A Comparative Assessment of Bacteria Associated with Fried and Dry-Smoked Fish in Some Locations in Makurdi, Nigeria.

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ABSTRACT

Thirty-two samples of fried and smoked fish were collected from four different locations (Watada, High-level, North bank and Wurukum) in Makurdi. Serial dilutions were prepared and appropriate dilutions inoculated onto Nutrient and Salmonella/Shigella agar. The bacterial load of the samples was determined and the colony forming unit per gramme (cfu/g) of fried fish samples ranged from 2.0×10^8 to 3.8×10^8 cfu/g while that of dried fish samples ranged from 4.3×10^8 to 7.8×10^8 cfu/g indicating that the dried fish samples were more contaminated. A total of 9 bacterila species were isolated from the 32 samples which includes, *E. coli*, *Staphylococcusaureus*, *Bacillus* spp, *Shigella* spp, *Salmonella* spp, *Klebsiella* spp, *Micrococcus* spp, *Listeriamonocytogenes*, and *Pseudomonas* spp. The percentage (%) occurrence of the bacteria isolates indicates that *E. coli*, *Staphylococcusaureus*, and *Bacillus* spp, showed 100%, *Micrococcus* spp 43.75%, *Klebseilla* spp 31.25%, *Shigella* spp and *Salmonella* spp 21.88% each, *Listeria monocytogenes* 18.75%, and *Psedomonas* spp 12.5% percentage frequency respectively. Results from this study showed that, samples collected from Wadata were the most contaminated, followed by those from North Bank, then those from Wurukum and those from High Level had the least contamination. The antimicrobial susceptibility test on the bacterial isolates showed that, 3 of the isolates (*Staphylococcus aureus*, *E. coli* and *Pseudomonas* spp) were multi-drugs resistance. Staphylococcus *aureus* isolates were resistance to Penicillins and Glycopeptides as it showed 60.7%, 62.5%, and 25.5% resistance to Oxacillin, Penicillin G, and Vancomycin respectively. *E. coli* and *Pseudomonas* spp isolates were both resistant to Tetracyclines and Penicillins. The resistance shown by *E. coli* isolates were 32.2% and 36.9% to Tetracycline and Ampicillin respectively while *Pseudomonas* spp isolates showed 54.5% and 79.3% to Tetracycline and Ampicillin respectively.

Key words: Fried fish, dried fish, bacterial load, antimicrobial, and susceptibility.

INTRODUCTION

Fish are aquatic animals that typically have paired fins, gills, a lengthy, scale-covered body, and are typically cold-blooded, some of which have no scales. Fish can be divided into three primary classes, groupings, or types: cartilaginous fish (Chondrichthyes), jawless fish (Agnatha), and bony fish (Osteichthyes).With over 33,000 different species, fish are the most varied group of vertebrates (Bales, 2023).**Ichthyology** is the branch of zoology devoted to the study of fish (Bond, 1999).

The flesh of fish is usually infected with a wide range of microbes present in the water body. While the precise source of the majority of microbes found in fish products remains unknown, some workers have linked microbial infections and/or contamination of fish and fish products to a variety of factors, including unfavourable fish system conditions, pollution, seasonal variations, and fish handling and processing, which frequently results in fish spoilage and nutritional deterioration (Mossel and Ingram,1995; Abolagba*et al.*, 2011).

Fish can be sold in various forms such as frozen fish, dried fish, smoked fish, fried fish, canned fish, pre-cooked fish, salt-cured fish, marinades fish, fermented fish products, and processed fish products such as fish balls ((NurSyahirah and Rozzamri, 2022).

