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Detection of *Listeria* Species and *Staphylococcus aureus* in Smoked Fish Sold Within Ahmadu Bello University Main Campus Samaru, Zaria

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Abstract
Hygiene practices in food processing plants are important determinants of food quality and safety. Poor hygiene practices may result in the contamination of foods and food products with pathogens, which means a serious risk to public health. This study was aimed at isolating and determining the antibacterial susceptibility profile of Listeria spp. and Staphylococcus aureus from smoked fish sold within Ahmadu Bello University Main Campus. A total of twenty-five (25) smoked fish samples were collected. Fifteen (15) samples, five each from Community market, Akenzua market and ICSA Ramat market were processed and inoculated on Mannitol Salt Agar for the isolation of Staphylococcus aureus. The remaining ten (10) samples were processed using stomacher and on Listeria Selective Agar (Oxford formulation) for the isolation of Listeria spp. The isolates were characterized based on their colonial morphology, Gram's and biochemical reactions. In addition, agglutination test was carried out to further identify Listeria spp. Antibacterial susceptibility patterns of the isolates was determined using disc diffusion method. Staphylococcus aureus was isolated from all the 15 samples analyzed, giving an occurrence of 100%. However, only one Listeria spp. (Listeria ivanovii) was isolated from the 10 samples analyzed, with a 10% occurrence. All the S. aureus isolates were susceptible to most of the antibiotics, but four were resistant to rocephin and eight to ampiclox. The Listeria ivanovii isolate was also resistant to most of the antibiotics and susceptible to only two. The Multiple Antibiotics Resistance Index (MARI) of S. aureus isolates ranges from 0.2 to 0.4 while it was 0.75 for the Listeria ivanovii isolate. The study demonstrated that smoked fish sold in Ahmadu Bello University Main Campus were found to be contaminated and its consumption is potentially regarded as a health hazard, as such measures should be adopted to control it.
Keywords: *Smoked fish, Isolation, Listeria spp., Staphylococcus aureus, Antibacterial susceptibility pattern*

INTRODUCTION

Poor hygiene practices in food processing plants may result in the contamination of food products with pathogens, which leads to a serious risk for the health of consumers. The complete elimination of pathogens from food processing environments is a difficult task, because bacteria can attach to food contact surfaces and form biofilms, where they survive even after cleaning and disinfection (Brooks and Flint, 2008; Yang, 2012).

Pathogenic bacteria such as *Vibrio* spp., *Escherichia coli*, *Clostridium* spp., *Listeria monocytogenes* and *Staphylococcus aureus* are occasionally detected in fish products (Herrera, 2006). Some of these bacteria can be present in the natural environment, but many others enter the food chain as a result of poor hygienic conditions during processing and storage. *S. aureus* is one of the major bacterial agents causing food-borne diseases in humans. It can cause food poisoning through the production of enterotoxins (Le Loir, 2003).

Species of the genus *Listeria* are Gram positive, facultatively anaerobic, non-spore-forming, rod-shaped bacteria with a low G + C content (Deepti *et al.*, 2015). The genus consists of different species such as *Listeria monocytogenes*, *Listeria ivanovii*, among others. *L. monocytogenes* is a primary human pathogen amidst rare reports of illnesses caused by *L. seeligeri*, *L. ivanovii*, and *L. innocua* (Perrin *et al.*, 2003). *L. monocytogenes* causes gastroenteritis varying from mild to severe illness reported in veterinarians, farmers and abattoir workers and circling disease which is a manifestation of basilar meningitis besides spontaneous abortions in animals.

The occurrence of *Listeria monocytogenes* in smoked fish products result either from contamination of raw material that is not eradicated during processing, or from cross-contamination during processing raw materials or from site and equipment within the factory (Vaz-Velho *et al.*, 2001). Evidence from outbreak of listeriosis has highlighted the importance of contamination of foods from factory sites by *Listeria monocytogenes* (Farber and Peterkin, 1991).

So far, the food chain is considered a potential route of transmission of many pathogens including antibiotic-resistant bacteria to humans which has been provoked due to widespread use of antibiotics. Thus, there is need for constant evaluation of the quality of food including meat and meat products to establish their safety levels for human consumption. Therefore, the aim of this study was to isolate and determine the antibacterial susceptibility profile of *Listeria* spp. and *Staphylococcus aureus* from smoked fish.

