

Microbial Profile of smoked fish sold in selected markets in Ibadan metropolis, Oyo State, Nigeria

Abstract

Aim

Smoked fish is a widely consumed food and protein source prepared in various cultures around the world. The aim of this study was to determine microbial load of smoked fish sold in select markets in Ibadan, Oyo State.

Methodology

A total of 36 samples of 4 different fish species; Mackerel (*Scomber scombrus*), Sardine (*Sardinella eba*), Panla (*Gadus morhua*) and Cat fish (*Clarias gariepinus*) were sourced from three different market locations. Three pieces of whole smoke-dried fish samples of each of these four species was collected at three different markets. These samples were purchased from Taska market, Adelabu market, and Molete market, all situated in Ibadan Metropolis.

Results

The study identified microorganisms; *Listeria monocytogenes* (15%), *staphylococcus aureus* (15%), *Vibrio paraheamolyticus* (10%), *Salmonella sp.* (15%), *Pseudomonas sp.* (15%), *Aeromonas sp* (15%), and *Escherichia coli* (15%) from the samples. The presence of these bacteria pose a health risk as some of them have been reported in previous studies as hazardous for human consumption. The Multiple Antibiotics Resistance (MAR) Index of bacterial isolates showed some isolates displayed high resistance (e.g., *Listeria monocytogenes* with a MAR Index of 1.0) and others showing lower resistance levels (e.g., *Vibrio parahaemolyticus* and *Salmonella sp.*, both with a MAR Index of 0.1).

Conclusion

The presence of these bacteria in smoked-fish sample is a cause for concern because it suggests that the fish is contaminated with pathogenic bacteria that have survived the smoking process.

27 Caution should be exercised in consuming smoked-dried fish displayed openly, reheating and
28 prolonged cooking may be necessary to deactivate such micro-organisms before consumption.

29 Keywords: *Microbial profile, Bacteria, Fish, Pathogenic microorganisms, Ibadan.*

30 **1. Introduction**

31 Fishes are a rich source of protein commonly consumed as an alternative source of protein due to
32 the higher cost of meat and other sources of animal protein [1]. Consumption of fish and fish
33 products are highly recommended due to good digestibility and the high content of
34 polyunsaturated fatty acids. Yet fish is a highly perishable food and so, many strategies have
35 been developed to limit its spoilage [1]. While there are various food preservation techniques to
36 improve microbial safety and extend shelf-life of fish including freezing, chemical preservation,
37 salting, smoking, frying and filleting, smoking still remains a popular method of fish processing
38 [2].

39 Smoking is one of such strategies used to preserved fish over a long period of time [3]. Smoked
40 fish is a widely consumed food item that has been prepared and enjoyed for centuries in various
41 cultures around the world [4]. The smoking process not only imparts unique flavors but also
42 provides a method of preservation, allowing fish to be stored for longer periods without spoilage
43 [5]. Consumption of smoked and smoke-dried fish both with and without further cooking is
44 common in Nigeria [5]. It has been reported that smoke-dried fish are often contaminated with
45 microorganisms such as bacteria, yeasts and mould from the processing units to market centers
46 [3]. It has also been noticed that good storage practices are not used by most wholesalers of
47 smoked and smoke-dried fishes [4]. Studies have also observed that post processing microbial
48 contaminations originates from poor handling practices, while some could be from the air, the
49 source of the fish, or from other degrading substances [5,6].

50 Food is unsafe when microbial pathogens or contaminants that can invade human body (e.g
51 *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, etc.) are present in the food. The presence
52 of toxin producing microbes such as *Staphylococcus aureus*, *Clostridium botulinum* and *Bacillus*
53 *cereus* are also injurious to human health [7, 8]. This study was set out to determine the
54 microbial profile of smoked fish sold in select markets within Ibadan which is the largest black
55 city in terms of land mass in West Africa with an estimated population of over 4 million
56 residents.

