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Research article

Identification of bacterial pathogens isolated from smoked blue whiting fish (*Micromesistius poutasou*) from Odeomu market in Osun state Nigeria

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Abstract

The presence of microorganisms is one of the major factors affecting the quality of smoked fish sold in the open markets. Smoked blue whiting fish (*Micromesistius poutasou*), commonly called (Panla) sold in the Odeomu market in Osun State, were analyzed for microbial contaminants. Isolates were identified using conventional biochemical methods, and antibiotics susceptibility testing was carried out using the disc diffusion method. The total bacterial counts (TBC) results showed that the fish samples had high bacterial counts, ranging from 2.1×10^3 to 9.2×10^3 colony-forming units (CFU)/g. Bacteria isolated from the fish samples were: *E. coli* (45.46%), *Enterobacter* spp. (1.01%), *Klebsiella* spp. (6.06%), *Proteus* spp. (9.09%), *Salmonella* spp. (7.07%), *Shigella* spp. (19.19%), *Bacillus* spp. (4.04%) and *Staphylococcus* spp. (8.08%). The antibiotic sensitivity pattern of Gram-Negative bacteria indicated that all the isolates were resistant to more than three antibiotics. All *E. coli* isolates were resistant to augmentin and ceftazidime, 82.2% were resistant to cefuroxime, 17.7% to gentamicin, and 6.7% to ofloxacin. Screening of resistance genes showed that all six selected multiple antibiotic-resistant *E. coli* isolates tested harbored TEM gene, and two isolates (33.33%) harbored the *aac* (3)-II gene. None of the isolates harbored *SHV*, *CTX-M*, and *qnr*B genes. Our results showed that smoked blue whiting fish may pose a significant risk of spreading antibiotic-resistant bacteria that contain multiple antibiotic-resistance genes, highlighting a serious public health concern.

Keywords: Antibiotics resistant, *E. coli*, *Micromesistius poutasou*, Resistant genes

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Introduction

In most of Nigeria, the open markets of smoked blue whiting fish (*Micromesistius poutasou*), commonly called Panla fish, are readily available. Fish serve as good animal protein sources that are more efficient than plant sources (Akinwumi and Adegbehingbe, 2015). Fish contain other essential elements necessary for the maintenance of good health, like calcium and phosphorus, and a great source of minerals, such as iron, zinc, iodine, magnesium, potassium, and vitamins that can lower blood pressure and help reduce the risk of a heart attack or stroke. This food item is also a rich source of omega-3 fatty acids and vitamins D and B2 (riboflavin). These essential nutrients are important for maintaining a healthy human heart and brain (Balami et al., 2019).

The digestibility and the concentrations of essential amino acids in food proteins are important determinants of the efficacy of protein absorption into the body (Mohanty et al., 2014). Fish has lower cholesterol content when compared to meat (Hagen et al., 2016), thus often recommended for consumption, especially among the adult population. The availability of smoked blue whiting fish makes them a preferable diet. It's important to note that fish is a perishable food that can spoil quickly after it's caught, especially in high temperatures. As a result, it is crucial to preserve it promptly and properly.

Various preservation methods are available for fish, including freezing, salting, sun-drying, oven-drying, fermentation, and smoking (Ayeloja et al., 2018). In Nigeria, fish are majorly roasted using smoke to pre-

vent spoilage (Ineyougha et al., 2015). As regards the conservation of protein value and decrease in moisture content, smoking proved a more effective way of fish processing (Akinwumi, 2014).

In developing countries, smoke-drying has been used to preserve fish; up to 70% of the catch is smoked (Akinwumi and Adegbehingbe, 2015). The smoked fish is in high demand due to its improved flavor and texture and the protection it provides against deteriorating microbiological, enzymatical, and chemical changes (Agu et al., 2013). Smoking of fish at high temperatures (>600°C) has been found to control microbial contamination in fish, although the bacteria spores may not be effectively controlled. For a good quality fish, the total bacteria counts should be less than 10^5 colony-forming units (CFU) per gram, while the fecal coliforms and coliforms should not exceed 10/g and 100/g, respectively (Dutta et al., 2018).

Food is considered microbiologically unsafe for human consumption when pathogenic microorganisms may invade the human body system, alter normal physiological and biochemical processes, and ultimately cause infections (Aladejana et al., 2021). Foodborne diseases affect the health of the individual as well as the economy. The presence of both Gram-positive and Gram-negative bacteria in smoked fish samples is an indicator of food microbiological safety. Fish are common vehicles for transmitting foodborne diseases. It has been confirmed to be a source of spreading cholera and other bacterial infections (Ayeloja et al., 2018).