MATERIALS AND METHODS

The study was carried out at in Ahmadu Bello University Main Campus, Samaru, Zaria, involving three different locations namely; Ahmadu Bello University Community Market, ICSA Ramat Hostel Market and Akenzua Hostel Market.

A total of twenty-five (25) Smoked fish samples were bought and used in this study using convenience sampling. Fifteen (15) samples, five each were from the three designated sampling areas. The other ten (10) samples were bought from only one of the sampling areas (Community Market). The samples (Smoked fish) was were collected from the vendors at the designated sampling areas, labeled appropriately and transported separately in clean polythene bags to the Department of Microbiology, Ahmadu Bello University, Zaria Main Campus for processing.

Mannitol salt agar (MSA) was used for the isolation of *S. aureus*. It was prepared according to manufacturers' instructions (Cheesbrough, 2006). On the other hand, *Listeria* enrichment broth and *Listeria* Selective Medium (oxford formulation) was used for the isolation of *Listeria* spp. and Mueller-Hinton agar was used for the antibiotic susceptibility assay. All the media were prepared according to manufacturers' instructions and were autoclaved at 121°C for 15 minutes.

The weighted samples (10g) each were homogenized in 90ml of peptone water followed by incubation at 37°C for 24hrs. After overnight incubation, 1ml of the homogenized sample was inoculated into 9ml of *Listeria* enrichment broth and incubated for 24hrs at 37°C. Mannitol salt agar (MSA) plates were streaked with loop full of the homogenized sample and incubated at 37°C for 24 hours for the isolation of *S. aureus*.

After incubation, suspected colonies that appeared yellow on MSA plates were considered as presumptive *S. aureus*. On the other hand, *Listeria* selective agar (LSA) plates were inoculated with the overnight incubation of *Listeria* enrichment broth and were incubated for 24 – 48 hrs at 37°C. The suspected colonies appeared black on the surface of LSM indicating aesculin hydrolysis. These colonies were considered as presumptive *Listeria* spp. Those with positive result were sub-cultured on nutrient agar slants and stored for further at refrigeration temperature characterization (Cheesbrough, 2006).

Gram staining was carried out to identify the isolates according to standard procedure. The slides were examined microscopically under oil immersion objective lens after adding a drop of oil immersion (Chessbrough, 2006). Isolates showing Gram positive and spherical cell morphology arranged in clusters were sub-cultured on freshly prepared nutrient agar slant as presumptive *S. aureus*, while isolates showing Gram positive reaction and rod-shaped morphology were presumptively identified as *Listeria* spp. and stored on nutrient agar slants. All isolates were stored in the refrigerator for further characterization by biochemical methods.

Biochemical characterization of the presumptive *S. aureus* isolates was carried out using the following biochemical tests: Catalase test, Coagulase test and DNAse test as described by Cheesbrough (2006). Catalase test, sugar fermentation test (Maltose, Mannitol and Xylose) and latex agglutination test were used to identify the *Listeria* spp. (Ghassan *et al.*, 1997).

Confirmed isolates were further subjected to Antibacterial susceptibility test using Kirby Bauer disc diffusion technique. Colonies of the isolates were taken from overnight culture and suspended in sterile distilled water and were adjusted to match 0.5 McFarland turbidity standard which correspond to 1.5×10^8 CFU/mL. One milliliter of the standardized inoculum of each isolate of *S. aureus* was separately and evenly inoculated onto agar plates and allowed to stand for some time for pre-diffusion before placing the following antibiotic disks equidistant from each other; Rocephin (25 µg), Ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg), Erythromycin (10 µg), Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg).

The inoculated plates were then incubated at 37°C for 24 hrs. Zones of inhibition were measured using a transparent ruler and recorded. The susceptibility, intermediate and resistant categories were assigned on the basis recommended by the Clinical and Laboratory Standards Institute (CLSI) (2012). The same procedure was used for the isolate of *Listeria ivanovii* except that the following antibiotics were tested against the isolate; Nalidixic Acid (30µg), Tetracyclin (30µg), Gentamycin (30µg), Ampicillin (10µg), Clindamycin (2µg), Amoxicillin (10µg), Kanamycin (30µg), Ciprofloxacin (5µg).

Multiple antibiotic resistance (MAR) index was calculated according to Apun *et al*. (2008) as shown below:

$$MAR = \frac{a}{b}$$

Where;

a = number of antibiotics to which the isolates were resistant

b = total number of antibiotics to which the isolate was exposed

RESULTS

The results of the study revealed that based on cultural, microscopic and biochemical characteristics two organisms were identified and these were *Staphylococcus aureus* and *Listeria ivanovii*.

