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Microbial Load and Antibiotic Resistance Pattern of Microflora Isolated from Smoked Catfish and Mackerel Sold in Ghanaian Markets: A Potential Health Threat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Smoked fish, particularly catfish and mackerel, plays a vital role in the Ghanaian diet, serving as a rich source of protein and essential nutrients. Smoking is a traditional preservation method widely used to extend the shelf life of fish, enhance flavor, and make it more palatable. However, despite the advantages of smoking, the process does not entirely eliminate the risk of microbial contamination, which can have significant health implications.
Aim of Study: This study examined the microbial flora contamination of the smoked fish sold in Ghanaian markets.
Methodology: The research was conducted in three markets: Kejetia Market, Ejura Market and Ejusu. A total of 75 different smoke-dried fish including Cat fish (Clarias gariepinus) and Mackerel (Scomber scombrus) from the markets. The fish samples were collected and kept in sterile polythene bags and transported to the laboratory for microbial analysis.
Result: The study revealed that out of 75 samples analyzed, approximately 23% (n=17) were found to be contaminated with microorganisms. *Escherichia coli* had the highest occurrence, detected in 35.2% (6/17) of the total samples. Additionally, *Shigella flexneri* and *Salmonella arizonae* were each found in 17.6% (3/17) of the total samples. *Klebsiella oxytoca* was 11.7% (2/17), and *Enterobacter aerogenes*, *Streptobacillus monilliforms*, and *Fusobacterium necrophorus* each constituted 5.8% (1/17) of the total samples. All the identified isolates were susceptible to gentamycin (100%), and all the isolates were resistant to lincomycin (100%). Overall resistance rates for each antibiotic for all the organisms identified are lincomycin (100%), penicillin (67%), ampicillin (81%), erythromycin (65%), tetracycline (63%), neomycin (61%), cloxaxillin (43%), kanamycin (24%), and sulphamethaxole (13%). All the isolates have 100% resistance to at least three antibiotics used except for *Salmonella arizonae*.
Conclusion: To Address these findings, a collaborative effort is required among regulatory authorities, food producers, and healthcare providers to implement stringent food safety protocols and mitigate the risks associated with contaminated fish consumption.

Keywords: Smoked fish; microbial load; microflora; Ghanaian markets; food safety; health threat.

1. INTRODUCTION

The presence of pathogenic microorganisms and antibiotic-resistant strains in smoked fish constitutes a significant public health hazard with far-reaching consequences for consumers [1]. Consumption of contaminated fish products can precipitate a spectrum of foodborne illnesses, ranging from mild discomfort to more severe and potentially life-threatening conditions [2]. Smoked fish, including catfish (*Clarias gariepinus*) and mackerel (*Scomber scombrus*), is a popular and widely consumed food product in Ghana, providing a significant source of protein and essential nutrients to the local population [3].

The safety of smoked fish products is often compromised due to poor handling, processing, and storage practices, leading to microbial contamination [4]. Improper storage conditions, such as inadequate temperature control and exposure to environmental contaminants can also contribute to the proliferation of harmful microorganisms in smoked fish products [4,5]. The journey of smoked fish from production facilities to market stalls presents additional opportunities for microbial contamination [6]. Cross-contamination during transportation and marketing activities, where fish products meet unclean surfaces, equipment, or hands, further compounds the risk of microbial proliferation [7]. Therefore, smoked fish products sold in Ghanaian markets may harbor a diverse array of pathogenic microorganisms, including bacteria, viruses, and parasites, which pose significant health hazards to consumers [8].

The ingestion of smoked fish contaminated with pathogenic microorganisms can lead to a range of foodborne illnesses, encompassing bacterial infections, gastrointestinal disorders, and, in severe cases, life-threatening conditions [9]. Gastrointestinal infections are among the most common consequences of ingesting tainted fish, manifesting as symptoms such as nausea, vomiting, diarrhea, and abdominal cramps [10]. These symptoms can be debilitating, leading to dehydration and electrolyte imbalances, particularly in vulnerable populations such as children, the elderly, and individuals with weakened immune systems [11]. Moreover, certain pathogens found in contaminated fish,

such as Salmonella spp. and Vibrio spp., have the capacity to cause severe systemic infections, including septicemia and meningitis, posing grave risks to affected individuals [12].

