

Bacteriological Quality and Multi-drug Resistant *Staphylococcus aureus* Isolated from Smoked Dried Catfish (*Clarias gariepinus*) in Kaduna Metropolis, Nigeria

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ABSTRACT

Smoked dried catfish is a major source of protein in resource-poor countries. Unhygienic processing and handling of fish are a source of foodborne pathogens such as *Staphylococcus aureus*. This study aimed to determine the bacteriological quality of ready to eat smoked dried catfish and the antimicrobial susceptibility pattern of *S. aureus* from catfish in Kaduna, Metropolis, Nigeria. Three hundred smoked catfish specimens were collected from 4 markets (Central, Mando, Kawo and Tudun wada) and processed. The smoked dried catfish were subjected to total aerobic and coliform count, isolation and antimicrobial susceptibility testing of *S. aureus*. The results were analysed with descriptive statistics and presented in tables. The overall mean total aerobic and coliform counts were 3.01×10^6 CFU/g and 1.41×10^6 CFU/g, respectively. The Central market had the highest aerobic count (5.63×10^6 CFU/g), and Mando market had the highest coliform count (2.7×10^6 CFU/g). The overall isolation rate of *S. aureus* from smoked catfish was 276 (92.0%). Mando market had the highest isolation of *S. aureus* 74 (98.7%), while Kawo and Tudun Wada markets had 67 (89.3%). Of the 276 *S. aureus* isolates, 225 (75.0%) were identified as coagulase positive. The isolate showed the lowest resistance to Ciprofloxacin 21 (9.3%), Gentamycin 21 (9.3%) and Chloramphenicol 39 (17.3%). Resistance to Ampicillin and Penicillin was 100%. Multi drug resistant *S. aureus* contamination of ready to eat smoked dried catfish pose a severe public health hazard to the consumers.

Keywords: Antimicrobial Susceptibility; Fish, Foodborne pathogens; Microbiology; MDR-Staphylococcus aureus; Resistance

INTRODUCTION

Smoked dried catfish is distributed through a market web created by fish processors, traders, and consumers at various retail levels (Agbebi and Fagbote, 2012; Fox *et al.*, 2018). Hard curing by salting and smoking permits lengthy preservation by removing moisture, essential for bacteriologic and enzymatic spoilage. An increase in awareness of the nutritional value of seafood has stimulated strong demand for fish and its products (Pigott and Tacker, 1990). Therefore, producing good quality and safe smoked fish is necessary to satisfy the increasing demand for fish.

Fish is susceptible to a wide variety of potentially pathogenic bacteria (Schmidt *et al.*, 2000). Contamination during the processing and the presence of these bacteria harmful to humans generally indicates poor sanitation in handling and processing (Pandey *et al.*, 2014; WHO, 2020b). Contamination with pathogenic micro-organisms may result in severe chronic or fatal health consequences for the population and reduced productivity for the country.

Some people eat smoked dried fish uncooked or washed with water only, which is a considerable public health risk. Most outbreaks of food poisoning are associated with fish-derived from consumption of raw or insufficiently heat-treated fish which may be contaminated with bacteria from water or environment (such as *Escherichia coli*, *Staphylococcus* spp, *Vibrio* spp, *Clostridium botulinum*, *Clostridium perfringes*, *Salmonella* spp) or fish products re-contaminated during processing (Khatib *et al.*, 1994). Hot smoking in mild conditions at a temperature in the fish not exceeding 65°C does not inactivate all pathogens or inhibit bacteria during storage (Novothy *et al.*, 2004). Omojowo and Ihuahi (2006) also reported that smoked fish samples from four local markets in the Kainji lake area of Nigeria were contaminated by gram-positive bacteria such as coagulase-positive *Staphylococci* and *Escherichia coli*. Besides, the human infection may be caused by bacteria endogenous to fish. Bacterial pathogens which may be transferred from fish to humans include *Aeromonas hydrophilia* (septicemia and diarrhoea) *Clostridium* spp, *Plesiomonas* spp (gastroenteritis), *Salmonella* spp, and *Vibrio*

parahaemolyticus (food poisoning and foodborne infection) (Bintsis, 2017; Mellou *et al.*, 2019; Imre *et al.*, 2020).