In most Nigerian markets, fish are commonly sold in fried and dry-smoke form. Frying is considered one of the popular processes used in the food industry to provide unique sensory appetite and change the physical properties of the food products (NurSyahirah and Rozzamri, 2022).Frying in oil is one of the conventional procedures for reducing water activity($_a$w), it is effective in fish preservation in the sense that the nature and gravity of fish spoilage depends proportionally on water activity ($_a$w), an important constituent of biological systems (Eyo, 2001).Smoking is one of the oldest methods of preserving fish. According to Arazu and Ogbeibu (2009), the preservation and processing of fish by smoking and drying are methods used by artisans' fishermen to avoid spoilage of their catches, and is still the most popular way of preserving fish in many communities in Nigeria today.

Fish spoilage still happens even though preservation techniques like frying, smoking, drying, and other methods help to prevent it. Microbial contamination of fish and contaminated fish products pose a serious threat to the world's growing demand for fish and fish products. Contamination and spoilage of fish due to bacteria is found to cause health hazards to man. Consequently, this work was aimed at examining the bacterial associated with both dried and fried fish sold in Makurdi, the Benue state, Nigeria as well as testing the antimicrobial susceptibility pattern of the bacteria isolates using some commercially available antibiotics.

MATERIALS AND METHODS

Sample Collection, Preparation and Inoculation

A total of 32 samples, 16 each of fried and smoked fish were collected from four different locations in Makurdi (Wurukum, North Bank, High Level and Wadata). The samples were collected in sterile polythene bags, labelled appropriately and taken to the laboratory for bacteria examination. Serial dilutions were made by dissolving 1g of the sample into 9ml of sterile distilled water, from 10^{-1} up 10^{-9}. The dilution factor of 10^{-5} to 10^{-9} were used to inoculate the nutrient agar and Salmonella/Shigella agar already prepared using spread plate method. All the plates were incubated at 37^0C for 24 hours. After 24 hours, the colonies on the plates were counted using colony counter (Infitek Inc, China) and the results recorded as colony forming unit per grammme (cfu/g). The selected isolates were sub-cultured to obtain a pure culture.

The isolates were identified using morphological characteristics such as Gram staining/microscopy and biochemical characteristics (Franco-Duarte *et al*., 2019; ASM, 2020).

Antimicrobial Susceptibility Test on the Bacteria Isolates

The disk diffusion susceptibility method was used, and the direct colony suspension method was used for preparing the inoculum from colonies grown for 24 hours. The test was performed by applying a bacterial inoculum of approximately 1–2×10^8cfu/ml to the surface of a Mueller-Hinton agar plate. Up to 6 for Gram-positive and 7 for Gram-negative commercially prepared, fixed concentration, paper antibiotic disks were placed on the inoculated agar surface. The antibiotics used for Gram-positive bacteria were; Cefazolin (30µg), Erythromycin (15µg), Gentamicin (10µg), Oxacillin (1µg), Penicillin G (10µg), and Vancomycin (30µg). That of Gram-negative bacteria were; Amikacin (30µg), Ampicillin (10µg), Cefoperazone (75µg), Cefazolin (30µg), Gentamicin (10µg), Tetracycline (30µg), and Tobramycin (10µg). Plates were incubated for 16–24 hours at 35°C prior to determination of results (Hudzicki, 2009; Reller *et al*., 2009; Bayot and Bragg, 2022).

RESULTS

The Results of the Total Bacteria Count from all the Samples

Table 1: The colony forming unit per grammme (cfu/g) of the bacteria from the fried fish.

cfu/g for fried fish samples				
Sample	1	2	3	4
A	3.3 x 10^8	2.3 x 10^8	2.31 x 10^8	2.3 x 10^8
B	2.8 x 10^8	2.3 x 10^8	2.2 x 10^8	2.4 x 10^8
C	2.0 x 10^8	2.8 x 10^8	3.8 x 10^8	3.7 x 10^8
D	3.6 x 10^8	2.4 x 10^8	2.0 x 10^8	2.4×10^{8}

Key: A= Wadata market, B= Wurukum market, C= North-Bank market, D=High-Level

Table 2: The colony forming unit per grammme (cfu/g) of the bacteria from the smoked-dried fish.