The presence of antibiotic-resistant bacteria in smoked fish exacerbates the threat to public health by undermining the efficacy of antibiotic treatment [13]. Antibiotics are cornerstone medications for combating bacterial infections, but the proliferation of antibiotic-resistant strains diminishes their effectiveness, leading to prolonged illness, increased healthcare costs, and elevated mortality rates [14]. Beyond the immediate health implications for consumers, the dissemination of antibiotic-resistant bacteria through the food chain poses broader societal challenges [15]. Resistant strains can persist and proliferate in the environment, facilitating the transmission of resistance genes to other bacteria and compromising treatment outcomes for various infectious diseases, not limited to those originating from foodborne pathogens [16,17]. This phenomenon erodes the effectiveness of antibiotics across healthcare settings, jeopardizing the success of medical interventions for both common and life-threatening infections [18]. Therefore, this study investigated the microbial load and antibiotic resistance pattern of microflora isolated from smoked Catfish and Mackerel in Ghanaian markets.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in three markets: Kejetia Market, Ejura Market and Ejusu Market from November 2023 to April 2024. Kejetia market (6.6666 ° N, 1.6163° W) is one of the largest and busiest markets in Ghana, located in Kumasi, the capital city of the Ashanti Region of Ghana. The market serves as a significant trading hub, attracting traders from various regions and neighboring countries. Kejetia Market offers a wide range of goods, including fresh produce, clothing, textiles, crafts and household items. Ejura Market (7.3856° N 1.3562° W) is a bustling trading center located in the town of Ejura; which is also situated in the Ashanti Region of Ghana. This market serves as a vital commercial hub for the local community and surrounding areas. Ejusu Market (6.53880 N, 0.26010 W) is another significant trading center in the Ashanti Region, located in the town of Ejusu. Although smaller in size compared to Kejetia Market; it plays a crucial role in meeting the daily needs of the local community [19].

Fig. 1. Map of the study area [19]

2.2 Sample Collection

A total of 75 different smoke-dried fish including Catfish (*Clarias gariepinus*) and Mackerel (*Scomber scombrus*) was purchased from Kejetia Market, Ejura Market and Ejusu Market. The fish samples were collected and kept in sterile polythene bags and transported to the laboratory for microbial analysis.

2.3 Preparation of Materials

The working tables were swabbed with ethanol to disinfect them. All the wares were washed and air-dried after which they were sterilized in hot air oven at 160^0C for 1hour. Culture media (nutrient agar) were prepared according to manufacturers' specifications and distilled water used for serial dilution, followed by sterilization took place in an autoclave at 121^0C for 15minutes before use [20].

2.4 Preparation of Agar Plate

The agar plates were prepared by first sterilizing the petri dishes. This was done by putting the petri dishes in Petri dish containers in the hot air oven at 160^0C for 1 hour. The sterilized plates were then left in petri dish container until required; the wire loop was sterilized by flaming in red-hot fire using a spirit lamp. The agar was prepared by dissolving 0.6g of the agar in 100 ml distilled water and sterilized it using a microwave at 121^0C for 15min and left to cool to 45^0C [21].

2.5 Preparation of Fish Samples for Microbiological Tests

Fish samples were collected from different markets. The fish were minced after serial dilutions were made. Total viable count was done, colonies on the plates were picked and sub-cultured for identification.