57 2. MATERIALS AND METHODS

58 2.1 Sample Collection and Processing

59 This study was carried out as a cross-sectional study within the city of Ibadan, Oyo state,
60 Nigeria. Samples of smoked fish sold were collected from three markets namely; *Taska market*,
61 *Adelabu market*, and *Molete market* within Ibadan. These markets were chosen due to their
62 popularity and significant presence in the city. They represent different areas within Ibadan
63 metropolis and provide a diverse range of smoked fish products.

64 A total of 36 samples of smoked fish was collected in sterile plastic bags and transported using
65 ice packs to the microbiology laboratory at *Lead City University, Ibadan* for microbial analysis.
66 The sample included three replicates of four different types of smoke-dried fish: mackerel
67 (*Scomber scombrus*), sardine (*Sardinella eba*), Panla (*Gadus morhua*), and catfish (*Clarias*
68 *gariepinus*).

69 O

70 2.2 Serial dilution, biochemical tests, Gram staining and cell morphology

71 ~~2.2.1 Serial dilution~~ The fish samples were collected in triplicate from each market, brought to the laboratory

74 According to the methods of Begum et al., 2021 10g of each fish sample was carefully weighed
75 aseptically and homogenized in 90ml sterile peptone water for serial dilutions. Serial dilution
76 was carried out with dilution factors 10^{-3} , 10^{-5} and 10^{-7} [21]. Diluents were then spread-plated on
77 plates of nutrient agar (for total viable counts); salmonella-shigella agar (for salmonella and
78 shigella species); Mannitol salt agar (for *staphylococcus spp*); listeria agar base (for *Listeria*
79 *monocytogenes*); and MacConkey agar (for *E. coli* and other enteric bacteria).

80 The agar plates were prepared in triplicates and incubated at 37°C for 24 hours. Total number of
81 cells per gram of samples was then estimated after counting the colonies on the plates. Distinct
82 colonies on the plates were then picked and sub-cultured on nutrient agar plates to ensure purity
83 of cultures. The different pure cultures were then transferred to nutrient agar slants. To confirm
84 the presence of bacteria, a series of biochemical tests were performed, including indole, methyl
85 red, voges-proskauer (VP), and citrate tests, as well as oxidase, hydrogen sulfide production,
86 lactose fermentation, gas production, catalase, sugar fermentation tests and coagulase tests.
87 Gram staining was also done to determine gram reaction while the cell morphology was
88 determined using microscopy.

89 **2.3 Pathogenicity test**

90 All the isolates within this study were subjected to pathogenicity test using blood agar. Tryptic
91 soy agar (TSA) was prepared and supplemented with 5% sheep blood and this was done following
92 manufacturer's instructions. Pure cultures of the bacterial strains under investigation were
93 obtained using sterile inoculating loop or needle to pick a colony from the fresh culture and
94 streak it onto the surface of the blood agar plate. Inoculated blood agar plates were put into the
95 incubator set at 37°C for all the bacteria isolates in this study. The plates were incubated for 18-
96 24 hours, to allow bacterial growth. After the incubation period, the blood agar plates were

97 examined for signs of hemolysis, this is indicated by changes in the appearance of the blood
98 surrounding the bacterial growth.