cfu/g for smoke-dried fish samples				
Sample	1	2	3	4
A	4.3 x 10^8	6.3 x 10^8	5.3 x 10^8	5.0 x 10^8
B	6.8 x 10^8	5.6 x 10^8	4.9 x 10^8	7.4 x 10^8
C	5.2 x 10^8	4.8 x 10^8	5.7 x 10^8	7.8 x 10^8
D	6.6 x 10^8	6.7 x 10^8	5.9 x 10^8	6.0×10^{8}

Key: A= Wadata market, B= Wurukum market, C= North-Bank market, D=High-Level

Results of Bacteria Isolated and Identified from all the Samples

Table3: Different bacteria species isolated from fried fish samples

S/N	**ISOLATE**	**A**				**B**				**C**				**D**			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	STA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	ECO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	BAC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	SHI	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
5	SAL	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
6	KLE	+	+	+	–	+	+	+	–	+	+	–	–	+	–	+	–
7	MIC	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
8	PSE	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–

Key: STA = *Staphylococcus aureus*, ECO = *E. coli*, BAC = *Bacillus* spp, SHI = *Shigella* spp, SAL = *Salmonella* spp, KLE = *Klebsiella* spp, MIC = *Micrococcus* spp, PSE = *Pseudomonas* spp., A = Wadata. B

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= North-Bank, C = Wurukum, and D = High-Level, + = present, – = absent

Table 4: Different bacteria species isolated from dried fish samples

S/N	ISOLATE	A				B				C				D			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	STA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	ECO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	BAC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	SHI	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–
5	SAL	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–
6	KLE	+	+	–	–	+	+	–	–	+	–	–	–	+	–	–	–
7	MIC	+	+	+	+	+	–	+	+	–	+	+	+	+	–	–	–
8	PSE	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–
9	LIS	+	–	–	–	+	+	–	–	+	–	–	–	+	–	–	+

Key: STA = *Staphylococcus aureus*, ECO = *E. coli*, BAC = *Bacillus* spp, SHI = *Shigella* spp, SAL = *Salmonella* spp, KLE = *Klebsiella* spp, MIC = *Micrococcus* spp, PSE = *Pseudomonas* spp., LIS = *Listeria monocytogenes*, A = Wadata. B = North-Bank, C = Wurukum, and D = High-Level, + = present, – = absent

Key: ECO = *E. coli*, STA = *Staphylococcus aureus*, BAC = *Bacillus* spp, MIC = *Micrococcus* sp, KLE = *Klebsiella* spp, SHI = *Shigella* spp, SAL = *Salmonella* spp, LIS = *Listeria monocytogenes*, and PSE = *Pseudomonas* spp.