2.6 Serial Dilution

10g of each fish sample was weighed aseptically and homogenized in 90ml sterile peptone water. Then, serial dilutions were made by mixing 1.0ml of the suspension in 9.0ml sterile peptone water to obtain 10-1 dilution. The dilution was then made to 10-2, and 10-3 diluents, then spread-plated on plates of nutrient agar (for total viable counts); Salmonella shigella agar (for Salmonella and Shigella species); Mannitol salt agar (for *Staphylococcus spp);* Listeria agar base (for *Listeria monocytogenes*); MacConkey agar (for *E. coli* and other enteric bacteria). The plates were triplicated and incubated at 37^0C for 24 hours. Total number of cells per gram of samples were then estimated after counting the colonies on the plates. Colonies on the plates were picked and sub-cultured on nutrient agar plates to ensure purity of cultures. The different pure cultures were transferred to nutrient agar slopes and identified [22].

2.7 Characterization and Identification of the Isolates

Bacterial isolates were characterized using routine microbiological procedures as described by Olutiola et al. (1991) after which they were identified using Bergey's Manual of Determinative Bacteriology [22].

The microbiological identification procedures used included the following:

2.7.1 Colony morphology

This involves the microscopic evaluation of the characteristics of bacteria colonies on the agar plates. The characteristics considered included the shape of the colony, elevation of the colony, edge of the colony, colony surface pigmentation and the optical characteristics [23].

2.7.2 Cell morphology

This involved staining of the isolates to show the cell shape and appearance. In this study, gram staining method was conducted on each isolate. This involved studying the isolates under the oil lens immersion microscope after gram-staining. A thin smear of each isolate was made and heat-fixed. The heat-fixed smears were covered with crystal violet for about 1 minute and immediately rinsed with clean water. The smear was then flooded with iodine for 1 minute and then rinsed immediately. The smear was decolorized for 10 – 30 sec using 95% ethyl alcohol. The alcohol action was terminated by rinsing the slide with clean water; the smear was counterstained with safranin for 30 seconds and it was rinsed off using clean water and after which it was allowed to air dry. The stained slides were examined under the microscope (with the aid of immersion oil) for results [24].

2.7.3 Motility test

This was carried out using hanging drop method (Fawole and Osho, 1995). Here, a clean

Fig. 2. Colonies of identified isolates

depression slide and cover glass was used. They were washed and rinsed to provide a grease-free slide. An exceedingly small amount of Vaseline was placed near each corner of the cover slide. Two loopful of the isolate was placed in contact with the cover glass with the depression slide put over the drop of suspended bacteria. The slide was quickly inverted and examined under the microscope; the motion of the organisms was observed [25].

2.8 Antibiotic Susceptibility Testing (AST)

"Agar diffusion technique on Mueller–Hinton agar (Kirby–Bauer modified disc diffusion technique) according to CLS I guidelines was used to determine the antibiotic susceptibility. The inhibition zone standards for antimicrobial susceptibility were consulted from tables of interpretative zone diameters of the Clinical and Laboratory Standards Institute [26]. The study tested 10 antibiotic discs of the most commonly used drugs to treat human and animal infections caused by bacteria. These include erythromycin (ERY) (5 µg), Trimethoprim-sulfamethoxazole (SXT) (25 µg), gentamicin (GEN) (10 µg), Kanamycin (KAN) (10 µg), tetracycline (TET) (30 µg), chloramphenicol (CHL) (30 µg) ampicillin (AMP) (10 µg), Neomycin (NEO) (10 µg), Penicillin (PEN) (30 µg) and Lincomycin (LIN) (30 µg)".

2.9 Statistical Analysis

Data from the microbial investigations was cleaned and entered into Microsoft Excel 2016, then exported into Statistical Package for Social Sciences (SPSS, version 26) for statistical analysis. Descriptive statistics, including frequencies and percentages, was used to summarized the prevalence of microbial contamination. Statistical inference was conducted using the chi-square test. A p -value of < 0.05 was considered statistically significant.

3. RESULTS

3.1 Proportion of Contaminated Fish Samples

The results of the current study revealed that out of the total 75 samples analyzed, approximately 23% (n=17) were found to be contaminated with various microorganisms. Out of these contaminated samples, (69%, n=11) were smoked catfish, while 31% (n=6) were smoked mackerel (Fig. 3).