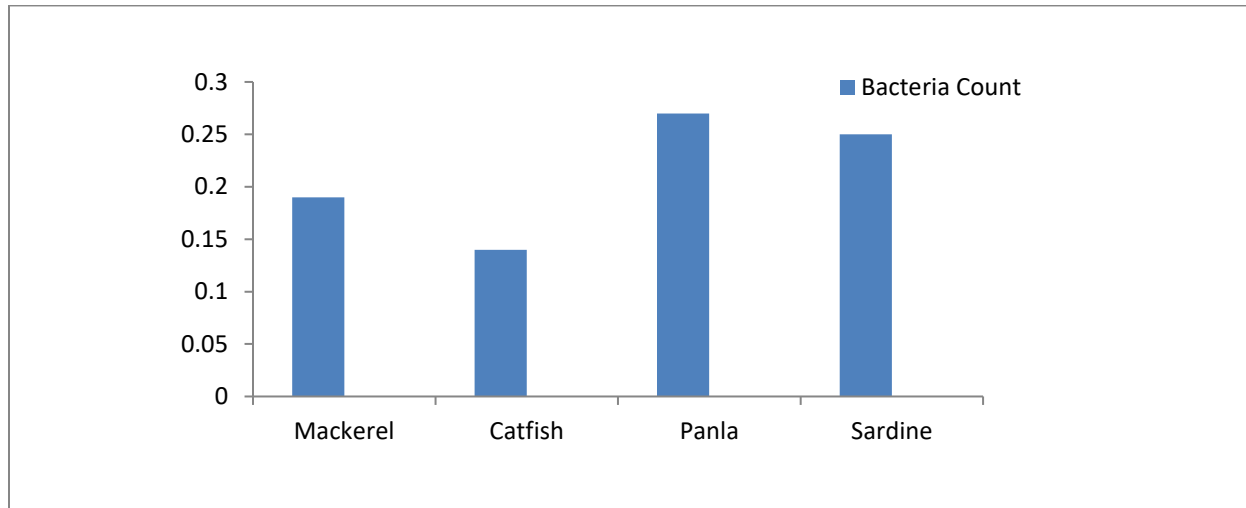
99 **2.4 Antibiotics susceptibility tests (AST)**

100 Disk diffusion (Kirby-Bauer) method was used to determine the susceptibility of bacterial isolates
101 to various antibiotics and identify multidrug-resistant strains. Bacterial isolates were spread on
102 Mueller-Hinton agar plates. Antibiotic-impregnated disks were placed on the surface, and plates
103 were incubated. Zones of inhibition around the disks were measured to determine susceptibility.
104 Multiple antibiotics resistance index (MAR index) was also done and calculated in this study.
105 MAR index is the ratio of number of antibiotics to which organism is resistant to total number of
106 antibiotics to which the organism is exposed. **Where the numerator is the aggregate antibiotic
107 resistance score of the isolate from the sample and denominator is the total number of antibiotics
108 used.**

109 **3. Results and Discussion**

110 The bacterial load on fish from different market locations in Ibadan metropolis are presented in
111 **Table 1**, displays the results of the bacterial load in fish samples obtained from various markets.
112 The highest bacterial load of $0.35 \pm 0.11 \times 10^3$ cfu/g was observed in fish samples from Taska,
113 followed by samples from Molete market with a count of $0.12 \pm 0.10 \times 10^3$ cfu/g. On the other
114 hand, the lowest bacterial load was recorded in fish samples from Adelabu, measuring 0.07 ± 0.04
115 $\times 10^3$ cfu/g. **Figure 1**, presents the bacterial load found in different fish types across the Ibadan
116 metropolis. The highest bacterial count was detected in panla (*Gadus morhua*) with a value of
117 $0.27 \pm 0.19 \times 10^3$ cfu/g, followed closely by sardine (*Sardinella eba*) with a value of $0.25 \pm 0.20 \times$
118 10^3 cfu/g. Conversely, the lowest bacterial count was observed in catfish (*Clarias gariepinus*)

119 samples with value $0.14 \pm 0.10 \times 10^3$ cfu/g. Nevertheless, statistical analysis using ANOVA
 120 revealed no significant difference ($p > 0.05$) in the microbial load among the various fish species
 121 sold in the Ibadan metropolis.