Table 1 revealed that *S. aureus* was isolated from each of the five samples collected from all the three sites with a percentage frequency of occurrence of 100%. However, only one isolate of *Listeria* spp. with percentage frequency of 10% was isolated from the 10 samples obtained from community market.

Table 1: Percentage Occurrence of *S. aureus* and *Listeria* spp. Isolated from Smoked Fish

Sample location	Number of samples analyzed	Samples Positive with *S. aureus*		Samples Positive with *Listeria* spp.	
		Number	%Occurrence	Number	%Occurrence
AK	5	5	100	-	-
CM	5	5	100	-	-
IR	5	5	100	-	-
CM*	10	-	-	1	10

Key: AK=Akenzua Market; **CM**=Community Market; **IR**=ICSA Ramat Market, *= Sample for *Listeria* spp. isolation

Table 2 shows that all the 15 isolates of *S. aureus* obtained were 100% susceptible to six of the antibiotics tested. However, for Rocephin, only eight isolates (53.3%) were susceptible, three (20.0%) were intermediately susceptible and four (26.7%) were completely resistant. Similarly, only seven (46.7%) of the 15 isolates were susceptible to Ampiclox and eight (53.3%) were completely resistant with no intermediately resistant isolates compared to Rocephin. Surprisingly, all the 15 isolates were completely resistant to Zinnacef and Amoxacillin.

Table 2: Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from the smoked fish

Antibiotic	Susceptibility status (n=15)		
	Susceptible No (%)	Intermediate No (%)	Resistant No (%)
Rocephin (25µg)	8 (53.3)	3 (20)	4 (26.7)
Ciprofloxacin (10 µg)	15 (100)	0 (0.0)	0 (0.0)
Streptomycin (30 µg)	15 (100)	0 (0.0)	0 (0.0)
Septrin (30 µg)	15 (100)	0 (0.0)	0 (0.0)
Erythromycin (10 µg)	15 (100)	0 (0.0)	0 (0.0)
Pefloxacin (10 µg)	15 (100)	0 (0.0)	0 (0.0)
Gentamycin (10 µg)	15 (100)	0 (0.0)	0 (0.0)
Ampiclox (30 µg)	7(46.7)	ND	8(53.3)
Zinnacef (20 µg)	0 (0.0)	0 (0.0)	15 (100)
Amoxacillin (30 µg)	0 (0.0)	0 (0.0)	15 (100)

Key: n = Total number of isolates screened; ND = Not determined

Table 3 revealed that the single isolates of *Listeria ivanovii* obtained was 100% resistant to all the antibiotics tested except for Kanamycin and Ciprofloxacin which were 100% effective against the isolate.

Table 3: Antibiotic Susceptibility Patterns of *Listeria ivanovii* Isolated from the Smoked fish

Antibiotic	Susceptibility status (n=15)		
	Susceptible No (%)	Intermediate No (%)	Resistant No (%)
Nalidixic Acid (30µg)	0(0.0)	0(0.0)	1(100)
Tetracyclin (30µg)	0(0.0)	0(0.0)	1(100)
Gentamycin (30µg)	0(0.0)	0(0.0)	1(100)
Ampicillin (10µg)	0(0.0)	0(0.0)	1(100)
Clindamycin (2µg)	0(0.0)	0(0.0)	1(100)
Amoxicillin (10µg)	0(0.0)	0(0.0)	1(100)
Kanamycin (30µg)	1(100)	0(0.0)	0(0.0)
Ciprofloxacin (5µg)	1(100)	0(0.0)	0(0.0)

Key: n = Total Number of Isolates Screened

Table 4 shows the Multiple Antibiotic Resistance Indices (MARI) of all the resistant isolates of *S. aureus* and *Listeria ivanovii*. The MAR index of the S. aureus isolates ranges between 0.2 to 0.4. However, the *Listeria ivanovii* isolate had the highest MAR index of 0.8 which is twice the highest MAR index of the *S. aureus* isolates (0.4). The resistance pattern of the *Listeria ivanovii* was TET, NA, GN, AMP, CLI, AM

Table 4: Multiple Antibiotic Resistance Indices (MARI) of *Staphylococcus aureus* and *Listeria ivanovii* Isolated from the Smoked Fish