3.2 Prevalence of Microbial Infection

The study found that, the prevalence of pathogenic microorganisms varied among the sampled smoked fish products. *Escherichia coli* had the highest occurrence, detected in 35.2% (6/17) of the total samples. Additionally, *Shigella flexneri* and *Salmonella arizonae* were each found in 17.6% (3/17) of the total samples, indicating potential issues with hygiene and improper processing practices during fish handling and preparation. *Klebsiella oxytoca* was 11.7% (2/17), and *Enterobacter aerogenes*, *Streptobacillus monilliforms*, and *Fusobacterium necrophorus* each constituted 5.8% (1/17) of the total samples. Although present in relatively small percentages, the presence of these pathogenic bacteria highlights the need for continuous monitoring and improved hygiene practices during fish processing and distribution.

3.3 Prevalence of Microbial Contamination among Fish Samples

The most identified microbial contamination in this study was *Escherichia coli*; it was predominantly found in catfish 23.5% (4/17), compared to mackerel 11.7% (2/17). Hever, a significant association was found between *Escherichia coli* and Mackerel ($X2 = 133.333$, $df = 1$, $p < 0.001$) (Table 1). *Shigella flexneri* was found in catfish 11.7% (2/17) more than in mackerel 5.8% (1/17), however, there was no

significant association between *Shigella flexneri* infection and any of the fish samples (Table 1). In this study, *Salmonella arizonae* was found to be significantly associated with catfish (X2 =89.653, df = 1, p < 0.001). The analysis of the current study reported *Enterobacter aerogenes* in only catfish, however, there was no significant association between the two (X2 =225.333, df = 1, p < 0.001). These findings were summarized in Table 1. A higher prevalence of *Escherichia coli* was commonly found in fish samples collected from Kejetia market (54%) compared to others. Microbial contamination of fish samples among study sites was summarized in Fig. 5.