122
 123 **Figure 1 : Microbial count on different types of fishes in Ibadan, oyo state (x 10³ cfu/g)**

124 **Table 1: Microbial load on fish from different market location in ibadan**

Market	Tvc (cfu/g)
Taska	$0.35 \pm 0.11 \times 10^3$
Adelabu	$0.07 \pm 0.04 \times 10^3$
Molete	$0.12 \pm 0.10 \times 10^3$

125 **F-statistic: 82.89**
 126 **P-value: 7.09×10^{-21}**

127 **Table 2: Biochemical characteristics of gram positive isolates**

Isolate code	Gram stain	Cell morphology	Catalase	Oxidase	Citrate	Lactose	Glucose	Arabinose	Sucrose	Mannitol	V.p tests
T3e	+	Rods	+	+	-	-	+	-	-	+	-
T2e	+	Cocci	+	+	-	-	+	-	-	+	-
Ad1av	+	Rods	+	+	-	-	+	-	-	+	-
T3v	+	Cocci	+	+	-	-	+	-	-	+	-
M4v	+	Cocci	+	+	-	-	+	-	-	+	-
Ad6e	+	Rods	+	+	-	-	+	-	-	+	-

128 Key: + = positive reaction, - = negative reaction, v = variable

129 Samples were coded based on the market the sample was collected from and the number of
 130 isolates which were grown from samples from that market

131 **Table 3: Biochemical characteristics of gram negative isolates**

Isolate code	Gram stain	Cell morphology	Catalase	Oxidase	Citrate	Lactose	Glucose	Arabinose	Sucrose	Mannitol	V.p tests	Indole
M3v	-	Curved Rods	+	+	+	+	+	+	+	+	+	V
M2e	-	rods	-	-	-	-	+	-	-	-	+	-
M6e	-	Rods	+	+	+	+	+	+	+	-	+	-
M6v	-	Rods	+	+	-	-	+	-	+	V	V	-

M5v	–	Rods	+	–	–	+	+	–	–	+	–	+
Ad3v	–	Rods	+	–	–	+	+	–	–	+	–	+
M4v	–	Rods	–	–	–	–	+	–	–	–	+	–
Ad6v	–	Rods	+	+	+	+	+	+	+	–	+	–
Ad6ae	–	Rods	+	+	–	–	+	–	+	V	V	–
Ad1bv	–	Rods	+	–	–	+	+	–	–	+	–	+
M3e	–	Rods	+	+	–	–	+	–	+	V	V	V
T3v	–	Rods	+	+	+	+	+	+	+	–	+	V
T3e	–	Rods	–	–	–	–	+	–	–	–	+	V
M3v	–	Curved Rods	+	+	+	+	+	+	+	+	+	V

132 Key: + = positive reaction, - = negative reaction, v = variable

133 **Table 4: Suspected identity of gram positive isolates based on biochemical tests**

Isolate code	Suspected isolate identity
T3e	<i>Listeria monocytogenes</i>
T2e	<i>Staphylococcus aureus</i>
Ad1av	<i>Listeria monocytogenes</i>
T5v	<i>Staphylococcus aureus</i>
M4v	<i>Staphylococcus aureus</i>
Ad6e	<i>Listeria monocytogenes</i>

135 **Table 5: Suspected identity of gram negative isolates based on biochemical tests**

Isolate code	Suspected isolate identity
M3v	<i>Vibrio parahaemolyticus</i>
M2e	<i>Samonella sp.</i>
M6e	<i>Pseudomonas sp.</i>
M6v	<i>Aeromonas sp.</i>
M5v	<i>Escherichia coli</i>
Ad3v	<i>Escherichia coli</i>
M4v	<i>Samonella sp.</i>
Ad6v	<i>Aeromonas sp.</i>
Ad6ae	<i>Pseudomonas sp.</i>
Ad1bv	<i>Escherichia coli</i>
M3e	<i>Aeromonas sp.</i>
T3v	<i>Pseudomonas sp.</i>
T3e	<i>Samonella sp.</i>
M3v	<i>Vibrio parahaemolyticus</i>

