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Research Article

Detection of Methicilin resistant *Staphylococcus aureus* in open displayed Ready-to-eat fish in some markets in Ilorin

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Abstract: Consumption of the open displayed ready-to-eat (RTE) fish is associated with health risks. This study investigated methicillin-resistant Staphylococcus aureus (MRSA) contamination of open displayed RTE fish samples in Oja Tuntun and Mandate markets in Ilorin Kwara State, Nigeria. Dried, smoked and fried fish samples obtained from the markets were subjected to bacteriological analysis to isolate and identify methicillin-resistant Staphylococcus aureus using standard methods. Samples obtained from Oja Tuntun market had a higher count than those obtained from Mandate market. The mean MRSA count ranged from $18.00 \pm 1.00 \times 108$ cfu/mg to $206.33 \pm 1.53 \times 108$ cfu/mg. Dried fish samples obtained from Oja Tuntun had the highest mean count of MRSA. While the lowest mean count was found in fried fish sample from Mandate market. In summary, the RTE fish samples examined were contaminated with MRSA, and this portends tendency of RTE foodborne spread of MRSA.

Keywords: Methicilin-resistant, *Staphyloccus aureus*, Ready-to-eat foods, Dried fish, Smoked fish, Fried fish

1 INTRODUCTION

Foods ready for immediate consumption at the point of sale are referred as ready-to-eat foods (RTEs). Such foods could be consumed raw or in already processed form (Akinwumi, 2014; Oje *et al.,* 2018). Various RTE food items and beverages are sold by hawkers or street vendors (Adiama *et al.,* 2022), they include: exotic dishes, fruit and vegetables, fermented drinks and beverages, and animal protein sources such as meat and various types of fishes (Ameko *et al*., 2012). In Nigeria and other developing countries, RTEs form a major source of food for the majority of the populace, because it is a convenient alternative to homemade foods (Orji *et al*., 2016).

Despite the benefit of RTEs, their consumption has been linked to the occurrence and outbreak of foodborne diseases (Beshiru *et al*., 2022). This could be due to the way such foods were processed, the hygiene condition of the handlers and the environments, and open display conditions for sale (Adu-Gyamfi and Nketsia-Tabiri, 2007). Microbial foodborne illnesses are often associated with RTEs or street vended foods (Mensah *et al.,* 2002; Feglo and Sakyi, 2012).

According to the Food and Agriculture Organization (1994), fish contributes about 60% of the world's supply of protein and more than half of the population of the developing world depend on fish as their main source of annual protein. Fish and fish products provide significant portion of protein need of man and livestock. It is particularly important as a cheap source of protein in riverine regions in developing countries (Oramadike *et al*., 2010). The nutritional and health benefits of fish consumption are well documented, fishes are low fat but rich in polyunsaturated fatty acids, and they had been claimed to have the capacity of lowering the occurrence of heart-related diseases (Amusan *et al*., 2010).

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In Nigeria, the fish processing industries are mostly small scale; hence, handling and processing of fish are such that it is prone to many forms of microbial contamination (Adelaja *et al*., 2013). Fish products are viable growth media for microbial pathogens; the growth process releases secondary metabolites and toxins that could cause food poisoning, infection diseases and other health hazards to the consumer (Barro *et al.,*2006). Recently, there has been an increase in the occurrence of foodborne diseases of microbial origin (da Silva, 2002; Novotny *et al*., 2004; Kate, *et al*., 2006). *Staphylococcus aureus*, is a Gram-positive cocci in clusters, it is a normal human commensal, and an opportunistic pathogen. Every human carries the bacterium on the superficial surfaces and the orifices. It is one of the leading causes of food-borne illnesses in humans.

Staphylococcus aureus has been reported to possess many virulent factors, notable among which are enterotoxins known as Staphylococcal Enterotoxins (SEs). Food poisonings due to *S. aureus* occur when foods containing one or more preformed SEs are consumed with food (Premarante *et al.*, 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* that are resistant to antibiotics with Beta lactam ring such as penicillins, cephalosporins and their derivatives. Human isolates of *S. aureus* are becoming resistant to the penicillins (De-Neeleng, 2007). The resistance is attributed to a mobile genetic element called a staphylococcal chromosomal cassete (SCCmec) (Sanabria-Rios *et al.,* 2022). Strains of MRSA pose a serious challenge to human and livestock health and have been of major concern to medical practitioners. Diseases caused by the bacterium are often difficult to treat because the organism is resistant to a wide range of commonly used antimicrobials (Lee, 2003; El-Deeb*et al.*, 2022).