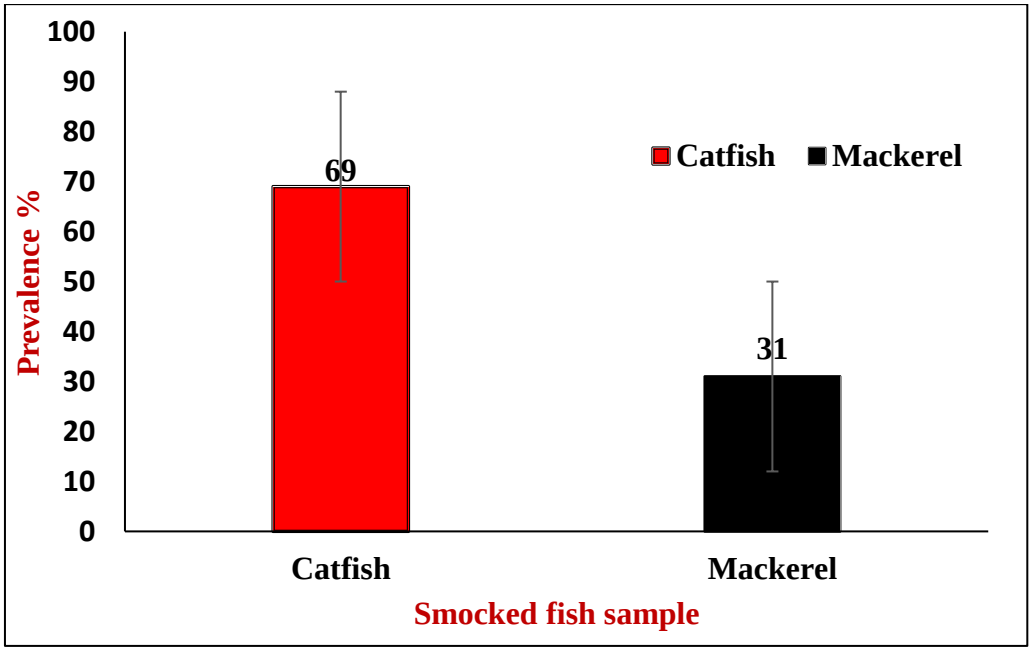

Fig. 3. Proportion of contaminated fish samples

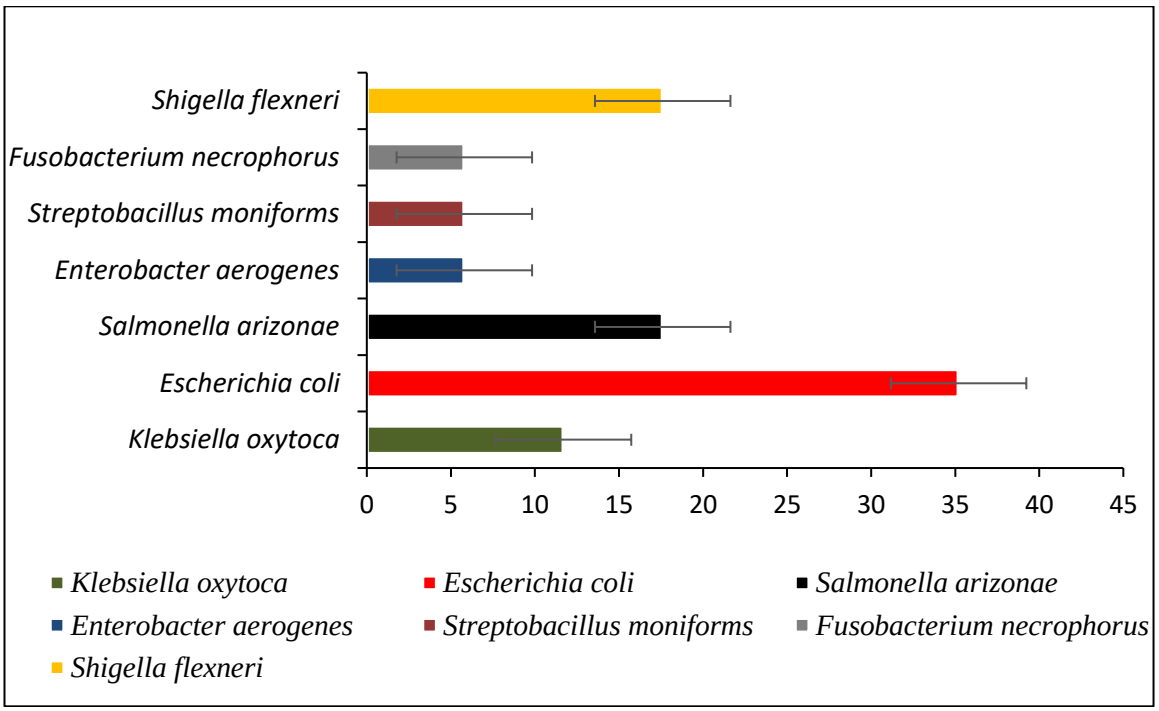

Fig. 4. Prevalence of bacterial infection among fish samples

Table 1. Prevalence of microbial contamination among fish samples

Microbial infection	Catfish	*p*-value	Mackerel	*p*-value
Escherichia coli	23.5% (4/17)	0.101*	11.7% (2/17)	< 0.001*
Shigella flexneri	11.7% (2/17)	0.027*	5.8% (1/17)	0.027*
Salmonella arizonae	17.6% (3/17)	< 0.001*	-	-
Klebsiella oxytoca	-			
	-		11.7% (2/17)	0.092
Enterobacter aerogenes	5.8% (1/17)	0.027*	-	0.160
Streptobacillus monilliforms	5.8% (1/17)		-	1.001
	0.027*			
Fusobacterium necrophorus			5.8% (1/17)	0.200
	-			