Isolate's Code	Number of Antibiotics Resisted	Resistance Patterns	MAR index
AK2	2	AM, Z	0.2
AK4	2	AM, Z	0.2
AK5	2	AM, Z	0.2
CM2	2	AM, Z	0.2
CM5	2	AM, Z	0.2
IR3	2	AM, Z	0.2
IR5	3	R, Z, M	0.3
IR4	3	APX, Z, AM	0.3
CM1	3	APX, Z, AM	0.3
CM3	3	APX, Z, AM	0.3
AK1	3	APX, Z, AM	0.3
IR1	3	APX, Z, AM	0.3
IR2	4	R, APX, Z, AM	0.4
AK3	4	R, APX, Z, AM	0.4
CM9*	6	TET,NA,GN,AMP,CLI,AM	0.8

Key: AK=Akenzua Market; **CM**=Community Market; **IR**=ICSA Ramat Market; **AM**=Amoxacillin; **APX**=Ampiclox; **R**=Rocephin; **Z**=Zinnacef; **MAR**=Multiple Antibiotic Resistance Index; **TET**=Tetracyclin; **NA**=Nalidixic Acid; **GN**=Gentamycin; **AMP**=Ampiclox; **CLI**=Clindamycin; *CM9=The only *Listeria ivanovii* isolate

DISCUSSION

The results obtained in this study shows that *Staphylococcus aureus* was isolated from all the smoked fish samples analyzed from the three sampling locations (Community Market, Akenzua Market and ICSA Ramat Market). The high occurrence of *S. aureus* observed in this study might be as a result of unhygienic practices and improper handling of the materials used in the processing of fish by the smoked fish vendors, and this is critical to the consumers since *S. aureus* has been implicated in food poisoning. This agrees with the findings of Abdullahi *et al.* (2005) who also reported high level of contamination with *S. aureus* in meat and meat-products and many other foods sold in Zaria. However, lower occurrence (12.5 %) of this organism in smoked fish was reported by Moshood *et al.* (2012) in Bauchi, Nigeria.

Only one *Listeria* spp. (*Listeria ivanovii*) was isolated from the ten (10) samples analyzed from Community Market, given an overall occurrence of 10%. This is in line with the findings of Kwaga *et al*. (2013) who also reported 9.5% occurrence of *L. ivanovii* in some locally made meat products sold in Zaria. The low occurrence of *Listeria* spp. in this study could be due the fact that *Listeria* spp. are generally psychrophilic in nature, requiring lower temperature for survival and thus, might have been destroyed during the fish processing and heat treatment.

The findings of this study reported 100% susceptibility to Ciprofloxacin, Streptomycin, Septrin, Erythromycin, Perfloxacin and Gentamycin by all the isolates. However, high level of resistance to Zinnacef (100%) and Amoxacillin (100%) was observed among all the *S. aureus* isolates. The result of this finding is similar to that of Lee *et al*. (2003) and Shi *et al*. (2018). It also agrees with the work of Kwaga *et al*. (2013) who reported significant resistance to Amoxacillin by *S. aureus* isolated from meat and meat-products sold in Zaria. The CDC (2016) explained that the major causes of antibiotic resistance have been linked to abuse and misuse of antibiotics in humans as well as in livestock and fish farming, poor infection control in health care settings, poor hygiene and sanitation, and absence of new antibiotics being discovered.

High susceptibility of *Listeria* spp. (*Listeria ivanovii*) to Kanamycin (100%) and Ciprofloxacin was observed in this study. The isolate was found to be resistant to Nalidixic acid (100%), Tetracyclin (100%), Gentamycin (100%), Ampicillin (100%), Clindamycin (100%) and Amoxillin (100%). This finding also agrees with the findings of Kwaga *et al*. (2013) who reported high resistance of *Listeria* spp. to Gentamycin antibiotic. The resistance of these isolates observed could be due to the indiscriminate use of such antibiotics in both human and Veterinary Medicine because of their availability and affordability.

The Multiple Antibiotic Indices for the *S. aureus* and *Listeria* spp. (*Listeria ivanovii*) isolates observed in this study is generally worrisome since the MAR indices of both *S. aureus* and *Listeria* spp. were above 0.2, indicating high risk sources of contamination as reported by Thenmozhi *et al*. (2014).

CONCLUSION

From the findings of this study, it was concluded that the smoked fish sold in Ahmadu Bello University Main Campus were found to be contaminated with *Staphylococcus aureus* and *Listeria* spp. (*Listeria inovanii*) which displayed high resistance to several antibiotics tested. The study revealed that consumption of such contaminated fish is potentially a health hazard; as such measures should be adopted to control it.