Cases of asymptomatic carriers of MRSA in local communities have been reported (Bale *et al*., 2018), and MRSA contamination of fish and other foods by such individuals can lead to cases of community acquired MRSA (CA-MRSA) (Feglo and Sakyi, 2012). This tendency of CA-MRSA through consumption of openly displayed ready-to-eat (RTE) foods cannot be overemphasized, yet, there are scanty studies in the subject. In the study, we explored MRSA contamination in openly displayed RTE fish products. The study uses two market in Ilorin to highlights the tendency of CA-MRSA and other microbial foodborne illnesses through openly displayed RTE food products.

2 MATERIALS AND METHODS

2.1 Sample Collection

A total of forty-eight samples of dried (roasted dry on low heat), fried (deep fried in oil) and smoked fish (smoke-dried on local kiln) used for this study were obtained from fish sellers at Mandate and Oja Tuntun markets in Ilorin, Kwara State, Nigeria. Eight samples (Two samples per seller) were collected for each fish type in clean sterile black polythene bags, and taken to the laboratory of the Department of Microbiology, Kwara State University, Malete, immediately for microbiological analysis

2.2 Isolation of Methicillin-Resistant *Staphylococcus aureus*

Ten grams of each of the fish samples was aseptically weighed and introduced into 90 ml of sterile distilled water and homogenized with an electric blender for 3 minutes. The resulting homogenate was used as a stock, and kept for further analysis. Isolation MRSA was carried out using modified mannitol salt agar (MSA). Modified mannitol salt agar was prepared by adding 0.004 g of cefoxitin antibiotic powder to sterilized mannitol salt agar. One millilitre of the resulting homogenate of each fish sample was diluted ten-fold via serial dilution. One millilitre of a solution from the serial dilution was aseptically inoculated in triplicates onto modified mannitol salt agar plates using pour plate techniques. The plates were incubated at 37 °C for 24 hours. The resulting colonies were counted manually and the means of the colony forming units per gram of fish sample were recorded. Data obtained were subjected to statistical analysis using SPSS (2010). Total number of occurrences of isolate in a sample. Total number of times an isolate occurs in a is recorded as frequency of occurrence. Percentage occurrence of MRSA was determined by the formula:

$$\text{Percentage Occurrence (\%)} = \frac{\text{Number of occurrence of MRSA in a sample} \times 100}{\text{Total Number of occurrences of MRSA in all samples}}$$

2.3 Characterization and Identification of the Isolates

The bacterial isolates were characterized and identified using conventional colonial morphology, Gram's reaction (Olutiola *et al.*, 2000), catalase and coagulase tests as described by Fawole and Oso (2004). Isolates that produced characteristic golden yellow colonies on MSA and were later characterized as Gram positive cocci in clusters, catalase-positive and coagulase-positive were considered as *S. aureus*.

3 RESULTS

3.1 Characteristics of Isolated Bacterial Colonies

Bacterial colonies obtained on the modified mannitol salt agar were large, round, raised and yellow, they produce yellowish pigment that disperses into the medium. Details of the characteristics of the colonies is summarised in Table 1. The observed featured suggested that the bacterial colonies are Methicillin Resistant *Staphylococcus aureus* (MRSA).

Table 1: Characteristics of Bacteria Colonies Isolated from Open Displayed RTE Fish Samples

Features	Observation
Colour on MSA	Yellow
Elevation	Raised
Colony shape	Round
Coagulase	Positive
Catalase	Positive
Motility	Non-motile
Cell-shape	Cocci
Cell Arrangement	Cluster
Gram Reaction	Positive

Table 2: MRSA counts isolated from RTE fish samples from Oja Tuntun and Mandate Market

S/N	Mean Count ± SEM × 10^8 cfu/mg					
	Ojatuntun Market			Mandate Market		
	Fried fish	Dried fish	Smoked fish	Fried fish	Dried fish	Smoked fish
1	51.67±2.08^a	137.33±2.52^b	ND	ND	80.00±1.00^a	ND
2	95.33±2.52^b	44.00±1.00^a	104.00±1.00^c	ND	18.00±1.00^a	20.00±1.00^b
3	ND	122.67±2.52^b	95.33±2.51^a	ND	ND	ND
4	104.00±1.00^a	ND	191.00±1.00^b	22.00±1.00^a	ND	37.00±1.00^b
5	54.00±1.00^a	ND	ND	44.00±1.00^a	95.33±2.50^b	ND
6	20.00±1.00^a	70.00±1.00^b	98.33±1.53^c	18.00±1.00^a	51.67±2.09^b	ND
7	122.67±2.52^b	206.33±1.53^c	88.23±2.52^a	ND	ND	50.00±1.00^a
8	ND	188.33±1.53^b	70.00±1.00^a	51.33±2.02^a	ND	54.00±1.00^a