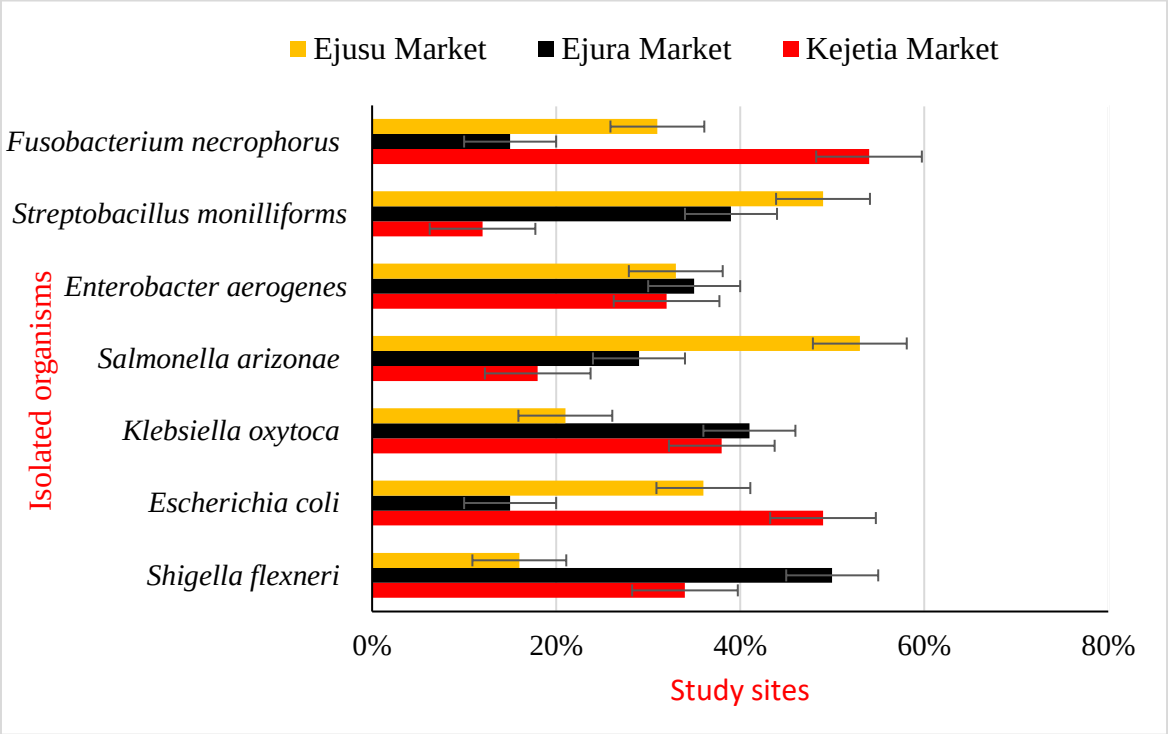

Fig. 5. Microbial contamination of fish samples among study sites

3.4 Antimicrobial Susceptibility and Resistance Testing

All the identified isolates were susceptible to gentamycin, and all the isolates were resistant to lincomycin. The isolated bacteria showed variable resistance rates to each antibiotic as shown in Table 2. Overall resistance rates for each antibiotic for all the organisms identified are lincomycin (100%), penicillin (67%), ampicillin (81%), erythromycin (65%), tetracycline (63%), neomycin (61%), cloxaxillin (43%), kanamycin (24%), and sulphamethaxole (13%) (Fig. 6). All the isolates have a 100% resistance to at least three antibiotics used except for *Salmonella arizonae* was 100% resistant to only two antibiotics. Therefore, all the bacterial isolates from fish sold at informal market were multidrug resistant except for *Salmonella arizonae*; A few species of *Klebsiella* were intermediate, and not considered resistant, but it was bunched in the susceptible group (Table 2).