Bacterial contamination reduces fish quality/shelf life. Fish contaminated with *Aspergillus flavus* make fish consumption hazardous to consumers' health due to the ability to produce carcinogenic aflatoxins that are not acceptable in the international markets (Ikutegbe and Sikolo, 2014). The global dissemination of pathogens through seafood is a significant public health problem. Improving seafood quality and safety is paramount for processing companies and exporters (Ababouch *et al.*, 2005). Foodborne bacterial pathogens are the principal cause of product rejection in the international seafood trade (Ababouch *et al.*, 2005). Therefore, there is a great demand for high-quality processed fish for the domestic and export market. This study aimed to determine the bacteriologic quality of smoked dried catfish (*Clarias gariepinus*) in Kaduna metropolis, Kaduna State of Nigeria.

MATERIALS AND METHODS

Study Area

The research was conducted in the Kaduna Metropolis of Kaduna State. The metropolis has an estimated land area of about 46,063km². The State lies between longitude 30⁰ East of the Greenwich meridian and between latitudes 090⁰ and 130⁰ North of the equator. Kaduna State occupies part of the central position of Nigeria (with Kaduna as its capital) and shares common borders with Katsina, Niger, Kano, Bauchi, and Plateau States. About 60% of the population, especially women, engage in small business ventures, including selling smoked fish in various retail outlets in the metropolis (Muhammad *et al.*, 2016).

Sample Collection Procedure

Four markets in Kaduna metropolis were selected using the homogenous convenience sampling technique (Jager *et al.*, 2017). The markets were selected because they are the most popular markets in the Metropolis. The smoked dried catfish were purchased; these included Kawo, Mando, Tudun-Wada and Central market. Five (5) samples were collected weekly for 15 weeks from each market. A total of 300 fish samples were collected during the study period. The samples were collected from the retailers in four markets of Kaduna metropolis. Seventy-five samples were obtained from each market (Kawo, Mando, Central, and Tudun Wada markets) and were subjected to total aerobic and coliform counts and were also analysed for the presence of *Staphylococcus aureus*.

Samples were transported to the Bacterial Zoonosis laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, ABU, Zaria in sterile polyethylene bags. Care was taken to avoid post-purchase contamination by not allowing the fish sample to be touched by an ungloved hand.

Processing of the Samples

About 10grams of the edible parts of each catfish sample were weighed, suspended in 90mls of 0.1% bacteriological peptone water, and blended separately to homogeneity by

using the Stomacher machine. Ten-fold serial dilutions of homogenates were made to give 10⁻² 10⁻³ and 10⁻⁴. Each dilution was plated using 0.1ml of inocula for bacteriological counts

Total Aerobic Counts

0.1ml from the 10⁻¹ dilution was aseptically transferred into nutrient agar plates from diluted samples. An aseptic glass rod spreader was used to spread the inocula. Plates were then incubated at 37°C for 24 hours. All colonies, including pinpoint-sized colonies, were counted on selected plates using a colony counter. Results from plates, which contained 30 to 300 colonies per plate, were recorded. Plates with more than 300 colonies could not be counted and were designated as TMTC (too many to count), while plates with fewer than 30 colonies were designated as TFTC (too few to count). The plate counts were expressed as a colony-forming unit of the suspension (CFU/ml) (Faisal and Ahmed, 2018), and the average for each sample was recorded as CFU/ml,

Total Coliform Counts

0.1ml from 10⁻¹ dilution was aseptically transferred into duplicate MacConkey agar plates from the diluted samples. The inocula were spread using an aseptic glass rod spreader, the plates were incubated at 37°C for 24hrs, and total coliform counts were made from the MacConkey agar plates. Lactose fermenting organisms on the MacConkey agar plates were enumerated and were recorded to determine coliform counts per gram (Lagier *et al.*, 2015).