137 **Table 6: Antibiotics susceptibility tests for gram positive isolates**

Antimicrobial	T3v	T2e	Ad1av	Ad3v	M4v	Ad6e
Agent						
Apx	0mm	0mm	14mm	0mm	0mm	14mm
Z	0mm	0mm	17mm	19mm	14mm	17mm
Am	15mm	0mm	16mm	17mm	17mm	16mm
R	17mm	0mm	18mm	18mm	15mm	18mm
Cpx	19mm	19.5mm	17mm	19mm	18.5mm	17mm
S	17mm	16mm	17mm	19mm	15mm	17mm
Sxt	17mm	20mm	14.5mm	18mm	14mm	14.5mm
E	14mm	18mm	16mm	13mm	12mm	16mm
Pef	15mm	19mm	14mm	18mm	15mm	14mm
Cn	20mm	19mm	19mm	17mm	16mm	19mm

138 **Key:** Apx: Ampicillin, Z: Azithromycin, Am: Amoxicillin, R: Rifampin, Cpx: Ciprofloxacin,

139 S: Sulfamethoxazole, Sxt: Trimethoprim-sulfamethoxazole, E: Erythromycin, Pef: Pefloxacin

140 Cn: Clindamycin

141 **Table 7: Antibiotics susceptibility tests for gram negative isolates**

Antimicrobial Agent	M3v	M2e	M6e	M6v	M5v	T3 e	M4v	Ad6v	Ad6ae
Apx	0	0	14mm	0	0	0	0	0	0
Z	0	0	17mm	19mm	14mm	0	0	0	17mm
Am	15mm	0	16mm	17mm	17mm	17mm	16.5m m	18mm	19mm
R	17mm	0	18mm	19mm	15mm	16mm	15mm	13mm	15mm
Cpx	19mm	19.5m m	17mm	19mm	18.5m m	16mm	16mm	14mm	17mm
S	17mm	16mm	17mm	19mm	15mm	15.5m m	17mm	18mm	16mm
Sxt	17mm	20mm	14.5m m	18mm	14mm	14mm	18mm	17mm	13mm
E	14mm	18mm	16mm	13mm	12mm	13mm	19mm	15mm	15mm
Pef	15mm	19mm	14mm	18mm	15mm	15mm	17.5m m	16mm	17mm
Cn	20mm	19mm	19mm	17mm	16mm	16mm	18mm	17mm	11mm

142 **Key:** Apx: Ampicillin, Z: Azithromycin , Am: Amoxicillin, R: Rifampin, Cpx: Ciprofloxacin,
 143 S: Sulfamethoxazole, Sxt: Trimethoprim-sulfamethoxazole, E: Erythromycin, Pef: Pefloxacin
 144 Cn: Clindamycin

145 **Table 8 : Antibiotics susceptibility tests for gram negative isolates**

Antimicrobial	Ad1bv	M3e	T5v	T3e	M3v
Agent					
Apx	0	14mm	0	15mm	0
Z	0	17mm	0	15mm	13mm
Am	12mm	15mm	12mm	14mm	15mm
R	13mm	16mm	16mm	16mm	16mm
Cpx	16mm	16mm	15mm	16mm	15mm
S	16.5mm	14mm	15mm	18mm	17mm
Sxt	14mm	15mm	14mm	17mm	16mm
E	15mm	15mm	14mm	14mm	14mm
Pef	16mm	16mm	13mm	16mm	15mm
Cn	15mm	14mm	11mm	15mm	15mm

146 **Key:** Apx: Ampicillin, Z: Azithromycin , Am: Amoxicillin, R: Rifampin, Cpx: Ciprofloxacin,
 147 S: Sulfamethoxazole, Sxt: Trimethoprim-sulfamethoxazole, E: Erythromycin, Pef: Pefloxacin
 148 Cn: Clindamycin

149 **Table 9: Pathogenicity test for gram positive isolates**

Isolate code	Isolate Name	Alpha Hemolysis	Beta Hemolysis	Nil Hemolysis
T3e	<i>Listeria monocytogenes</i>	-	+	-
T2e	<i>Staphylococcus aureus</i>	-	-	-
Ad1av	<i>Listeria monocytogenes</i>	-	-	-
T3v	<i>Staphylococcus aureus</i>	-	+	-
M4v	<i>Staphylococcus aureus</i>	-	-	-
Ad6e	<i>Listeria monocytogenes</i>	-	+	-

