Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

ARCHIVES OF PHARMACEUTICAL SCIENCES AND BIOTECHNOLOGY JOURNAL

VOLUME 4 ISSUE 2, DECEMBER, 2024

ISSN 2971 – 611X

Published by the Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

MOLECULAR DETECTION AND ANTIBIOGRAM OF *LISTERIA* SPECIES FROM SMOKED FISH SOLD IN ZARIA METROPOLIS, KADUNA STATE, NIGERIA

[1]*Danraka, F.N., [1]Bashir, S.B., [2]Hussaini, I.M,[2]Tijjani, M.B., [3]Danraka R.N.and John Ndubuisi[4]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Kaduna State University.
[2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria
[3]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria
[4]Department of Medical Laboratory Science, Federal University, Lafia, Nasarawa
*Corresponding Author: Email: fndanraka@gmail.com Phone number: +2347061603142

ABSTRACT

Aim: This study evaluated the presence of *Listeria* species in smoked fish sold within Zaria metropolis and determined their antibiogram. **Method**: A total of two hundred (200) smoked fish sampleswere collected from Samaru, SabonGari TudunWada and Zaria City markets.Polymerase chain reaction was used to detect the presence of *prs* and *hlyA* genes in the *Listeria* isolates. Antibiotic susceptibility test was carried out in order to determine the level of resistance of the isolates. **Result**:Out of the 15 isolates of *Listeria* species, 3 (20.0%) were *L. seeligeri* while 12 (80.0%) were *L. ivanovii*. Multiple antibiotic resistant indices of the isolates were found to be ≥ 0.69, indicating that the isolates werepre-exposed to antibiotics. **Conclusion**: Smoked fish in Zaria metropolis, contain clinically significant bacteria, which are of public health importance and arenot safe for human consumption.

Keywords: *Listeria species*, smoked fish (*clarius gariepinus*)

INTRODUCTION

*Listeria*s pieces. has been found in a great variety of fish, even in smoked fish (1). *Listeria monocytogenes* and *Listeria innocua* are the most frequently isolated strains in food processing plants. However, most of the cases of listeriosis transmitted through food are due to the contamination of raw or cooked foods with *Listeria monocytogenes* (2). *Listeria monocytogenes* can develop at low temperatures in a wide pH range, with a high concentration of sodium chloride and reduced water activity. Hence, it can survive and multiply in a great variety of food products (3). *Listeria* has been involved in a large number of food-borne outbreaks transmitted through food all over the world. The most hazardous products are those "ready to eat" foods which are stored at room temperature for long periods of time (4). Because of the ubiquitous nature of *Listeria monocytogenes*. all food contact surfaces can be a source of contamination and the bacteria can survive the acidity of the stomach enter the small intestine, cross the epithelial barrier and then spread to the liver, spleen, central nervous system and the fetus in pregnantwomen (5). The mortality rate of listeriosis is from 20 to 30 and 25%

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

worldwide and can be up to 70% in immunocompromised persons (5).

The use of PCR in combination with traditional culture, to detect the presence or absence of *Listeri*a has also been explored in recent years by amplifying *Listeria* specific genes through PCR and quantifying them by the detection of a fluorescent probe attached to the DNA fragments, even low numbers of the bacteria can be detected within a few hours (after enrichment) as opposed to the several days it takes to complete traditional plating techniques. (6).

METHODS

Detection of *prs* and *hlyA* Genes by polymerase chain reaction

DNA Extraction

Extraction of the DNA using the boiled-cell method as performed as described by (7), which is a modification of the method by (8). For the detection of these genes, isolates of the presumptive colonies were sub-cultured in 6ml tubes containing *Listeria* enrichment broth and incubated at 37^{o}C for 24h. The tubes were centrifuged at 10,000 rpm for 2 min to pellet the cells. The harvested cells were washed with 500 µl of sterile distilled water and vortexed to re-suspend the pellets. Tubes were heated at 100^{0}C for 10 min and allowed to cool at -20°C for 5 min, followed by a centrifugation at 12,000 rpm for another 5 min. The clear supernatant was used as template DNA in PCR assay.

Polymerase Chain Reaction (PCR)

DNA amplification was done in a 50µL reaction mixture containing 10 µL of 5x PCR buffer (50mM NaCl, 50mM Tris-HCl, pH 9.0) 250µM each deoxynucleotide triphosphate; 2mM MgCl2; and 1 U of GoTaq Flexi DNA polymerase (Promega U.S). Each primer was added at the final concentrations of 15µL. Five microlitre of template DNA was also added to each reaction mixture. The final volume of the reaction mixture was thenadjusted to 50µL with sterile deionized distilled water. Amplification was done in thermal cycler (Applied Biosystems, U.S) following standardized conditions consisting of initial denaturation of 94^{o}C for 3minutes, final denaturation at 94^{o}C for 1minute, primer annealing at 60^{o}C for 2minutes, primer extension at 72^{0}C for 1minute and final extension at 72^{o}C for 15minutes.