Microbiologists believe that the presence of *E. coli* in food indicates the probability of contamination of the food products with sewage from human or animal origin (Ofred, 2009). Antibiotic-resistant bacteria in food samples are of great health concern (Aladejana et al., 2021). Many authors have reported the microbial quality of several smoked/dried fish species across Nigeria (Adebayo-Tayo et al., 2008; Daniel et al., 2013; Odu and Imaku, 2013; Akinwumi and Adegbehingbe, 2015; Ineyougha et al., 2015; Ayeloja et al., 2018). However, there is a lack of information about antimicrobial susceptibility testing and the presence of resistant genes. Hence, the current study aimed to test the antimicrobial susceptibility and determine if the resistant bacterial isolates harbor resistant genes.

Materials and methods

Sample collection

Fish previously smoked overnight and brought to the market using available public transportation by fish mongers for sale at the open market at Obada market in Odeomu Osun State, Nigeria, were used for this study. The samples were collected in March 2022. One fish sample was picked at random from ten different mongers; the species of the smoked fish sampled were smoked blue whitening fish (Panla). The fish were purchased in batches and brought to the Department of Microbiology laboratory, Kings University, Odeomu, Osun State, Nigeria, for microbiological analysis.

Isolation of bacteria

One gram of the dried fish samples was weighed, and serial dilution was done using standard procedure. The serial dilution of 10^3 was plated in duplicates using nutrient agar. The agar plates were incubated at 37°C for 24 hours for total bacterial count and further identification of bacteria isolates. The pour plate method of Harrigan (1976) was used for isolation. Distinct colonies were picked and sub-cultured by successive streaking on freshly prepared nutrient agar and incubated at 37°C for 24 hours to obtain pure isolates. These were stored on a nutrient agar slant and kept in the refrigerator at 4°C for further use.

Biochemical characterization of the isolates

The biochemical characteristics conducted were carried out as described by Cheesbrough (2006) and included catalase, oxidase, Methyl Red Voges-Proskauer (MRVP), Simmons citrate agar, Triple sugar iron (TSI), urease and motility tests (Oxoid Ltd., Hampshire, UK) (Olutiola et al., 1991; Aladejana et al., 2021).

Antimicrobial susceptibility testing and multiple antimicrobial resistant index

In the current study, commercially available antimicrobial discs have been used (Abtek Biological Ltd., UK) to determine the isolated strains' drug sensitivity and resistance pattern. The antimicrobial susceptibility testing of each isolate was carried out as described by the disc diffusion method (Bauer et al., 1966) using 0.5 MacFarland's standard turbidity and interpreted according to the Committee for Clinical Laboratory Standards (CLSI, 2021). An appropriate quantity of Muller-Hinton agar was weighed, and distilled water was used to dissolve it. The solution was sterilized in the autoclave for 15 minutes at 121°C. A 24-hour-old mixture was mixed in saline solution and standardized by comparing its turbidity to 0.5 McFarland solution, and it was then streaked evenly on each plate using a sterile swab stick and left to dry.

The antimicrobial discs were placed on the streaked agar plate using a pair of sterile forceps. The plates were incubated at 37°C for 24 hours. The eight different antibiotics used for bacterial isolates organisms were gentamicin (GEN) 10 μg, ceftazidime (CAZ) 30 μg, ofloxacin (OFL) 5 μg, augmentin (AUG) 30 μg, nitrofurantoin (NIT) 300 μg, ciprofloxacin (CPR) 5 μg, cefixime (CXM) 5 μg, and cefuroxime (CRX) 30 μg (Hi-Media, Vadhani, India). Reading and interpretation of results were done after overnight incubation. The results were recorded by measuring the diameter of the zone of inhibition, which is the area with no growth in millimeters using a calibrated ruler.