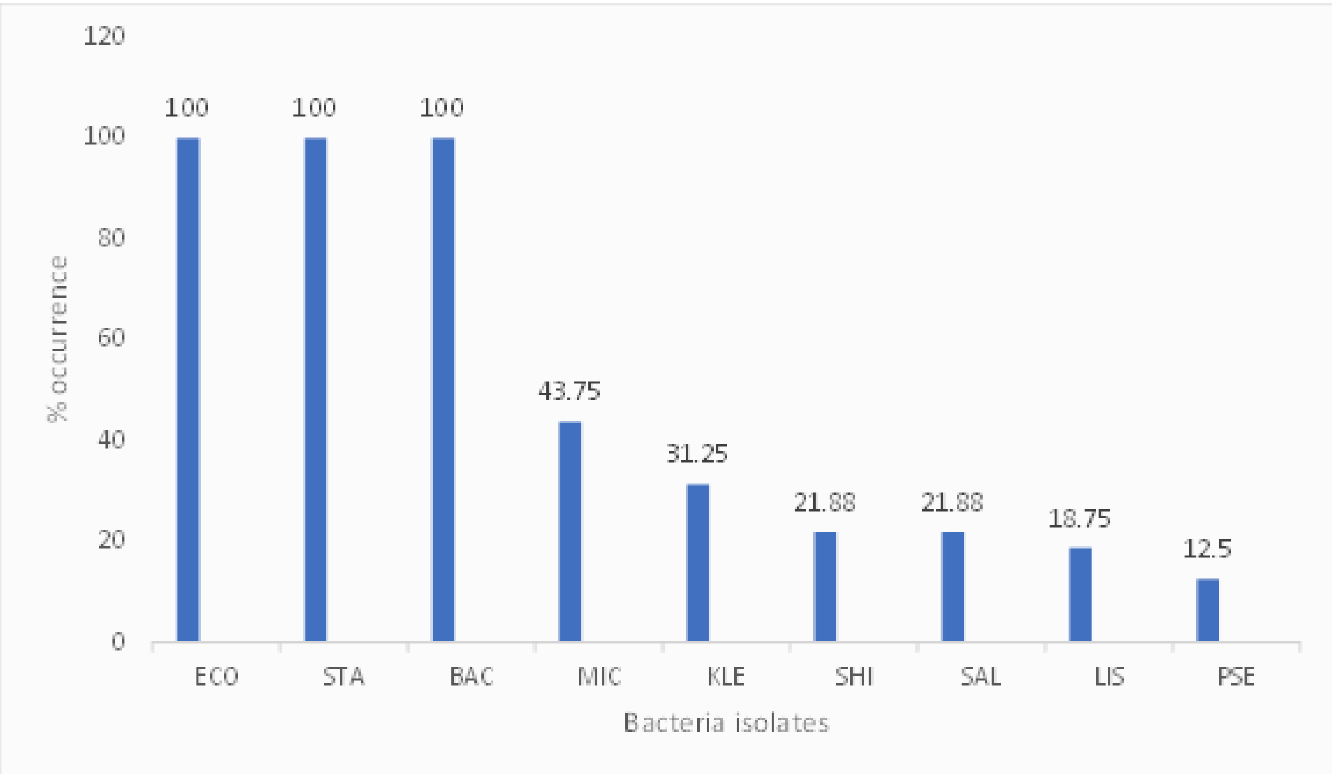

Figure 1: Percentage (%) occurrence of the bacteria isolates from the samples

Results of Antimicrobial Susceptibility Test on the Bacterial Isolates

Table 5: Antimicrobial susceptibility pattern of Gram-positive bacterial isolates from the fish samples

ANTIBIOTIC	ISOLATE (% frequency of sensitivity and resistance)			
	STA	MIC	BAC	LIS
	S R	S R	S R	S R
Cefazolin (30µg)	100, 0	100, 0	100, 0	100, 0
Erythromycin (15µg)	100, 0	100, 0	100, 0	100, 0
Gentamicin (10µg)	100, 0	100, 0	100, 0	100, 0
Oxacillin (1µg)	39.3, 60.7	100, 0	100, 0	100, 0
Penicillin G (10µg)	37.5, 62.5	100, 0	100, 0	100, 0
Vancomycin (30µg)	74.5, 25.5	100, 0	100, 0	100, 0

Key:STA = *Staphylococcus aureus*, BAC = *Bacillus* spp, MIC = *Micrococcus* sp, LIS = *Listeria monocytogenes*, S = susceptible, and R = resistance

Table 6: Antimicrobial susceptibility pattern of Gram-negative bacterial isolates from the fish samples

ANTIBIOTIC	ISOLATE (% frequency of sensitivity and resistance)				
	ECO	SHI	SAL	KLE	PSE
	S R	S R	S R	S R	S R
Amikacin (30µg)	100, 0	100, 0	100, 0	100, 0	100, 0
Cefoperazone (75µg)	100, 0	100, 0	100, 0	100, 0	100, 0
Cefazolin (30µg)	100, 0	100, 0	100, 0	100, 0	100, 0
Gentamicin (10µ)	100, 0	100, 0	100, 0	100, 0	100, 0
Tetracycline (30µg)	67.8, 32.2	100, 0	100, 0	100, 0	45.5, 54.5
Tobramycin (10µg)	100, 0	100, 0	100, 0	100, 0	100, 0
Ampicillin (10µg)	63.1, 36.9	100, 0	100, 0	100, 0	20.7, 79.3