Agarose Gel Electrophoresis

The amplified DNA fragments were resolved by agarose gel electrophoresis, stained with ethidium bromide (0.5µg/ml) and visualized with an UV transilluminator (9). The set of primers used for the amplification is as presented on the Table 1.

Table 1: Set of Primers used for Polymerase Chain Reaction

Primer	Primer sequence (5'-3')	target gene	amplicon(bp)	Reference
PrsF	GCT GAA GAG ATT GCG AAA GAA G	*Prs*	370	(10)
PrsR	CAA AGA AAC CTT GGA TTT GCG G			
***HlyA*F**	CCT AAG ACG CCA ATC GAA	*hlyA*	702	(11)
***HlyA*R**	AAG CGC TTG CAA CTG CTC			

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

Antibiogram of *Listeria* species Isolated from Smoked Fish Samples

The antibiotic susceptibility of the isolates was determined using disc-diffusion method as recommended by Clinical Laboratory Institute Standard (12). The isolates were grown for 24h at 37^{o}C on surface of nutrient agar, after which the isolates were suspended in 5ml normal saline and the turbidity was adjusted to match McFarland Standard of 0.5 (equivalent to 1.5×10^{8}CFU/ml). Aliquot of 0.1ml of the bacterial suspension was dispensed on the surface of freshly prepared Mueller Hinton agar and spread evenly using a sterile bent glass rod and allowed to air dry for 5 minutes.The antibiotic discs containing Amoxicillin (25µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Erythromycin (15µg), Gentamicin (30µg), Streptomycin (10µg), Tetracycline (30µg), Trimethoprim (5µg) and Oxacillin (1µg) were placed on the surface of the agar plate and incubated at 37°C for 24 hrs. The zones of inhibition were measured in millimeter and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines (12). 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 to (a) by the total number of antibiotics to which the isolate was tested (b) (13).

MAR index

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.

RESULTS

Genes detected in the *Listeria* species isolated

The *prs* gene, which is a housekeeping gene was detected in the isolates screened by PCR, this confirms that the isolates are *Listeria* species. The virulence gene *hlyA* was detected in all the isolates screened (Plate I) below:

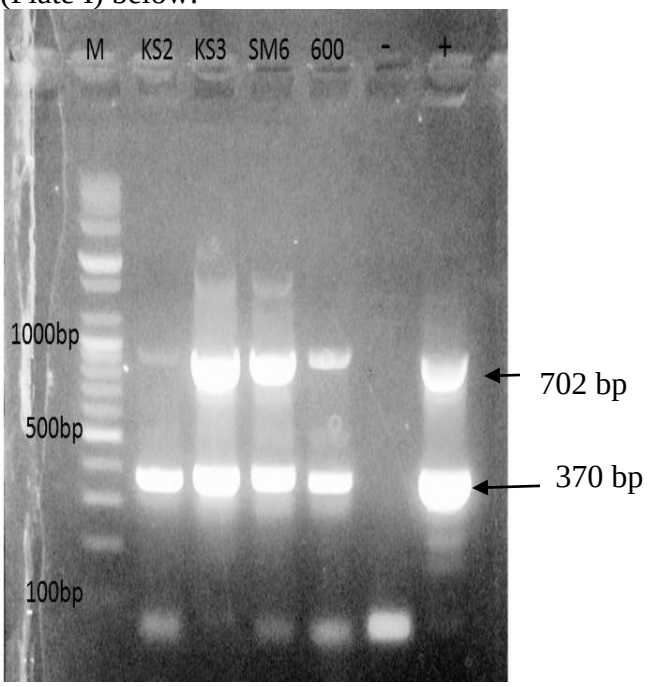

Plate1: Amplicons of *prs* gene (370bp) and *hlyA* gene (702bp) from *Listeria* species isolated from smoked fish

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

Lane M is 100 bp molecular ladder.
Lane 1 to 4 are samplesKS2, KS3, SM6, 600(SM4) respectively
Lane 5 negative control
Lane 6 positive control

Multiple Antibiotic Resistance Index and Resistance Pattern of *Listeria* species isolated from smoked fish

The MARI and Resistance Patterns of the *Listeria* species isolated from smoked fish sold within Zaria metropolis is presented on the Table 2and Table 3 below respectively. MAR indices of the isolates were found to be between 0.69 for isolates resistant to 6 classes of antibiotics and 0.89 for isolates resistant to 8 classes antibiotics

.