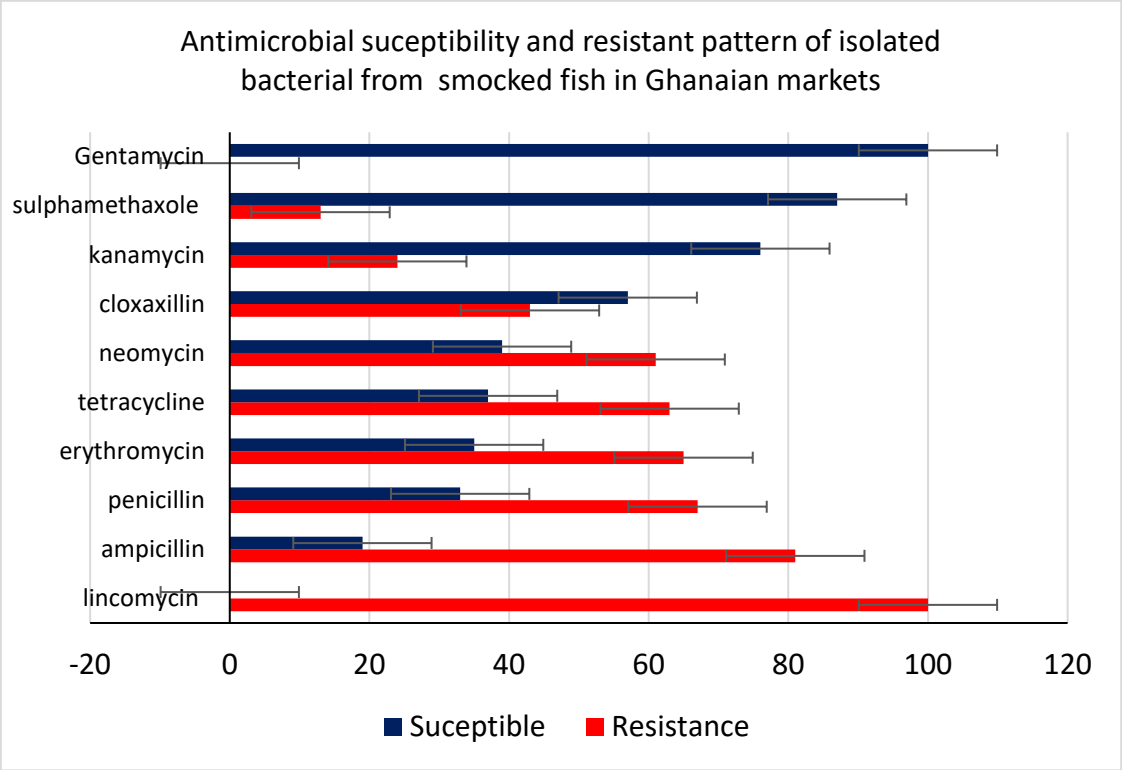

Fig. 6. Antimicrobial susceptibility and resistance pattern of isolated bacterial

Table 2. Resistance rate of bacteria to antibiotics

Bacteria	PEN	SXT	ERY	GEN	NEO	KAN	CLO	AMP	TET	LIN
Escherichia coli	100	0	49	0	100	0	50	88	67	100
Shigella flexneri	0	100	100	0	0	100	0	0	0	100
Salmonella arizonae	0	0	0	0	100	0	0	0	0	100
Klebsiella oxytoca	67	0	100	0	100	0	0	100	67	100
Enterobacter aerogenes	100	22	100	0	100	0	100	100	100	100
Streptobacillus monilliforms	100	0	50	0	100	0	50	50	50	100
Fusobacterium necrophorus	0	0	100	0	0	0	100	100	0	100