Key:
Values are means of replicates ± SEM
ND = Notdetected
SEM = Standard error of mean
Values within the same row having different superscripts are significantly different at $P < 0.05$

3.2 Colony counts of MRSA from the RTE Fish Samples

The colony count of MRSA isolated from the collected open displayed RTF fish samples are presented in Tables 2. The count ranged from 20.00±1.00×10^8cfu/mg to 206.33±1.53×10^8cfu/mg. Table 3. compares the mean count of MRSA isolated from the ready-to-eat fish samples obtained from Mandate and Oja Tuntun market. Among the fish samples collected, dried fish sample obtained from Oja Tuntun market had the highest MRSA count (206.33±1.53×10^8cfu/mg) and the lowest MRSA count was found in fried fish sample obtained from Mandate market (18.00±1.00×10^8cfu/mg). Generally, the total MRSA count on samples obtained from mandate market was significantly lower than those from Oja Tuntun market.

Table 3. Comparison of MRSA counts from samples from Mandate and Oja Tuntun markets

Samples	Mean count ± SEM × 10^8cfu/mg	
	Mandate market	Ojatuntun market
Fried fish	33.83±1.52 [a]	74.61±2.52 [b]
Dried fish	63.00±1.00 [a]	128.00±1.00 [b]
Smoked fish	40.25±1.52 [a]	107.82±1.52 [b]

Key;
Values are means of replicates ± SEM
SEM = Standard error of mean
Values within the same row having different superscripts are significantly different at $P < 0.05$

3.3 Frequency and Percentage Occurrence of MRSA

The frequency and percentage of occurrence of MRSA isolated from ready-to-eat locally processed fish samples are presented in Tables 4. The values with the spreads are the frequency while the values in parentheses are the percentages. Among the samples obtained from Oja Tuntun market, dried fish sample has the highest frequency of occurrence of MRSA (11.10%) followed by smoked fish sample (10.27%) as shown in Table 2. Table 3 shows the frequency and percentage of occurrence of methicillin resistant *Staphylococcus aureus* isolated from ready-to-eat locally processed fish samples obtained from Mandate market with dried fish sample having highest percentage frequency of occurrence (17.61%) and the lowest percentage of occurrence was observed in fried fish sample (3.33%).

Table 4: Frequency and Percentage Occurrence of MRSA Isolated from RTE Fish Samples obtained from Oja Tuntun and Mandate Market

S/N	Frequency and Percentage occurrence (%)					
	Ojatuntun Market			Mandate Market		
	Fried fish	Dried fish	Smoked fish	Fried fish	Dried fish	Smoked fish
1	51.67±2.08(2.78)	137.33±2.52(7.40)	104.00±1.00(5.60)	22.00±1.00(4.06)	80.00±1.00(14.78)	20.00±1.00(3.70)
2	95.33±2.52(5.13)	44.00±1.00(2.37)	95.33±2.51(5.13)	44.00±1.00(8.13)	18.00±1.00(3.33)	37.00±1.00(6.84)
3	104.00±1.00(5.60)	122.67±2.52(6.60)	191.00±1.00(10.27)	18.00±1.00(3.33)	95.33±2.50(17.61)	50.00±1.00(9.24)
4	54.00±1.00(2.90)	70.00±1.00(3.77)	98.33±1.53(5.30)	51.33±2.02(9.48)	51.67±2.09(9.55)	54.00±1.00(9.98)
5	20.00±1.00(1.08)	206.33±1.53(11.10)	88.23±2.52(4.75)			
6	122.67±2.52(6.60)	188.33±1.53(10.13)	70.00±1.00(3.77)			