Determination of multiple antibiotic resistance index

The multiple antibiotic resistance (MAR) index was determined for each isolate using the formula "$MAR = a/b$", where "a" represents the number of antibiotics to

Table 1: Primers used for sequencing of resistant genes (Bebe et al., 2020)

Target genes		Primers Sequence 5'-3'	Amplicon size (bp)
SHV	*SHV*-F	CGC CTG TGT ATT ATC TCC CT	293
	SHV-R	CGA GTA GTC CAC CAG ATC CT	
TEM	TEM-F	CTC ACC GGC TCC AGA TTT ATC	440
	TEM-R	CCG CAT ACA CTA TTC TCA GAA TG	
CTX-M	*CTX-M*-F	CGC TGT TGT TAG GAA GTG TG	874
	CTX-M-R	GGC TGG GTG AAG TAA GTG AC	
aac(3)IIa	*aac*(3)IIa-F	ATA TCG CGA TGC ATA CGC GG	877
	aac(3)IIa-R	GAC GGC CTC TAA CCG GAA GG	
*qnr*B	*qnr*B-F	GGC TGT CAG TTC TAT GAT CG	488
	*qnr*B-R	SAK CAA CGA TGC CTG GTA G	

which the test isolate depicted resistance, and "b" represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility.

DNA extraction

The isolates' DNA was extracted using the boiling method described by Bebe et al. (2020). Three colonies of each isolate were emulsified in 100 μl of sterile distilled water in an Eppendorf tube, heated for 15 minutes, then centrifuged in a micro-centrifuge at 10,000 rpm for five minutes. After centrifugation, the supernatant was transferred to a new Eppendorf tube and utilized as template DNA for polymerase chain reaction (PCR). Detection of resistant genes for the extended-spectrum Beta-lactamase genes (*TEM*, *SHV*, and *CTX-M*) and the cephalosporin-resistant isolates were screened by PCR, using primers as previously described (Wang et al., 2017; Ungo-Kore et al., 2019; Bebe et al., 2020).

Amplification reactions were carried out using gene-specific primer pairs listed in Table 1 under the following conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C in case of TEM, *SHV*, and *CTX-M* for 30 s and extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Also, for aminoglycosides, amplification of *aac* (3)-II and *qnr*B genes using PCR experiments was performed with crude lysates obtained after boiling, according to under standard conditions (Arpin et al., 2003; Marti and Balcázar, 2013; Soleimani et al., 2014).

For the *aac* (3)-II amplification, conditions used were 94°C for 5 min and 35 subsequent cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final step at 72°C for 10 min. For the *qnr*B gene, the amplification conditions were the initial denaturation step at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 2 minutes, and final extension step at 72°C for 10 minutes. *Klebsiella pneumoniae* 27 ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

PCR products (amplicons) were visualized by loading 10 μl of each PCR reaction into 1.5% agarose gel in Tris-acetate EDTA (TAE) buffer. The DNA bands were visualized with a fluorescence ultraviolet light transilluminator and analyzed using a photo documentation system.

Results

This study used ten smoked blue whiting fish (*Micromesistius poutasou*) samples collected from different sellers in the market. They were labelled 1A, 2A, 3A, 4A, 5A 6A, 7A, 8A. 9A 10A from different sources. They were gotten from Obada market in Odeomu, Osun State, Nigeria. The Total Bacterial Count (TBC) results showed that smoked blue whiting fish had a higher bacterial count ranging from 2.2×10^3 to 9.2×10^3 CFU/g. (Table 2).

A total of 99 bacterial isolates were identified as *E. coli* (45.46%), *Enterobacter* spp. (1.01%), *Klebsiella* spp. (6.06%), *Proteus* spp. (9.09%), *Salmonella* spp. (7.07%), *Shigella* spp. (19.19%), *Bacillus* spp. (4.04%) and *Staphylococcus* spp. (8.08%) as shown in Table 3. These consist of 87 Gram-negative and 12 Gram-positive bacterial isolates. The antibiotic susceptibility of the Gram-negative isolates shows that they were resistant to more than three antibiotics, as shown in Table 4. All isolates displayed different antibiotic susceptibility patterns, as shown in Table 5. The result of the resistant genes shows that all six *E. coli* isolates tested harbored the TEM gene (100%), and two carried the *aac*(3)IIa gene (33.33%). The other genes *SHV*,*CTX-M*, and *qnr*B screened were absent in the *E. coli* isolate (Table 6.

The Multiple Antimicrobial Resistant Index (MRA Index) showed that all (100%) isolates have an MRA index greater than 0.2. The results ranged from 0.375 to 1.0.

Discussion

Microorganisms can threaten human health by causing food poisoning and related illnesses. Certain cases of food poisoning occur due to a higher concentration of

Table 2: Average total bacterial count in smoked blue whiting fish samples.