Isolation and Identification of *Staphylococcus aureus*

Ten grams of each sample was aseptically added to 90mls of bacteriological peptone water (Oxoid) in a polythene bag and homogenised for five minutes in a stomacher (Coldworth Stomacher Seward London) and incubated at 37°C for 24h. A loop full of the homogenates was picked using an aseptic inoculating wire loop (Lee, 2003). The inocula were streaked onto a Baird Parker agar (Oxoid Ltd Basingstoke, England), and the plates were incubated at 37°C for 24 hours for isolation of *Staphylococcus*. Typically, tiny shining black colonies on BPA suggestive of *S. aureus* were picked and streaked onto nutrient agar slant, incubated for 24hours, and subsequently stored 4°C in the refrigerators before identification by biochemical methods (Cowan and Steel, 1993).

Biochemical identification of *Staphylococcus aureus*

Colonies presumptively identified as *S. aureus* were identified by gram staining, coagulase test, catalase test, and hemolysis on 5% sheep blood agar, rabbit blood agar, and horse blood agar (Becker *et al.*, 2015).

Antimicrobial Susceptibility Testing of *Staphylococcus aureus* Isolates

Antimicrobial susceptibility testing was carried out using a panel of 10 antimicrobial agents by the disk diffusion method (Bauer *et al.*, 1966) following CLSI guidelines (Humphries *et al.*, 2018) and cultured on Mueller Hinton agar. The sterile nutrient broth was inoculated with test isolate and incubated at 37°C for 24hours. The broth culture was adjusted with 2mls of sterile saline to obtain turbidity optically comparable to 0.5

McFarland standards. Mueller Hinton agar was inoculated with 0.1ml of the nutrient broth culture and spread over the entire sterile agar surface. The drug impregnated disks used (Oxoid) contained sulphamethoxazole/trimethoprim (25µg), Gentamicin (30µg), Erythromycin ciprofloxacin (5µg), Chloramphenicol (30µg), Oxacillin, Ampicillin (10µg), Streptomycin (25µg), Tetracycline (30µg) and Penicillin G (10units) were placed individually on the surface of inoculated agar plate using a dispenser (Oxoid Lt, Basingstoke, England) and incubated at 37°C for 18hours. The zones of inhibition were measured to the nearest millimetre using a transparent metre ruler.

Data Analyses

Descriptive statistics, including tables, and graphs, were used for analysing and presenting the data obtained from the

different markets sampled. The isolation rate was determined by taking the ratio of positive isolates to the total sample collected and expressed as a percentage.

RESULTS

Total Aerobic Counts and Total Coliform Counts

The results of the TAC showed that Central market Kaduna had the highest total aerobic count, 5.63×10^6 , followed by Mando 3.37×10^5 , while Kawo had the least count of 2.96×10^6 . The overall mean total aerobic count was 3.01×10^6 . Mando had the highest total coliform count, 2.70×10^6 , followed by Tudun –Wada 2.68×10^6 , with Kawo having the least count, 2.37×10^6 (Table 1).

Table 1: Total Aerobic Counts and Coliform Counts Smoked Dried Catfish in Kaduna Metropolis, Nigeria

Markets	Mean Total Aerobic Counts/ cfu/g	Total Coliform counts/cfu/g
Mando	3.37×10^5	2.70×10^6
Kawo	2.96×10^6	2.37×10^5
Central	5.63×10^6	2.44×10^6
Tudun-Wada	3.11×10^6	2.68×10^5
Total	3.01×10^6	1.41×10^6

Isolation and Characterisation of *Staphylococcus species* from Smoked Dried Catfish

Three hundred samples of smoked dried catfish were analysed, which yielded 276 isolates of *Staphylococcus species* by conventional biochemical tests giving an overall prevalence rate of 92.0%. Catfish obtained from Mando market had the highest prevalence of 98.7%, followed by Central market catfish 90.7%, Kawo yielded the least with 89.3%, and Tudun Wada 89.3% (Table 2)

Detection of *S. aureus* in Smoked Dried Catfish

Out of 300 samples examined bacteriologically on BPA, 276 (92.0%) were found to be contaminated with *Staphylococcus species* (Table 3). Two hundred and twenty-five (81.5%) were coagulase-positive, while 51 (18.5%) were coagulase-negative. Samples from the central market Kaduna had sixty-one (22.1%) coagulase-positive and 7 (2.5%) coagulase-negative.