Table2: Antibiotic Susceptibility Pattern of *Listeria* Species Isolated from Smoked Fish

Antibiotic (n = 15)	Susceptible (%)	Intermediate (%)	Resistant (%)
AML	0(0.0)	0(0.0)	15(100.0)
AK	0(0.0)	0(0.0)	15(100.0)
C	0(0.0)	0(0.0)	15(100.0)
CAZ	0(0.0)	0(0.0)	15(100.0)
CIP	0(0.0)	9(60.0)	6(40.0)
CRO	0(0.0)	14(93.3)	1(6.7)
CN	0(0.0)	12(80.0)	3(20.0)
DO	0(0.0)	0(0.0)	15(100.0)
E	0(0.0)	0(0.0)	15(100.0)
ENR	0(0.0)	7(46.7)	8(53.3)
IPM	4(26.7)	11(73.3)	0(0.0)
NA	0(0.0)	0(0.0)	15(100.0)
TOB	0(0.0)	0(0.0)	15(100.0)

Keys: AK-Amikacin, AML- Amoxicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CRO-Ceftriaxone, C-Chloramphenicol, CN-Gentamicin, DO-Doxycycline, E-Erythromycin, ENR-Erofloxacin, IPM-Imipenem, NA-Nalidixic acid, TOB-Tobromycin

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

Table3: Multiple Antibiotic Resistance Index and Resistance Pattern of *Listeria* species Isolated from Smoked Fish

Isolate code	Resistance pattern	*MAR Index
SM4	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
SM6	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
SM8	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
SM17	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
SB3	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
KS2	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
KS3	AML, AK, C, CAZ, DO, E, NA, TOB	0.67
SM7	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
SM12	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
SM14	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
SM18	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
KS1	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
TW3	AML, AK, C, CAZ, DO, E, ENR, NA, TOB	0.78
SM13	AML, AK, C, CAZ, CN, DO, E, ENR, NA, TOB	0.78
SM16	AML, AK, C, CAZ, CRO, CN, DO, E, ENR, NA, TOB	0.89

Key: AML – Amoxycillin,AK – Amikacin, C – Chloramphenicol, CAZ – Ceftazidine, CIP – Ciprofloxacin, CN – Gentamicin, CRO – Ceftrioxone, DO – Doxycycline, E – Erythromycin, ENR – Erofloxacin, IPM – Imipenem, NA – Nalidixic acid, TOB – Tobromycin, Ks – Kasuwa (Zaria city) Sm – Samaru Sb – Sabon gari, Tw – Tudun wada. *MAR Index wascalculated as number of antibiotic classes resisted/number of antibiotic classes tested.

DISCUSSION

Molecular confirmation of the isolates revealed that all the isolates were *Listeria* species because the *prs* gene was detected in all of them which is the house keeping gene that is constitutively expressed in order to maintain cellular function.This suggest that the phenotypic identification (biochemical test and microbact) was specific. Contrary to this finding, (14) reported that only 77% of *Listeria* species isolates characterized phenotypically were confirmed to be *Listeria* species molecularly by detection of *prs* gene in their study.

All the isolates were positive for *hlyA* gene which is a virulence gene that codes for listeriolysin O. Listeriolysin O is essential for lysis of the phagosomal membrane. The presence of this gene implies that these isolates are pathogenic. According to (15), *Listeria ivanovii* and *Listeria seeligeri* also harbour virulence gene cluster just like *Listeria monocytogenes*, however these virulence genes are presumably not expressed in a correct or functional fashion. Our findings agree with this report of virulence gene in *Listeria ivanovii* and *Listeria seeligeri*. (16) also reported the presence of *hly* gene in *Listeria seeligeri.*

High level of resistance (100%) was observed to Amikacin, Amoxicillin, Chloramphenicol, Doxycycline, Nalidixic acid, Tobromycin, Ceftazidine and Erythromycin. The isolates also had reduced susceptibility to Ceftriaxone, Gentamicin, Erofloxacin and Ciprofloxacin. Susceptibility of the isolates to Imipenem was recorded as 26.7%. The high antibiotic resistance rate observed in *Listeria* species

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

isolated from smoked fish is of medical and public health concern since the isolates are resistant to commonly used and prescribed antibiotics.

The frequent use of antibiotics in the prevention and treatment of diseases in fish and other animals as well as growth promoter might be linked to the high resistance rate observed in this study to the antibiotics tested.

MAR index values ≤ 0.20 indicate that the isolates originate from samples with low risk where antibiotics are not commonly used while MAR index values > 0.20 indicate that the isolates originate from a high-risk source where antibiotics are commonly used or abuse (17). The MAR indices of the *Listeria* species isolated in this study were higher than 0.2 (0.67and 0.89) this indicating that the isolates originated from higher risk source where they are constantly exposed to antibiotics. Similar finding was reported by (17) and all isolates of *Listeria* species screened had MAR indices greater than 0.2.