S. No	Samples	Total bacterial count/g
1	1A	3.5×10^3
2	2A	8.7×10^3
3	3A	2.1×10^3
4	4A	5.2×10^3
5	5A	4.0×10^3
6	6A	7.2×10^3
7	7A	8.4×10^3
8	8A	8.6×10^3
9	9A	9.2×10^3
10	10A	2.2×10^3

Table 3: Occurrence and percentage frequency of bacteria isolated from of smoked blue whiting fish samples.

S. No	Isolates	Number of isolates	Frequency (%)
1	*E. coli*	45	45.46
2	*Enterobacter* spp	1	1.01
3	*Klebsiella* spp	6	6.06
4	*Proteus* spp	9	9.09
5	*Salmonella* spp	7	7.07
6	*Shigella* spp	19	19.19
7	*Staphylococcus* spp	4	4.04
8	*Bacillus* spp	8	8.08
Total		99	100

bacteria present in the consumed food. A bacterial infection can cause gastroenteritis, which is characterized by symptoms such as nausea, vomiting, diarrhea, abdominal pain, paralysis, malaise, and low-grade fever (Shane et al., 2017).

The present study investigated the occurrence of microbiological contamination in smoked blue whiting fish (Panla) sold in the local markets of Odeomu, Osun State, Nigeria. Detecting these microorganisms is a significant concern for food safety and could pose health risks to consumers. The presence of these microorganisms could be a result of food handlers' lack of knowledge of basic food hygiene. Additionally, using unclean water during fish preparation and processing may have contributed to the spread and growth of these microbes (Jung et al., 2014). Smoking was believed to lower the number of microorganisms in the fish sample, but spores may not be eliminated due to the low intensity of heat used during the process. Moreover, the contamination could be due to unhealthy handling, transportation, and other contaminants from the surrounding environment in the open market.

It has been noted that the TBC in fish is quite high. This could be attributed to the higher levels of moisture found in the blue whiting fish samples. One of the primary factors that impact the safety and quality of dried fish is the presence of bacteria and fungi that lead to contamination. For this study, the fish used were smoked and dried but still contained moisture that could promote the growth of bacteria. Additionally, the smoking process was conducted in unhygienic conditions. According to Agu et al. (2013), smoked fish should not be considered ready-to-eat food because *Salmonella*, *Staphylococcus aureus*, and *Shigella sonnei* were found. The authors concluded that smoked fish can be a potential vehicle for transmitting pathogens.

Results from this study evidenced the presence of different bacterial species, including *E. coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., and *Klebsiella* spp. This indicates that purchasing smoked fish in the open market does not guarantee food safety. Packaging material, storage conditions, and exposure time have been observed to play a pivotal role in the quality of the final dried fish. Faparusi and Adewale also confirm the presence of *Shigella* in blue whitening fish (Panla) samples from Ilaro Yewa-South, Nigeria, which was said to make it unsafe for direct human consumption (Faparusi and Adewole, 2018).

The number of Gram-negative bacteria (n=87) was quite high compared to the Gram-positive bacteria

Table 4: Multiple resistant pattern of Gam negative isolates from blue whiting fish.

Isolate (n)	Resistance pattern*	Frequency	No. of antibiotics	MAR index*
E. coli (n=45)	CAZ-CRX-AUG	18	3	0.375
	CAZ-CRX-CXM-AUG	8	4	0.5
	CAZ-CRX-CRP-AUG	8	4	0.5
	CAZ-CRX-OFL-AUG	1	4	0.5
	CAZ-CRX-GEN-AUG	2	4	0.5
	CAZ-CRX-CRP-OFL-AUG	1	5	0.625
	CAZ-CRX-GEN-CXM-AUG	3	5	0.625
	CAZ-CRX-GEN-CRP-AUG	3	5	0.625
	CAZ-CRX-GEN-CRP-OFL-AUG	1	6	0.75
Enterobacter spp (n=1)	CAZ-CRX-GEN-CXM-AUG	1	5	0.625
Klebsiella spp (n=6)	CAZ-CRX-AUG	1	3	0.375
	CAZ-CRX-CRP-AUG	1	4	0.5
	CAZ-CRX-GEN-CXM-AUG	1	5	0.625
	CAZ-CRX-GEN-CXM-OFL-AUG	3	6	0.75
Proteus spp (n=9)	CAZ-CRX-AUG	2	3	0.375
	CAZ-CRX-CRP-AUG	3	4	0.5
	CAZ-CRX-CXM-AUG	3	4	0.5
	CAZ-CRX-CXM-AUG-CPR	1	5	0.625
Salmonella spp (n=7)	CAZ-CXM-AUG	1	3	0.375
	CAZ-CRX-AUG	1	3	0.375
	CAZ-CRX-CXM-AUG	1	4	0.5
	CAZ-CRX-CXM-GEN-AUG	1	5	0.625
	CAZ-CRX-CRP-OFL-AUG	1	5	0.625
	CAZ-CRX-CXM-CRP-AUG	1	5	0.625
	CAZ-CRX-CXM-CPR GEN-AUG-OFL-NIT	1	8	1.0
Shigella spp (n=19)	CAZ-CRX-CXM-AUG	2	4	0.5
	CAZ-CRX-CRP-AUG	1	4	0.5
	CAZ-CRX-CXM-OFL-AUG	1	5	0.625
	CAZ-CRX-GEN-CXM-AUG	12	5	0.625
	CAZ-CRX-GEN-CXM-OFL-AUG-CPR	3	7	0.875