REFERENCES

Abdullahi, I.O., Umoh, V.J., Ameh, J.B. and Galadima, M. (2005). Comparative assessment of the microbiological quality of three local meat products as sold in Zaria, Nigeria. *Nigerian Journal of Scientific Research*, **5**: 55-60.

Apun, K., Chong, Y.L., Abdullahi, M.T. and Micky, V. (2008). Antimicrobial Susceptibilities of *Escherichia coli* Isolates from Food Animals and Wildlife Animals in Sarawak, East Malaysia. *Asian journal of Animal Veterinary Adventure*, **3**(6): 409-416.

Brooks, J.D., and Flint, S.H. (2008). Biofilms in the food industry: problems and potential solutions. *International Journal of Food Science and Technology*, **43**: 2163-2176

Centers for Disease Control and Prevention (2016). "Staphylococcal Food Poisoning." Food Safety. http://www.cdc.gov/foodsafety/diseases/staphylococcal.html.

Cheesbrough, M. (2006). *Biochemical tests to identify bacteria: District Laboratory practice in tropical countries*. Part 2, second edition Cambridge University press.

Clinical and Laboratory Standards Institute (CLSI) (2012): Performance for antimicrobial disk Susceptibility test; approved standard, 11th edition, CLSI document. Wayne (PA), USA., **32**(1): 1-76.

Deepti, N. Nayak, Savalia, C.V., Kalyani, I. H., Kumar, R., Kshirsagar, D. P. (2015). Isolation, identification, and characterization of *Listeria* spp. from various animal origin foods. *Veterinary World*, **8**(6): 695-701

Farber, J.M. and Peterkin, P. I. (1991). *Listeria monocytogenes*: a foodborne pathogen. *Microbiological Reviews*, **55**: 476-511.

Ghassan, M.M., Peggy, S.H., William, F.B., Bala, S. (1997). Listeriolysin O-Based Latex Agglutination Test for the Rapid Detection of Listeria monocytogenes in

Foods. *Journal of Food Protection*, **60**(9): 1038-1040.

Herrera, F.C., Santos, J.A., Otero, A. and García-López, M.L. (2006). Occurrence of food-borne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *Journal of Applied Microbiology*, **100**:527-536.

Kwaga, J.K.P., Ndahi, M.D., Bello, M., Kabir, J., Umoh, V.J., Yakubu, S.E. and NOK, A.J. (2013). Prevalence and antimicrobial susceptibility of *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* strains from raw meat and meat products in Zaria, Nigeria. *Letters in Applied Microbiology*, **58**: 262-269

Le Loir, Y., Baron, F., and Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetic and Molecular Research*, **2**: 63-76.

Lee, J. H. (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied Environmental Microbiology*, **69**: 6489-6

Moshood A. Y., Tengku Haziyamin, A.T.A.H., Ibrahim, H. (2012) Isolation and Identification of Bacteria Associated with Balangu (Roasted Meat Product) Sold in Bauchi. Nigeria. *IOSR Journal of Pharmacy*, **2**(6): 38-48

Perrin, M., Bremer, M. and Delamare, C. (2003) Fatal cases of *Listeria innocua* bacteria. *Journal for Clinical Microbiology*, **41**: 5308-5309

Shi, W., Jiahui, H., Qingping, W., Jumei, Z., Feng, Z., Xiaojuan, Y., Haoming, W., Haiyan, Z., Moutong, C., Yu, D., Juan, W., Tao, L., Shuhong, Z., Liang, X. (2018). *Staphylococcus aureus* Isolated from Retail Meat and Meat Products in China: Incidence, Antibiotic Resistance and Genetic Diversity. *Frontiers in Microbiology*, **9**: 2767

Thenmozhi, S., Rajeswari, P., Suresh, K.T., Saipriyanga, V. and Kalpa, M. (2014). Multi-drug Resistant Patterns of Biofilm Forming *Aeromonas hydrophila* from Urine Samples. *International Journal of Pharmaceutical Science Research*, **5**(7): 2908-2918.

Vaz-Velho, M., Duarte, G., McLauchlin, J., Gibbs, P. (2001). Characterization of Listeria monocytogenes isolated from production lines of fresh and cold-smoked fish. *Journal of Applied Microbiology*, **91**: 556-562

Yang, L. (2012). Combating Biofilms. *FEMS Immunology and Medical Microbiology*, **65**: 146-157.