Values are means of replicates ± SEM
Key;
SEM = Standard error of mean

4 DISCUSSION

Foodborne diseases are serious threat in the food and medical industries globally (Tambekar *et al.,* 2008). The presence of MRSA in the selected locally processed ready-to-eat fish samples can be due to poor sanitary practices of the food handlers and could also be as a result of cross contamination (Thwala *et al.*, 2021). Foodborne disease has been associated with the consumption of foodborne *Staphylococcus aureus* (Anderson *et al.*, 2020). Consumption of food contaminated with MRSA can result in a very serious health hazard (Fahim *et al.*, 2019) principally poisoning. Encounter with the organism from other sources can cause boils, impetigo, folliculitis and in some cases infections of the bones and wounds. Growth of microorganisms on food can lead to its decomposition and spoilage. Pathogenic microbes may result in the transmission of diseases. Also, the appearance, flavour, colour, and other properties of food may change as a result of the degradative action of spoilage microorganisms (Adelaja *et al*., 2013). It was observed in this study that the fish samples obtained from Oja Tuntun market were more contaminated than those obtained from Mandate market. This could be due to many factors such as the population of the market in terms of vendors and consumers patronizing the market, storage conditions and surfaces for displaying the fish, low sanitary condition of the market, time between production and purchase, moisture content of the fish samples after processing among others. This observation is similar to the report of Andargie *et al*. (2014) on the fact that foods sold in dirty environments are more prone to contamination.

Among the fish samples obtained from the two markets, it was observed that dried fish samples were more contaminated than smoked and fried fish samples. This can be as a result of the relatively high moisture content of the dried fish after processing. The moisture content is usually within the requirement for staphylococcal growth. Low processing temperature allows bacterial contaminants to survive and metabolize the fish and also the unhygienic practices of the handlers which could lead to cross contamination of wares. This goes in line with the report of Abolagba and Melle (2013) who observed high microbial counts in dried fish samples as a result of cross contamination. Also similar to this observation is the findings of Akinwumi (2014) who reported that outbreaks of foodborne illness have been linked to improper food handling practices. Montville *et al*. (2001) reported that uncontaminated food can be contaminated by microorganisms brought from another place. Smoked fish samples obtained from the two markets were also found to be more contaminated with MRSA than the fried fish samples. Smoked fish could be contaminated from sources similar to those earlier mentioned though the difference in count could be traceable to the method of processing and retailing. Kate *et al.* (2006) reported that processed fish are easily naturally contaminated through handling, during processing and if the post-processing is not properly done under hygienic conditions. Open-air markets have been implicated in direct transfer of *S.aureus* during handling between traders and customers of ready-to-eat smoked, dried and fried fish as reported by (Amusan *et al*. (2010). The quality of smoked products is dependent on several factors, including the preparation of raw material, the nature of the wood and the type of the smoking procedure employed (Kwaghvihi *et al.*, 2020).

On a general note, low frequency and percentage of occurrence of MRSA was observed in fried fish samples obtained from the two markets and this can be as a result of the heat of processing and the method of displaying fried fish in enclosed glazed box for sale. This observation is similar to the findings of William (2011), that processing and treatment reduces microbial load in foods. The findings in this research show that most of the fish samples were contaminated with MRSA beyond the acceptable limit in food. Fish because of its high protein content is usually prone to bacterial and fungal contaminants, which could result in food spoilage, infection and intoxication. Poor hygiene practices during processing and handling of food enhance the process (Olayemi *et al.,* 2012). Locally processed ready-to-eat fish samples are less expensive and commonly available protein food for the masses; hence, they must not carry pathogen or their toxic metabolites. Proper processing and storage of these fish samples is also necessary because poor storage

methods and unhygienic handling of the items are known to make them susceptible to contamination by MRSA. This researchprovided evidence that the fish samples were heavily contaminated with MRSA, a finding which is in agreement with the reports of Albuquerque *et al*. (2007), who found increasing emergence of antibiotic resistance in bacterial isolates originating from fish and fish handlers.

5. Conclusion

The results obtained from the study showed a high rate of contamination of the locally processed ready-to-eat fish samples with MRSA. The highest occurrence was in dried fish samples while the least was in fried fish samples. Samples from Oja Tuntun market were more contaminated than those from Mandate Market. This indicated that the locally processed ready-to-eat fish samples are produced and marketed under unhygienic conditions. The presence of these pathogenic organisms in the locally processed ready-to-eat fish samples is a matter of serious health concern. This calls for adopting adequate sanitary measures and hygienic practices during handling, processing and distribution of the ready-to-eat fish samples, to ensure safety of the unsuspecting consumers. It is recommended that there should be close monitoring along with ensuring the safety standard of the locally processed ready-to-eat fish samples sold to the public by both the State and Federal Ministries of Health and other food safety related agencies as a way of curbing or reducing the health hazards that its consumption may cause.

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