*Abbreviations: MAR= multiple antibiotic resistance, GEN= gentamicin, CAZ= cefiazidime, OFL= ofloxacin, AUG= augmentin, NIT= nitrofurantoin, CPR= ciprofloxacin, CXM= cerixime, CRX= cefuroxime.

Table 5: Percentage of antibiotic susceptibility pattern of isolates from smoked blue whiting fish.

Isolates		Class of antibiotics no (%)*							
		AUG	CAZ	CRX	CPR	GEN	NIT	OFL	CXM
E. coli	Resistant	45 (100)	45 (100)	45 (100)	9 (20)	8 (17.7)	0 (0)	3 (6.7)	0 (0)
	Susceptible	0 (0)	0 (0)	0 (0)	36 (80)	37 (17.7)	45 (100)	42 (93.3)	45 (100)
Enterobacter spp	Resistant	1 (100)	1 (100)	1 (100)	0 (100)	1 (100)	1 (100)	0 (0)	0 (0)
	Susceptible	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Klebsiella spp	Resistant	6 (100)	6 (100)	6 (100)	1 (16.67)	4 (66.7)	0 (0)	3 (50)	4 (66.7)
	Susceptible	0 (0)	0 (0)	0 (0)	5 (83.3)	2 (44.3)	6 (100)	5 (83.3)	2 (44.3)
Proteus spp	Resistant	9 (100)	9 (100)	9 (100)	0 (0)	0 (0)	1 (11)	0 (0)	4 (44.4)
	Susceptible	0 (0)	0 (0)	0 (0)	9 (100)	9 (100)	6 (89.9)	9 (100)	5 (55.6)
Salmonella spp	Resistant	7 (100)	7 (100)	6 (85.7)	1 (14.2)	2 (28.5)	1 (14.2)	2 (28.5)	0 (0)
	Susceptible	0 (0)	0 (0)	1 (14.3)	6 (85.8)	5 (71.5)	6 (85.8)	5 (71.5)	7 (100)
Shigella spp	Resistant	7 (36.8)	7 (36.8)	7 (36.8)	1 (5.2)	2 (10.5)	0 (0)	2 (10.5)	6 (31.5)
	Susceptible	12 (63.2)	12 (63.2)	12 (63.2)	18 (94.8)	17 (89.5)	18 (100)	17 (89.5)	13 (65.8)

*Abbreviations: GEN= gentamicin, CAZ= cefiazidime, OFL= ofloxacin, AUG= augmentin, NIT= nitrofurantoin, CPR= ciprofloxacin, CXM= cerixime, CRX= cefuroxime.

(n=12) isolated in this study. They are all sources of contamination to humans. The food eaten has a direct influence on health. Therefore, food inspectors, manufacturers, and handlers must keep food safe from pathogenic microorganisms, especially when food is consumed without further processing, i.e., fast foods or "ready-to-eat" foods. There are various microorganisms that can negatively impact the quality of food, which can be dangerous if consumed. Unfortunately, many locally sold food products are found to be contaminated with *Bacillus*, *Staphylococcus*, and other bacterial species (Olaleye and Abegunde, 2015).

Table 6: Summary of the genes present in the multiple antibiotics resistant *E. coli* isolated from smoked blue whiting fish.

