbiology*, 83: 325-330.

Aygun, P., Pehlivanlar, S. (2006). *Listeria* species in the raw milk and dairy products in Antakya,Turkey. *Journal of Food Control*, 17: 599–682.

Brooks, G. F., Butel, S.J., Moorse, A.S., Jawetz,Melnic and Aldelberg's (2001). *Medical Microbiology*, 22nd edition, John-Wiley and Son, London, 502.

Chasseignaux, E., Gerault, P., Toquin, M.-T., Salvat, G., Colin, P. and Ermel, G. (2002). Ecology of *Listeria monocytogenes* in the environment of raw poultry meat and raw pork meat processing plants. *FEMS Microbiology Letter*, **210**: 271-275.

Chen, Y.Q., Edwards, J.J., Kridel, S.J., Thornburg, T., and Berquin I.M. (2007). Dietary Fat gene interactions in cancer. *Cancer Metastasis Revised*. **26**: 535-551.

Chen, C.Y. and Chen, H.M. (2001): Heavy metal concentrations in nine species in fishes caught in coastal waters off Ann- Ping, s. w Taiwan. *Journal of food anddrug analysis*, 9: 107- 114.

Clinical and Laboratory Standards Institute (CLSI). (2013). Performance standards for antimicrobial susceptibility testing twentieth informational supplement. Document M100-S20. (ISBN 1-56238-716-20). 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-188 USA.

Colburn, K.G., Kaysner, C.A., Abeyta,C Jr. and Wekell, M.M. (1990). *Listeria* spp. in a California Coast estuarine environment. *Applied Environmental Microbiology*,**56**: 2007-11.

Dijkstra RG (1982) The occurrence of *Listeria monocytogenes* in surface water of canals and lakes, in ditches of one big polder and in the effluents and canals of a sewage treatment plant,*ZblBakt Hygiene*. *176*: 20-25.

Food and Agricultural Organization (FAO) (2007) http://www.fao.org/docrep/field/003/ac578e/AC578E09.htm,Retrieved on July 8, 2016.

Gudbjörnsdóttir, B., Suihko, M.-L., Gustavsson, P., Thorkelsson, G., Salo, S., Sjöberg, A.-M., Niclasen, O. and Bredholt, S. (2004): The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. *Food Microbiology*,**21**: 217-225.

Hanchun, Y., Sheng, C., David, G. W., Shaohua, Z., Patric, 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.

Harakeh, S., Yassine, H., Gharios, M., Barbour, E., Hajjar, S., and El-Fadel M. (2005): Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon. *Scientific Total Environment*,341:33–44.

Hitchins, A.D. (1995). *Listeria monocytogenes*. Food and Drug Administration Bacteriological Analytical Manual. 8th edition. *Association of Official Analytical Chemists*, 10:01–13.

Hoffman, A., Gall, K.L., Norton, D.M. and Wiedmann, M. (2003): *Listeria monocytogenes*contamination patterns for the smoked fish processing environment andfor raw fish. *Journal of Food Protection*, 66: 52-60.

Ieren, I. I., Bello, M., Kwaga, J. K. P. (2013).Occurrence and antibiotic resistance profile of *Listeria monocytogenes*in salad vegetables and vegetable salads sold in Zaria, Nigeria. *African Journal of Food Science*, 2: 334-336.

ISO 11290-1 (1996): Microbiology of food and animal feeding stuffs-Horizontal method of detection and enumeration of *Listeria monocytogenes*. Part I: Detection method.

Janzten, M.M., Navas, J., Corujo, A., Moreno, R., López, V., and MartínezSuárez, J.V., (2006). Review of Specific detection of *Listeria monocytogenes* in foods using commercial methods: from chromogenic media to real-time PCR. *Spanish Journal of Agricultural Research*,4:235-247.

Jemmi, T. and Stephan,R. (2006). *Listeria monocytogenes*: Food-borne pathogen and hygiene indicator. *Revised Science Technology*, 25: 571-580.

Kaduna State Government (KDSG): (2008). Kaduna state achievement: Investment opportunities in Kaduna State of Nigeria. 2007 Ed, pp 1-48

Karunasagar,I.K., (2000). *Listeria* in tropical fish and fishery products: *International Journal of Food Microbiology*, 62: 177-81.

Liu, D. (2006). Identification, subtyping and virulence determination of *Listeria monocytogenes*: an important foodborne pathogen. *Journal of Medical Microbiology*, 55:645-659.

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

Olasupo, N.A., Alabi, S.A., Akinyemi, K.A., andOmonigbehin, E. A., (2002a): The antimicrobial susceptibility pattern of bacteria agents isolated from patients with diarrhea. *Biomedical Letter*, 60: 77-82.

Olasupo, N.A., Smith, S.I., and Akinside, K.A., (2002b): Examination of the microbial status of selected indigenous fermented foods in Nigeria. *Journal ofFood Safety*,22:85-93.

Pourshaban, M., Ferrini, A.M., Cannoni, V., Oliva, B., and Aurelli, P., (2002): Transferable tetracycline

resistance in *Listeriamonocytogenes*from food in Italy. *Journal of MedicalMicrobiology*, 51(7), 564-566

Richet, H. M. J., Mohammed, L. C., mcdonald and Jarvis, W. R., (2001): Building communication networks: International network for the study and prevention of emerging antimicrobial resistance. *Emerging Infectious Diseases*, 7: 319-322.

Rocourt, J., Jacquet, C.H., and Reilly, A. (2000) Epidemiology of human listeriosis and sea foods. *International Journal of Food Microbiology*,62: 197-209.

Safdara,A., and Armstrong, D., (2003). Antimicrobial activities against 84 *Listeria monocytogenes*isolates from patients with systemic listeriosis at a comprehensive cancer center. *Journal of Clinical Microbiology*, 41, 483-485.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., and Wort, A.J.*et al.*, (1983). Epidemic listeriosis – evidence for transmission by food, *England Journal of Medicine*, 308: 203-206.

Shewan, J.M. (2000). Fish as Food: *The Microbiology of Sea Water Fish*. 1, New York Academic press, 487.

Shinkafi, S. A.,andUkwaja, V.C.(2010): Bacteria associated with fresh tilapia fish (Oreochromisniloticus) sold at Sokoto central market in Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Sciences*,18: 217-221.

Singh, S., Yadav, A., Singh, S. and Bharti, P. (2010).Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Journal of Food Research International*, 43: 2027-2030.

Thimothe, J., Kerr Nightingale, K., Gall, K., Scott, V.N. and Wiedmann, M. (2004) Tracking of *Listeria monocytogenes* in smoked fish processing plants. *Journal of Food Protection*,67: 328-341.

USDA (2008): Food-borne illness: What consumers need to know, Retrieved May 23, 2016 from http://www.fsis.usda.gov/factsheets

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., and Kreft, J. (2001) *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviewed* 14: 584-640.

Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A.A. and Meng, J. (2007): Characterization of *Listeria monocytogenes*isolated from retail foods. *International Journal of Food Microbiology*, 113:47-53

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.