Key: ECO = *E. coli*, SHI = *Shigella* spp, SAL = *Salmonella* spp,KLE = *Klebsiella* spp, PSE = *Pseudomonas* spp., S = susceptible, and R = resistance

DISCUSSION

The results from this investigation demonstrated that samples taken from each location contained bacteria (Table 1 and 2).Overall, smoked-dried fish samples had a greater bacteria load than fried fish. The fried fish samples showed a range of 2.0×10^{8} to 3.8×10^{8} colony forming units per gram (cfu/g), whereas the smoked-dried fish samples showed a range of 4.3×10^{8} to 7.8×10^{8} cfu/g (Table 1 and 2). According to Gutema and Hailemichael (2021), the high bacteria load in fish and fish products is probably due to poor handling. Many findings have been documented on the bacteria load of both dried and fried fish. According to Ebeniro and Nwosu (2020), the bacteria load of dried fish of samples collected from 3 local markets in Ngor-Okpara in Ebonyi state, Nigeria, ranged from 5×10^{4} to 1.8×10^{5} cfu/m. Various fried fish samples were observed to have cfu/g per plate ranging from 86×10^{5} to 165×10^{5} (Abolagba and Uwagbai, 2011).

A related finding was reported by Gutema and Hailemichael (2021), who found that samples of dried fish had a total viable bacterial count ranging from 4.20×10^{5} to 3.08×10^{7}.A range of 1.08×10 to 2.43×10^{3} cfu/g was similarly found by Ohalete *et al*. (2019) for 24 fried fish samples that were taken from 8 different

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locations in Owerri, Imo state, Nigeria. Similar findings were made by Ahmad *et al* (2024), who examined 40 fried fish samples that were collected from various sites in Katsina, Nigeria, and found that the cfu/g ranged from 1.5×10^6 to 7.2×10^6.

Table 3 and 4 lists the nine species of bacteria that were isolated from the 32 samples. The bacteria that were isolated included *E. coli*, *Staphylococcusaureus*, *Bacillus* spp, *Shigella* spp, *Salmonella* spp, *Klebsiella* spp, *Micrococcus* spp, *Listeriamonocytogenes*, and *Pseudomonas* spp. All 32 samples had *E. coli*, *Staphylococcusaureus*, and *Bacillus* spp., however only 7 samples contained *Salmonella* spp. and *Shigella* spp. Ten samples had *Klebsiella* spp, 14 samples included *Micrococcus* spp, 4 samples contained *Psudomonas* spp, and 4 samples contained *Listeria monocytogenes*.

The isolation of bacterial species from smoke-dried and fried fish agreed with some earlier findings. Gutema and Hailemichael (2021), reported the presence of coliforms and other bacteria such as *S. aureus* and *Salmonella* spp in dried fish products. Similarly, Anihouvi *et al* (2019), also isolated Enterobacteriaceae, *E. coli*, *Bacillus cereus*, and *Clostridium perfringens* from smoked and smoke-dried fish. From dried fish, Hassan *et al* (2021), also isolated *Salmonella* spp., *Bacillus* spp., *E. coli*, and some unknown bacteria.

There have also been reports of the isolation of many bacteria from fried fish. For instance, *Staphylococcus aureus*, *E. coli*, *Micrococcus* spp., and *Salmonella* spp. were identified among the bacteria isolated from fried fish (Amarachi, 2020). From dried fish, Begun and Rhaman (2021), isolated seven distinct types of bacteria, including *E. coli*, *Vibrio* spp., *Staphylococcus*spp., *Pseudomonas* spp.,*Salmonella* spp, *Shigella* spp., and *Klebsiella* spp. Similarly, Ohalete *et al* (2019), reported 10 genera of bacteria *Pseudomonas*spp, *E. coli*, *Aeromonas*spp, *Salmonella* sp, *Proteus* spp, *Vibrio* spp, *Bacillus* spp *Micrococcus* spp, *Streptococcus* spp, and *Staphylococcus*spp from fried fish.