CONCLUSION

The *Listeria* isolates were confirmed molecularly by detecting the *prs* gene and *hlyA* gene in all the isolates screened. High level of resistance was observed to most commonly used antibiotics and the MAR indices of the isolates were found between 0.61 to 0.85 for isolates resistance to 8 and 11 antibiotics respectively.

REFERENCES

1. El Marnissi, B., Bennani, L., Cohen, N., Lalami, A. and Belkhou,R. (2013). Presence of *Listeria monocytogenes* in raw milk and traditional dairy products marketed in the north-central region of Morocco. *African Journal of Food Science*, 7:87-91.
2. World Health Organization (2007). Critically important antimicrobials for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use: report of the second WHO Expert Meeting, Copenhagen, 29-31.
3. Gonzalez-Rodriguez, M.N., Sanz, J.J., Santos, J.A., Otero, A. and Garcia-Lopez, M.L. (2002). Numbers and types of microorganisms in vacuum-packed cold-smoked fresh water fish at the retail level. *International Journal of Food Microbiology*, 77:161-168.
4. Phraephaisarn, C., Khumthong, R., Takahashi, H., Ohshima, C., Kodama, K., Techaruvichit, P., Vvesaratchavest, M., Taharnklaew, R. and Keeratipibul, S. (2017). A novel biomarker for detection of Listeria species in food processing factory.*Food Control*,73: 1032-1038. DOI:10.1016/j.foodcont.2016.10.001
5. Haase, J., Didelot, X., Lecuit,M., Korkeala, H. and Achtman,M.(2014). The ubiquitous nature of *Listeria monocytogenes* clones: a large-scale Multilocus Sequence Typing study. *Environmental Microbiology*, 16:405-416.
6. Selamawit, M. (2014). The Prevalence, Risk Factors, Public Health Implication and Antibiogram of *Listeria monocytogenes*, In Sheep Meat Collected from Municipal Abattoir And Butcher Shops In Addis Ababa.
7. Chai, L.C., Robin, T., Usha, M.R., Jurin, W.G., Fatimah, A.B., Ghazali, M.F., Radu, S., Pradeep, M.K., 2007.

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 4 Issue 2 December, 2024 ISSN: 2971_611X
https://doi.org/10.47514/10.47514/APSBJ.2024.4.2.012
Page 132 - 139 Submitted 20/10/2024 Accepted 09/1/2025
APSBJournal

Thermophilic *Campylobacter spp*. in salad vegetables in Malaysia. *International Journal of Food Microbiology*117: 106–111.

8. Marian, M.N., SharifahAminah, S.M., Zuraini, M.I., Son, R., Maimunah, M., Lee, H.Y.,Wong, W.C., Elexson, N., 2012. MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. Food Control 28:309–314.
9. Bindu, C.K. and Krishnaiah, N. (2010). Detection of *Escherichia coli* O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique. *Veterinary World*, 3(1):13-16.
10. Doumith, M., Cazalet, C., Simoes, N.,Frangeul, L., Jacquet, C., Kunst, F., Martin,P., Cossart, P., Glaser, P., Buchrieser, C.,2004 b. New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. *Infection and Immunity*, 72:1072–1083.
11. Ciccio, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A., Ianieri, A., (2012). Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *International Journal FoodMicrobiology*,128: 497–500.
12. Clinical and Laboratory Standards Institute (2017). Performance Standards for Antimicrobial Susceptibility testing. *Eighteenth Supplement*. M100-S18.28(1).
13. 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.
14. Ezeonu, I.M., Ononugbo, C.M. and Ike, A.C. (2018). Molecular characterization of *Listeria monocytogenes* isolated from a ready-to-eat fermented milk and cereal product, *Fura-de-Nunu. African Journal of Microbiology Research*, 12(19): 448-455
15. Muller, A.A., Schmid, M.W., Meyer, O. and Meussdoerffer, F.G. (2010). *Listeria seeligeri* Isolates from Food Processing Environments Form Two Phylogenetic Lineages. *Applied and Environmental Microbiology*, 76(9): 3044–3047.
16. Osman, K.M., Samir, A., Abo-Shama, U.H., Mohamed, E.H., Orabi, A. and Zolnikov, T. (2016). Determination of virulence and antibiotic resistance pattern of biofilm producing *Listeria* species isolated from retail raw milk. *BMC Microbiology*. 16(263)
17. Bilung, L.M., Chai, L.S., Tahar, A.S., Ted, C.K. and Apun, K. (2018). Prevalence, Genetic Heterogeneity, and Antibiotic Resistance Profile of *Listeria* spp. And *Listeria monocytogenes* at Farm Level: A Highlight of ERIC- and BOX-PCR to Reveal Genetic Diversity. *BioMed Research International*, 2018: 1-12 https://doi.org/10.1155/2018/3067494

.