Isolate	*TEM*	*aac*(3)-II	*CTX-M*	*SHV*	*qnr*B
1	+	-	-	-	-
2	+	-	-	-	-
3	+	+	-	-	-
4	+	-	-	-	-
5	+	+	-	-	-
6	+	-	-	-	-

Based on the results of antibiotic susceptibility testing, it was found that all isolates were resistant to more than three types of antibiotics. Moreover, many isolates exhibited high levels of resistance and multidrug resistance to six, seven, or even eight antibiotics. Numerous studies have been conducted concerning bacterial resistance and transmitting resistant bacteria to consumers through contaminated food (Acharjee and Sultana, 2019; AcharjeeIsrat et al., 2019; Akter et al., 2021). So far, numerous researchers have attempted to gather information about drug-resistant bacteria and how they spread in the environment by analyzing bulk water, fish, fruit juices, milk, various street food samples, and patients.

Antibiotic resistance was highly pronounced in this study, as seen in all Gram-negative bacterial isolates. This is alarming because it could lead to an infection outbreak for those who consume raw smoked fish without further cooking it. It is important to address this issue and take necessary precautions. The MAR index of greater than 0.2 recorded in this study for all the bacteria isolates showed that they are likely to be from high-risk sources and originated from an environment where several antibiotics have been used. According to Sandhu (2016), the MAR indexing method is a cost-effective, fast, and easy way to track antimicrobial resistance compared to other methods like genotypic characterization.

β-lactamases mainly mediate the resistance of Gram-negative bacteria to cephalosporins and ampicillin. It was also found that the TEM gene was the most common gene present in the tested samples of ready-to-eat smoked fish in the studied area. Additionally, the presence of the *acc*(3)IIa gene was observed in two out of the six tested isolates (33.33%). Ryu et al. (2012) also detected the *bla*(TEM) gene in isolates from fish and seafood 15 (21.4%), although lower than the one discovered in this study. According to this study's findings, smoked fish sold commercially may serve as a breeding ground for bacteria resistant to multiple drugs. This may lead to the spread of genes that cause resistance, which is a significant health concern.

Conclusion

Smoked blue whiting fish are potential sources of microbial contaminants; fish processors and mongers should be well informed on observing good hygiene. It's crucial to use clean and potable water while processing smoked fish and to ensure that they are properly covered when being presented for sale. It is not recommended to consume smoked fish bought from open markets due to potential contamination from handling and dust particles. The present study highlights the importance of fishmongers following proper hygiene practices and authorities monitoring and regulating the production of smoked fish to ensure its safety for consumption in the area. The significant quantity and the antimicrobial resistance rate are major health concerns. The fact that these bacteria can carry resistant genes is alarming, as it suggests that commonly used antimicrobial drugs may not effectively treat infections caused by these bacteria.

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References

Acharjee, M., Sultana, R., 2019. Consequences of γ-irradiation on the dissemination of microorganisms among sea fish in Bangladesh. EC Microbiology 15.8, 784–794.

AcharjeeIsrat, M., Islam, I., Noor, R., 2019. Effects of gamma irradiation on the propagation of microbial growth in commonly available meat in Bangladesh. International Food Research Journal 26, 1211–1218.

Adebayo-Tayo, B.C., Onilude, A.A., Patrick, U.G., 2008. Mycofloral of smoke-dried fishes sold in Uyo, Eastern Nigeria. World Journal of Agricultural Sciences 4, 346–350.

Agu, K.C., Ukponmwan, I.O., Orji, M.U., Awah, N.S., Anaso, C.I., UdemezueInter, O.I., 2013. Prevalence of pathogenic bacteria in smoked fish sold in major retail markets in Benin,

Nigeria. International Journal of Applied Sciences and Engineering 1, 1–4. URL: http://www.ijapscengr.com/pdf-files/Volume-1-no-1-2013/1-4.pdf.

Akinwumi, F.O., 2014. Effects of smoking and freezing on the nutritive value of African mud catfish, *Clarias gariepinus* Burchell, 1822. Journal of Agricultural Studies 6. 10.5539/jas.v6n11p143.

Akinwumi, F.O., Adegbehingbe, K.T., 2015. Microbiological analysis of three of smoked fish obtained from the Ondo State, Nigeria. Food and Public Health 5, 122–126. 10.5923/j.fph.20150504.04.