Each of the isolated bacteria is a human pathogen with a history of being linked to food-borne illnesses. For instance, it is known that six different types of *E. coli* can infect humans when they are found in food: diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) (Begum, 2024). Food poisoning is frequently caused by *Staphylococcus aureus*, which can produce seven different types of toxins (Krause, 2017). *Bacillus* spp like *B. cereus* causes two distinct food poisoning syndromes: a rapid-onset emetic syndrome characterized by nausea and vomiting, and a slower-onset diarrhoeal syndrome (Turnbull, 1996). According to Nunez (2014), the skin of warm-blooded animals, including humans, is a main reservoir for *Micrococcus* strains, which frequently contaminate foods of animal origin and can cause opportunistic infections. *Klebsiella pneumoniae* is not only a major hospital-acquired pathogen but also an important food-borne pathogen that can cause septicemia, liver abscesses, and diarrhoea in humans (Zhang *et al*., 2018).Shigellosis is a form of bacterial diarrhoea caused by the *Shigella* species. It is common in developing countries and is transmitted via the ingestion of contaminated food (Aslam and Okafor, 2022). According to Lee (2023), *Salmonella* is a genus of bacteria that commonly cause foodborne illness. An infection by the bacteria is called salmonellosis and could be got by consuming contaminated food products. Foodborne listeriosis is one of the most serious and severe foodborne diseases. It is caused by the bacteria *Listeria monocytogenes*(WHO, 2018). *Pseudomonas aeruginosa* is a leading human opportunistic pathogen and a prevalent causative agent of food infection (Gao *et al*., 2023).

The percentage (%) occurrence of the bacterial isolates indicates that *E. coli*, *Staphylococcusaureus*, and *Bacillus* spp, showed (100%), *Micrococcus* spp(43.75%), *Klebsiella* spp(31.25%), *Shigella* spp and *Salmonella* spp (21.88%) each, *Listeria monocytogenes*(18.75%), and *Pseudomonas* spp (12.5%) (Fig. 1).This is comparable to other researchers' past findings. The percentage of isolated bacteria from fried fish, for instance, was documented by Amarachi (2020), as follows: 100% were *Staphylococcus aureus*, 85% were *E. coli*, 20% were *Micrococcus* spp, and 70% were *Salmonella* spp. Similarly, in their study, Begum *et al* (2021), identified seven different types of bacteria and the percentage occurrence from dried fish, including *E. coli* (21.43%), *Vibrio* spp (18.45%), *Staphylococcus* sp (17.86%), *Pseudomonas* spp (17.86%), *Salmonella* spp (12.5%), *Shigella* spp (8.93%), and *Klebsiella* spp (2.97%).

The 100% occurrence of *E. coli*, *Staphylococcusaureus*, and *Bacillus* spp in all the samples is a source of concern among the perceived consumers.Finding *E. coli*, *Staphylococcus aureus*, and *Bacillus* spp in all samples does indeed suggest a high level of contamination. *E. coli* is commonly found in faecal matter and indicates possible contamination by human or animal waste (Carson, *et al*., 2001). *Staphylococcus aureus* can cause various infections, and its presence suggests poor hygiene or improper food handling (Bencardino *et al*., 2021). *Bacillus* spp is a broad category of bacteria, some of which can be harmful if present in high quantities (Dilandro and Zundel, 2023). While finding these bacteria in all samples raises concerns, further analysis is necessary to determine the severity of the contamination and its potential risks to human health.