From Kawo market, 51 (18.5%) were coagulase-positive, and 16 (5.8%) were coagulase-negative. Sixty-three (22.8%) were coagulase-positive and 11 (4.0%) coagulase-negative from Mando market, while 50 (18.1%) coagulase-positive and 17 (6.2%) coagulase-negative were from Tudun Wada market (Table 3).

Haemolysis Patterns of the *S. aureus* Isolates Using Blood from Different Animal Species

The results of the haemolysis pattern showed that in the rabbit blood, 14 (5.1%) showed α haemolysis, 248 (89.8%) showed

β -haemolysis, and 14 (5.1%) showed δ haemolysis. In sheep blood, 83 (30.1%) showed α -haemolysis 27 (9.8%) β -haemolysis 166 (60.1%) showed δ - haemolysis. On horse blood, no α -haemolysis was observed; 235 (85.1%) showed β haemolysis, 41(14.9%) showed δ haemolysis. In humans, 96 (34.8%) had α -haemolysis, 14 (5.1%) showed β -haemolysis, and 166 (60.1%) showed δ - haemolysis. (Table 4).

Susceptibility of *S. aureus* Isolates to Commonly used Antimicrobials

All the 225 isolates tested were resistant to one or more antibacterial agents. The coagulase-positive *Staphylococcus aureus* was 100% resistant to ampicillin and penicillin. Likewise, 21 (9.3%) isolates were resistant to ciprofloxacin and gentamycin. Thirty-nine (17.3%) of the isolates were resistant to chloramphenicol. One hundred and four (50.7%) of the isolates were resistant to erythromycin. Two hundred and nineteen (97.3%) isolates were resistant to Oxacillin. One hundred and sixty (71.1%) isolates were resistant to Streptomycin. One hundred and ninety-eight (88.0%) isolates were resistant to sulphamethoxazole/trimethoprim. One hundred and fifty-two (67.6%) of the isolates were resistant to Tetracycline. All of the 225 (100%) of the *S. aureus* showed multiple drug resistance as they were resistant to ampicillin and penicillin. Only 6 (2.7%) of the isolates were susceptible to Oxacillin. (Table 5).

Table 2: Prevalence of *Staphylococcus spp* in Smoked Dried Catfish in Kaduna Metropolis, Nigeria

Markets	No. of samples Collected	No of <i>Staphylococcus</i> species isolated (%)
Central	75	68 (90.7)
Kawo	75	67 (89.3)
Mando	75	74 (98.7)
Tudun/Wada	75	67 (89.3)
Total	300	276 (92.0)

Table 4: Isolation of Coagulase-positive and Coagulase-negative *Staphylococcus aureus* in smoked dried catfish in Kaduna Metropolis, Nigeria

Market	No isolated (%)	Coagulase + (%)	Coagulase - (%)
Central	68 (22.7)	61 (22.1)	7 (2.5)
Kawo	67 (22.3)	51(18.5)	16 (5.8)
Mando	74 (24.7)	63 (22.8)	11 (4.0)
T/wada	67 (22.3)	50 (18.1)	17 (6.2)
Total	276 (92.0)	225 (81.5)	51 (6.2)

Table 5: Haemolysis Pattern of the *S. aureus* Isolates from smoked dried catfish in Kaduna Metropolis, Nigeria

Blood source	- α -Alpha (%)	β -Beta (%)	δ -Delta (%)
Rabbit	14 (5.1)	248 (89.8)	14 (5.1)
Sheep	83 (30.1)	27 (9.86)	166 (60.1)
Horse	0 (0.0)	235 (85.1)	41 (14.9)
Human	96 (34.8)	14 (5.1)	166 (60.1)

Table 6: Susceptibility of *S. aureus* Isolates from Smoked Dried Catfish to 10 commonly used Antimicrobials

Antibiotic	Disk Concentration (μ g)	No. Sensitive (%)	No. Resistant (%)
Ampicillin	10	0 (0.0)	225 (100.0)
Chloramphénicol	30	186 (82.7)	39 (17.3)
Ciprofloxacin	5	204 (90.7)	21 (9.3)
Erythromycin	15	111 (49.3)	114 (50.7)
Gentamicin	30	204 (90.7)	21 (9.3)
Oxacillin	5	6 (2.7)	219 (97.3)
Penicillin	10	0 (0.0)	225 (100.0)
Streptomycin	25	65 (28.9)	160 (71.1)
Sulphamethoxazole/ Trimethoprim	25	27 (12.0)	198 (88.0)
Tetracycline	30	73 (32.4)	152 (67.6)