151 **Table 10: Pathogenicity test for gram negative isolates**

Isolate code	Isolate Name	Alpha Hemolysis	Beta Hemolysis	Nil Hemolysis
M3v	<i>Vibrio paraheamolyticus</i>	-	-	-
M2e	<i>Samonella sp.</i>	-	-	-
M6e	<i>Pseudomonas sp.</i>	-	-	-
M6v	<i>Aeromonas sp.</i>	-	+	-
M5v	<i>Escherichia coli</i>	-	-	-
T3 e	<i>Escherichia coli</i>	-	+	-
M4v	<i>Samonella sp.</i>	-	-	-
Ad6v	<i>Aeromonas sp.</i>	-	-	-
Ad6ae	<i>Pseudomonas sp.</i>	-	-	-
Ad1bv	<i>Escherichia coli</i>	-	+	-
M3e	<i>Aeromonas sp.</i>	-	-	-
T3v	<i>Pseudomonas sp.</i>	-	-	-
T3e	<i>Samonella sp.</i>	-	+	-

M3v

Vibrio

-

-

-

paraheamolyticus

152

153 **Table 11: Multiple Antibiotics Resistance Index of isolates in this study**

S/N	Isolate code	Suspected isolate identity	MAR Index
1	T3e	<i>Listeria monocytogenes</i>	1.0
2	T2e	<i>Staphylococcus aureus</i>	0.4
3	Ad1av	<i>Listeria monocytogenes</i>	1.0
4	T5v	<i>Staphylococcus aureus</i>	0.2
5	M4v	<i>Staphylococcus aureus</i>	0.2
6	Ad6e	<i>Listeria monocytogenes</i>	1.0
7	M3v	<i>Vibrio paraheamolyticus</i>	0.1
8	M2e	<i>Samonella sp.</i>	0.1
9	M6e	<i>Pseudomonas sp.</i>	0.4
10	M6v	<i>Aeromonas sp.</i>	0.1
11	M5v	<i>Escherichia coli</i>	0.1
12	Ad3v	<i>Escherichia coli</i>	0.1

13 M4v *Samonella sp.* 0.1

154

155

156 **Table 12: Multiple Antibiotics Resistance Index of isolates in this study (Cont.)**

S/N	Isolate code	Suspected isolate identity	MAR index
14	Ad6v	<i>Aeromonas sp.</i>	0.2
15	Ad6ae	<i>Pseudomonas sp.</i>	0.1
16	Ad1bv	<i>Escherichia coli</i>	0.2
17	M3e	<i>Aeromonas sp.</i>	1.0
18	T3v	<i>Pseudomonas sp.</i>	0.2
19	T3e	<i>Samonella sp.</i>	1.0
20	M3v	<i>Vibrio paraheamolyticus</i>	0.1

157

158 MAR index is calculated as the ratio of number of antibiotics to which organism is resistant to total
159 number of antibiotics to which the organism is exposed. Where the numerator is the aggregate antibiotic
160 resistance score of all isolates from the sample and denominator is the total number of antibiotics used.

161 Smoking fish is a preservation method that involves exposing the fish to smoke and heat to
162 extend its shelf life. However, smoking alone might not be sufficient to eliminate all potential
163 pathogens [14]. Smoked fish that is improperly processed, stored, or handled can become
164 contaminated with bacteria, including those with pathogenic potential [15]. Consuming smoked

165 fish contaminated with pathogenic bacteria can lead to foodborne illnesses in humans, causing
166 symptoms such as gastroenteritis, nausea, vomiting, diarrhea, and abdominal cramps [20].