4. DISCUSSION

The results of the study reveal a concerning prevalence of pathogenic microorganisms in sampled smoked fish products, indicating potential risks to consumer health. *Escherichia coli*, a common indicator of fecal contamination and poor hygiene practices, exhibited the highest occurrence, detected in 35.2% of the total samples. This finding suggests a significant lapse in sanitary conditions during fish processing and handling, as the presence of *E. coli* indicates possible fecal contamination either from the aquatic environment, processing equipment, or personnel [27]. The detection of *Shigella flexneri* and *Salmonella arizonae* in 17.6% of the total samples each indicates the potential for foodborne illness transmission associated with consumption of these contaminated fish products [28]. *Shigella flexneri* is a known cause of shigellosis, a diarrheal disease characterized by abdominal pain, fever, and bloody stools [29], while *Salmonella arizonae* is a serotype of Salmonella associated with gastroenteritis and systemic infections in humans [30].

The presence of these pathogens suggests lapses in hygiene practices and inadequate processing techniques, posing significant risks to consumer health [27]. Furthermore, the identification of *Klebsiella oxytoca*, *Enterobacter*

aerogenes, Streptobacillus monilliforms, and *Fusobacterium necrophorus*, albeit in smaller percentages, shows the diverse array of potential contaminants present in smoked fish products. While these organisms may not be as commonly associated with foodborne illness as *E. coli, Shigella*, or *Salmonella*. Their presence still warrants attention due to their potential pathogenicity and implications for consumer safety [30].

In the current study, all the identified isolates were susceptible to gentamycin, and all the isolates were resistant to lincomycin. Gentamicin is an aminoglycoside antibiotic commonly used to treat bacterial infections, including those caused by Gram-negative organisms such as *Escherichia coli, Salmonella spp.,* and *Klebsiella spp*., which are frequently encountered in foodborne illnesses [31]. The susceptibility of all identified isolates to gentamicin suggests that this antibiotic remains an effective treatment option for infections caused by these bacteria [31]. This finding is reassuring from a clinical perspective, as it indicates that gentamicin can still be relied upon to combat infections associated with the consumption of contaminated fish products. However, continued monitoring of antibiotic susceptibility patterns is necessary to detect any emerging resistance trends and ensure the continued efficacy of gentamicin in clinical practice [32].

In contrast, the resistance of all isolates to lincomycin is concerning and has important implications for both veterinary and human medicine. Lincomycin, a lincosamide antibiotic is used in both human and veterinary medicine to treat various bacterial infections. It includes those affects the skin, respiratory tract, and bones [33]. The widespread resistance observed in the isolated bacteria suggests that lincomycin may not be an effective treatment option for infections associated with these organisms [33].

Multidrug resistance refers to the ability of bacteria to withstand the effects of multiple antibiotics, compromising the effectiveness of treatment options [34]. In this study, all isolates, except *Salmonella arizonae*, demonstrated 100% resistance to at least three antibiotics. This findings suggest a widespread exposure of these bacteria to antibiotics, due to indiscriminate antibiotic use, leading to the selection and proliferation of resistant strains [35]. The presence of multidrug-resistant bacteria in fish sold at the study markets poses a significant public health risk, as it increases the likelihood of foodborne disease outbreaks and exacerbates the burden of antibiotic-resistant infections [35]. Individuals who consume contaminated fish products are at heightened risk of developing infections that are difficult to treat, leading to prolonged illness, hospitalization, and potentially fatal outcomes [5]. Vulnerable populations, including children, the elderly, pregnant women, and individuals with compromised immune systems, are particularly susceptible to the adverse effects of multidrug-resistant infections, highlighting the need for stringent food safety measures and targeted interventions to protect public health [36-39].

5. CONCLUSION

In conclusion, this study sheds light on the alarming prevalence of pathogenic microorganisms in smoked fish products sampled from Ghanaian markets, highlighting significant risks to consumer health. The emergence of multidrug resistance among the isolated bacteria indicates the urgent need for enhanced surveillance and intervention measures to safeguard public health. Addressing these findings requires collaborative efforts among regulatory authorities, food producers, and healthcare providers to implement stringent food safety protocols and mitigate the risks associated with contaminated fish consumption.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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