Akter, T., Ishma, T., Razzeb, S.R., Uddin, S., Islam, M., Saha, S., Shomi, F., Feroz, F., Acharjee, M., 2021. Evaluation of microbial contamination level and the drug susceptible pattern of the isolates cultivated from famous dessert food. Journal of Food Science URL: https://www.myfoodresearch.com/uploads/8/4/8/5/84855864/_54__fr-2020-452_akter.pdf.

Aladejana, O.M., Famurewa, O., Onyia, I., Onyia, C.I., Thonda, O.A., Ogidi, C.O., Olawoye, A.A., 2021. Isolation and multiple antibiotic resistance pattern of enteric bacteria isolated from feacal samples of *Thryonomys swinderianus* in Sekona Osun State, Nigeria. Asian Journal of Research in Animal and Veterinary Sciences 7, 23–28. URL: http://stmdigitallib.com/id/eprint/329/.

Arpin, C., Dubois, V., Coulange, L., André, C., Fischer, I., Noury, P., Grobost, F., Brochet, J.P., Jullin, J., Dutilh, B., Larribet, G., Lagrange, I., Quentin, C., 2003. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in community and private health care centers. Antimicrobial Agents and Chemotherapy 47, 3506–3514. 10.1128/AAC.47.11.3506-3514.2003.

Ayeloja, A., George, F., Jimoh, W., Shittu, M., Abdulsalami, S., 2018. Microbial load on smoked fish commonly traded in Ibadan, Oyo State, Nigeria. Journal of Applied Sciences and Environmental Management 22, 493. 10.4314/jasem.v22i4.9.

Balami, S., Sharma, A., Karn, R., 2019. Significance of nutritional value of fish for human health. Malaysian Journal of Halal Research 2, 32–34. 10.2478/mjhr-2019-0012.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45, 493–496. 10.1093/ajcp/45.4_ts.493.

Bebe, T., Odetoyin, B., Bolarinwa, R., 2020. Occurrence of multidrug-resistant uropathogens implicated in asymptomatic bacteriuria in adults with sickle cell disease in Ile-Ife, Southwest Nigeria. Oman Medical Journal 35, e109. 10.5001/omj.2020.27.

Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries. 2nd (pt. 2) ed., Cambridge University Press, Cambridge.

CLSI, 2021. Performance Standards for antimicrobial susceptibility testing, CLSI supplement M100. 30th ed., Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, USA.

Daniel, E.O., Ugwueze, A.U., Igbegu, H.E., 2013. Microbiological quality and some heavy metals analysis of smoked fish sold in Benin City, Edo State, Nigeria. World Journal of Fish and Marine Sciences 5, 239–243. URL: https://www.cabdirect.org/globalhealth/abstract/20133252446.

Dutta, M., Majumdar, P.R., Islam, M.R.U., Saha, D., 2018. Bacterial and fungal population assessment in smoked fish during storage period. Journal of Food Microbiology, Safety & Hygiene 03, 127. 10.4172/2476-2059.1000127.

Faparusi, F., Adewole, A., 2018. *Salmonella* and *Shigella* assessment of smoked atlantic horse mackerel (*Trachurus trachurus*) and blue whiting (*Micromesistius poutasou*) sold in Ilaro, Yewa-South, Nigeria. Zenodo URL: https://zenodo.org/record/2652606, 10.5281/zenodo.2652606.

Hagen, I.V., Helland, A., Bratlie, M., Brokstad, K.A., Rosenlund, G., Sveier, H., Mellgren, G., Gudbrandsen, O.A., 2016. High intake of fatty fish, but not of lean fish, affects serum concentrations of TAG and HDL-cholesterol in healthy, normal-weight adults: A randomised trial. The British Journal of Nutrition 116, 648–657. 10.1017/S0007114516002555.

Harrigan, W.F., 1976. Laboratory Methods in Food and Dairy Microbiology. Revised ed., Academic Pr, London.

Ineyougha, E.R., Orutugu, L.A., Izah, S.C., 2015. Assessment of microbial quality of smoked *Trachurus trachurus* sold in some markets of three South-south States, Nigeria. International Journal of Food Research 2, 16–23. URL: http://www.bluepenjournals.org/ijfr/abstract/2015/June/Ineyougha_et_al.php.

Jung, A.V., Le Cann, P., Roig, B., Thomas, O., Baurès, E., Thomas, M.F., 2014. Microbial contamination detection in water resources: Interest of current optical methods, trends and needs in the context of climate change. International Journal of Environmental Research and Public Health 11, 4292–4310. 10.3390/ijerph110404292.