167 The findings of this study show the microbial load of fish samples collected from three markets
168 locations in Ibadan, Oyo state. The result shows no statistical difference in the amount of
169 microorganisms present from the different markets which were sampled. This indicates that
170 generally the samples from different markets had microbial loads which were similar. The
171 Codex guidelines for fish and fishery products (Codex Standard 244-2007) provide microbial
172 limits for various types of fish, including smoked fish, and these codes are used globally.
173 According to this standards the recommended limit of $< 10^6$ CFU/g (colony-forming units per
174 gram) is the limit acceptable for smoked fish and based on this recommendation, the samples in
175 this study are still well within acceptable limits for health and safety as values for microbial load
176 of samples ranged from 0.12 to 0.35 10^3 CFU/g [19].

177 Figure 1 shows the microbial load on various types of smoked fish collected from markets in
178 Ibadan, Oyo State. The microbial counts are expressed as colony-forming units per gram (cfu/g),
179 multiplied by 10^3 , and panla (*Gadus morhua*) can be clearly seen to carry the highest microbial
180 load followed by sardine (*Sardinella eba*),, mackerel (*Scomber scombrus*), and then lastly catfish
181 (*Clarias gariepinus*). The elevated microbial loads can impact shelf life and pose health risks.
182 The results of our study underscore the need for improved sanitary measures in fish handling and
183 storage at the markets, as well as routine microbiological assessments to ensure consumer safety.
184 Regular monitoring and stricter control measures during smoking and post-processing are
185 recommended to reduce microbial contamination and enhance the quality of smoked fish sold in
186 these markets.

187 The study identified suspected microorganisms that were both gram positive and gram negative
188 isolates associated with smoke-dried fishes sold in different markets in Ibadan, using
189 biochemistry, Gram staining and microscopy to include; *Listeria monocytogenes*,
190 *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella species*, *Pseudomonas species*,
191 *Aeromonas species*, and *Escherichia coli*. The presence of these bacteria in the fish samples is an
192 issue of concern as some of these isolates pose significant health risks and have been reported in
193 previous studies as hazardous for human consumption.

194 The Codex guidelines for fish and fishery products (Codex Standard 244-2007) recommended
195 the total absence of *Salmonella spp.* as it is a harmful pathogen, yet unfortunately it was
196 identified as one of the isolates in our sample [19]. The same can be said for *Listeria*
197 *monocytogenes* which was present in our samples and has been seen to be particularly dangerous
198 to the health of people within the vulnerable population such as pregnant women, infants and the
199 elderly. *Staphylococcus aureus* was present in our samples although we did not calculate the
200 levels of the isolate within our samples [13].

201 Studies [9,10] have reported that the spoilage of fish is primarily caused by the activity of
202 psychotropic gram-negative bacteria such as *pseudomonas species*. Similarly, studies have also
203 reported that fish and fish products can spoil due to specific spoilage organisms that vary
204 depending on the treatment, preservation, and storage conditions, including temperature [11, 12
205]. These findings of this study suggest that the presence of some of the organisms implicated in
206 causing spoilage of fish such as *pseudomonas sp.* *Aeromonas sp.*, *vibrio sp.* and
207 *enterobacteriaceae* which were identified in the fish samples can lead to spoilage and potential
208 health hazards if consumed.

209 Examples of specific spoilage organisms (SSO) commonly found in different fish and fish
210 products include *pseudomonas*, *Aeromonas hydrophila*, *vibrionaceae*, *enterobacteriaceae*, yeast,
211 and moulds etc. were similar to the isolates identified within our study [9]. According to studies
212 which were conducted in artisanal fishery, freshly caught fish are often covered with damp sacks
213 or mixed with wet grass or water weeds to lower the temperature [17]. This method can increase
214 the risk of contamination with microorganisms such as *Salmonella sp* and other microorganisms
215 meaning that fish spoilage can begin just after a fish has been caught or even while it is still
216 within the aquatic ecosystem.