The antimicrobial susceptibility test of the bacterial isolates was also determined using Gram-positive and Gram-negative commercial antibiotics disks (Table 5 and 6).From Table 5, *Staphylococcus aureus*isolates were 100% susceptible to Cefazolin,Gentamicin, and Erythromycin. The investigation of Munawar *et al.* (2021) is comparable to this one in that they found that *Staphylococcus aureus* was susceptible to the following drugs: Ceftriaxone, Ciprofloxacin, Clindamycin, Augmentin, Erythromycin, and Cotrimoxazole.The *Staphylococcus aureus*isolates wereresistant to Oxacillin(60.7%), Penicillin G(62.5%), and Vancomycin(25.5%). Because the *Staphylococcus aureus* isolates were resistant to more than one class of antibiotics, including Glycopeptides (Vancomycin) and Penicillins (Oxacillin and Penicillin G), they are categorized as multidrug resistant(Bharadwaj *et al*., 2022). According to Nwankwo and Nasiru (2011), *Staphylococcus aureus* develops resistance very quickly and successfully to different antimicrobials over a period of time.

Micrococcus spp isolates were 100% susceptible to all the antibiotics used. The antimicrobial susceptibility pattern of *Micrococcus* spp isolates here is similar to that reported by Munawar *et al* (2021), with Gentamicin, Erythromycin, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, and Augmentin. *Bacillus* sppand *Listeria monocytogenes* isolates were also 100% susceptible to the antibiotics used.

According to Table 6, the *E. coli* isolates were completely susceptible to Amikacin, Cefoperazone, Cefazolin, Gentamicin, and Tobramycin, while they were resistant to Ampicillin (36.9%) and Tetracycline (32.2%). The isolates can also be described as multi-drugs resistance hence their resistance to Tetracyclines and Penicillins.*E. coli* has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer (Poirel *et al*., 2018).This outcome is comparable to that of Aabed *et al*. (2021), who found that isolates of *E. coli* had a percentage resistance of (70.8%) to both ampicillin and amoxicillin. It is however, at variance with Hassan *et al* (2023), that reported resistance to Amikacin (60.9%), Gentamicin (30%), Cefepime (51.8%), Cefotaxime (100%), Ceprofloxacin (77.8%), Ofloxacin (91%), Meropenem (11.4%), and Colistin (0.4%) respectively from *E. coli* isolates. The isolates of *Pseudomonas* spp. from Table 6 also showed 100% sensitivity to Amikacin, Cefoperazone, Cefazolin, Gentamicin, and Tobramycin, but resistance to Ampicillin (79.3%) and Tetracycline (54.5%). According to Lister *et al* (2009), *Pseudomonas* spp can develop resistance to antimicrobials either through the acquisition of resistance genes on mobile genetic elements or through mutational processes that alter the expression of chromosomally encoded mechanisms. *Shigella* spp, *Salmonella* spp, and *Klebsiella* spp isolates bothexhibited100% sensitivity to all the antibiotics used.

CONCLUSION

The findings of this study showed the presence of pathogens from all the samples. However, smoke-dried fish samples had higher bacterial load compared to the fried fish samples. The overall bacterial load exceeded the Food and Agriculture Organization's (FAO) suggested threshold of 10^5 cfu/g. Most bacterial isolates are likely to originate from the fish handlers as they are of feacal origin and members of Enterobacteriaceae. Therefore, in order to prevent such incidents, it is important to assign agencies in charge of public and personal hygiene to assist in educating vendors about safe handling practices for fish and fish products.The antimicrobial susceptibility test showed 3 of the bacterial isolates (*Staphylococcus aureus*, *E. coli* and *Pseudomonas* spp) to be multi-drugs resistance.Therefore, it's crucial to implement strict infection control measures to prevent the spread of these resistant strains within healthcare facilities and the community. This can include practices such as proper hand hygiene, isolation protocols for infected patients, and prudent antibiotic use to minimize further development of resistance.

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