Marti, E., Balcázar, J.L., 2013. Real-time PCR assays for quantification of *qnr* genes in environmental water samples and chicken feces. Applied and Environmental Microbiology 79, 1743–1745. 10.1128/AEM.03409-12.

Mohanty, B., Mahanty, A., Ganguly, S., Sankar, T.V., Chakraborty, K., Rangasamy, A., Paul, B., Sarma, D., Mathew, S., Asha, K.K., Behera, B., Aftabuddin, M., Debnath, D., Vijayagopal, P., Sridhar, N., Akhtar, M.S., Sahi, N., Mitra, T., Banerjee, S., Paria, P., Das, D., Das, P., Vijayan, K.K., Laxmanan, P.T., Sharma, A.P., 2014. Amino acid compositions of 27 food fishes and their importance in clinical nutrition. Journal of Amino Acids 2014, 269797. 10.1155/2014/269797.

Odu, N.N., Imaku, L.N., 2013. Assessment of the microbiological quality of street-vended ready-to-eat Bole (roasted plantain) fish (*Trachurus Trachurus*) in Port Harcourt Metropolis,Nigeria. Researcher 5, 9–18.

Ofred, J.M.M., 2009. Microbiology and Spoilage Trailin Nile Perch (*Lates niloticus*), Lake Victoria, Tanzania. Master thesis. Faculty of Food Science and Nutrition, School of Health Sciences University of Iceland Reykjavik, Iceland.

Olaleye, O., Abegunde, T., 2015. Microbiological safety assessment of selected smoked fish in Lagos metropolis. British Microbiology Research Journal 6, 1–5. 10.9734/{BMRJ}/2015/17161.

Olutiola, P., Famurewa, O., Sontag, H., 1991. An Introduction to General Microbiology: A Practical Approach. Hygiene Institut der Universitat Heidelberg, Heidelberg, Germany. URL: https://www.scienceopen.com/document?vid=4fd92eda-2c64-4b36-a2a9-6a32b48d809c.

Ryu, S.H., Park, S.G., Choi, S.M., Hwang, Y.O., Ham, H.J., Kim, S.U., Lee, Y.K., Kim, M.S., Park, G.Y., Kim, K.S., Chae, Y.Z., 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* strains isolated from commercial fish and seafood. International Journal of Food Microbiology 152, 14–18. 10.1016/j.ijfoodmicro.2011.10.003.

Sandhu, R., 2016. Evaluation of multiple antibiotic resistance (MAR) index and doxycycline susceptibility of *Acinetobacter* species among inpatients. International Journal of Infectious Diseases 3, 299–304. 10.1016/j.ijid.2016.02.710.

Shane, A.L., Mody, R.K., Crump, J.A., Tarr, P.I., Steiner, T.S., Kotloff, K., Langley, J.M., Wanke, C., Warren, C.A., Cheng, A.C., Cantey, J., Pickering, L.K., 2017. Infectious Diseases Society of America Clinical Practice Guidelines for the diagnosis and management of infectious diarrhea. Clinical Infectious Diseases 65, e45–e80. 10.1093/cid/cix669.

Soleimani, N., Aganj, M., Ali, L., Shokoohizadeh, L., Sakinc, T., 2014. Frequency distribution of genes encoding aminoglycoside modifying enzymes in uropathogenic *E. coli* isolated from Iranian hospital. BMC Research Notes 7, 842. 10.1186/1756-0500-7-842.

Ungo-Kore, H., Bulus, M., Nuhu, T., Olowookere, A., Saadatu, M., Alhasan, M., Ibrahim, A., Shuaibu, A., 2019. Genotypic detection of extended-spectrum beta-lactamases from selected bacterial isolates in the specialist hospital Sokoto, Nigeria. Journal of Applied Sciences and Environmental Management 23, 1573. 10.4314/jasem.v23i8.23.

Wang, M.Y., Geng, J.L., Chen, Y.J., Song, Y., Sun, M., Liu, H.Z., Hu, C.J., 2017. Direct detection of *mec*a, bla_{SHV} , bla_{CTX}-m , bla_{TEM} and bla_{OXA} genes from positive blood culture bottles by multiplex-touchdown PCR assay. Letters in Applied Microbiology 64, 138–143. 10.1111/lam.12676.