217 During the smoke drying process, the use of smoking kilns and overcrowding of fish on trays can
218 lead to improper processing, which promotes fungal growth. Furthermore, inadequate storage
219 practices, such as poor ventilation and pest infestation, during the storage of smoked dried fish
220 products can further contribute to microbial contamination [13]. The environment in which fish
221 are displayed in the market is often unhygienic, providing another pathway for microbial
222 contamination. It is common to find retailers displaying smoke-dried fish samples in open trays
223 near gutters or refuse heaps. This practice encourages the growth of fungi and bacteria, which
224 can lead to the production of toxins.

225 Bacteria isolates within our study such as *listeria monocytogenes* and *staphylococcus aureus*
226 displayed beta hemolysis positive in the pathogenicity test while bacteria isolates such as
227 *Aeromonas sp.*, *Salmonella sp.*, and *Escherichia coli* showed beta hemolysis as Gram negative
228 isolates. The presence of hemolytic bacteria in a smoked-fish sample is a concern because it
229 suggests that the fish may be contaminated with harmful bacteria with virulent factors and
230 characteristics and yet, that have survived the smoking process. The presence of beta-positive

231 gram-positive bacteria in a smoked-fish sample may indicate a need for closer inspection of the
232 fish processing and handling practices.

233 In our study Multiple Antibiotics Resistance (MAR) Index of bacterial isolates from smoked fish
234 in markets were evaluated to determine the resistance level of the isolates to various antibiotics.
235 The MAR Index is a valuable indicator in assessing the resistance patterns in bacteria and
236 highlights the potential health risks posed to consumers. The MAR Index values of the isolates
237 ranged significantly, with some isolates displaying high resistance (e.g., *Listeria monocytogenes*
238 with a MAR Index of 1.0) and others showing lower resistance levels (e.g., *Vibrio*
239 *parahaemolyticus* and *Salmonella sp.*, both with a MAR Index of 0.1). Notably, isolates such as
240 *Staphylococcus aureus* had variable resistance profiles, with MAR Index values of both 0.2 and
241 0.4 across different isolates, indicating inconsistency in resistance patterns among different
242 strains within the same species.

243 High MAR Index values, such as those observed for *Listeria monocytogenes* and *Pseudomonas*
244 *sp.* with a MAR Index of 1.0, suggest that bacteria in our study is highly resistant to multiple
245 antibiotics, posing a greater risk of persistence. These results indicate the need for strict
246 monitoring of antibiotic resistance in smoked fish to safeguard public health, as well as the
247 importance of judicious antibiotic use in food production and processing environments. Many of
248 the bacteria isolated within this study can be harmful to humans and cause foodborne diseases,
249 hence their presence in smoked fish samples is quite concerning.

250 Notably, *vibrio parahaemolyticus*, which was present within the samples in this study has been
251 frequently linked to seafood contamination and can result in gastroenteritis when consumed by
252 people [16]. If correct food safety procedures are not followed, the study has corroborated the

253 findings of other studies that contamination will occur while the fish is being processed, handled,
254 or stored. A lack of precautions could allow bacteria found in raw fish to survive the smoking
255 process, continue alive, and endanger the health of consumers [17, 18].

256 **Conclusion**

257 The study findings revealed that smoked-dried fishes in Taska, Adelabu, and Molete markets in
258 Ibadan, Oyo state, are contaminated with microorganisms. However, the microbial load observed
259 still falls within the recommended limits for ready-to-eat foods, indicating that the fish sold in
260 different markets within Ibadan metropolis is safe for human consumption. Therefore in order to
261 prevent contamination of smoked fish products, it is recommended that fish sellers be educated
262 on processing and handling of their fish wares.

263 **Recommendation**

264 There is need for sensitization on post- processing handling of the smoked catfish products on
265 how to ensure that they are well packed in well ventilated baskets and transported in proper
266 sanitized trucks. The adoption of good processing practice and the use of controlled temperature
267 in processing and preserving of the smoked catfish are highly recommended.

268 **Disclaimer (Artificial intelligence)**

269 **Authors hereby declare that No generative AI technologies such as large language models such**
270 **as ChatGPT and COPILOT etc. as well as text to image generators were used in the writing or**
271 **editing of this manuscript.**

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