AMP= Ampicillin CIP=Ciprofloxacin C= Cloramphenicol E=Erythromycin CN= Gentamicin OB=Oxacillin P= Penicillin S=Streptomycin RL= Sulphamethoxazole/Trimethoprim TE= Tetracycline

DISCUSSION

The mean total aerobic count (TAC) of 3.01×10^6 CFU/g obtained in this study is higher than the mean total aerobic count of 3.1×10^5 CFU/g obtained in Zaria by Whong *et al.* (2003) in smoked dried fish in Zaria Nigeria and 3.05×10^4 CFU/g obtained by Olonitola *et al.* (2006) in some dried fish species in the same study area. The maximum microbiological limit for the TAC, which separates the good quality products from unacceptable quality, is 5×10^5 cfu/g (ICMSF, 1986; Sanjee and Karim, 2016). The mean TAC of the studied samples was 3.01×10^6 cfu/g, which was above the maximum permissible limit by the International Commission of Microbiological Specification for Food (ICMSF). So the samples of dried fish in this study did not

meet the acceptable limit specified by ICMSF, which points out the bad quality of dried fishes sold to consumers in the study area. Conversely, Sanjee and Karim (2016) reported lower aerobic bacterial contamination of frozen fish in Bangladesh.

Mean coliform counts 1.41×10^6 CFU/g obtained in this study is also higher than that obtained by Olonitola *et al.* (2006), who reported a mean coliform count of 4.53×10^2 CFU/g in some dried fish species in Sabon Gari Zaria, Nigeria. The coliform count in this study may also be due to continuous exposure to dust, overstay due to poor sales in the market and constant touching by buyers and sellers (Whong *et al.*, 2003).

Two hundred and seventy-six *Staphylococcus aureus* were isolated from 300 samples of smoked dried catfish, using conventional biochemical tests, giving a total isolation rate of 92%. This isolation rate was higher than the detection rate of 41% reported by Agu *et al.* (2013) in smoked fish sold in major retail markets in Benin, Nigeria. The prevalence of 62% reported by Sokari (1991) from ready-to-eat meat, fish and vegetables in Nigeria. Olonitola (2007) also observed a high percentage of *Staphylococcus aureus* isolated from some dried fish spp in Zaria 2007. The high isolation rate obtained in this study could be because the smoking process was done under unhygienic conditions; The most common way of contamination of fish is by contact with fish handlers' hands, especially in the cases where the fish is handled before or after cooking. Prolonged storage without refrigeration allows the bacteria to grow and form toxins. Since some toxins are heat-stable, the incriminated fish may also cause food poisoning even if it is further heat treated (Agu *et al.*, 2013)

Based on the location Mando market had the highest rate (24.7%) of isolation of *Staphylococcus* species followed by Central market Kaduna (22.7%), with Kawo and Tudun-Wada having the least isolation rate of (22.3%) respectively. The primary reason for the high isolation rate in the Mando market may be the rate of sales. When the sale is low, the fish continues to be in the market for a longer time and exposes the fish product to the factors mentioned above.

Beta (β) hemolysis, also called complete hemolysis on the rabbit and horse blood was observed in over 80% of the isolates in this study. This finding is significant concerning public health safety. Hemolysis is one of the virulence factors for pathogenic *Staphylococcus* species. Therefore, humans who consume smoked dried catfish without further heat treatment risk acquiring pathogenic *Staphylococcus* infection. Hemolytic *Staphylococcus* species are responsible for various health conditions in humans and animals. Examples include mastitis, pneumonia, infective endocarditis, septicemia and many other diseases (Salgado-Pabon *et al.*, 2014; Zhang *et al.*, 2016). It was observed that people working in different food sectors, such as fish traders, are exposed to different food pathogens causing various foodborne diseases such as hemolysin functionality, fever, diarrhoea, and abdominal cramps, which may lead to death (Das *et al.*, 2014). Other hemolytic patterns reported in this study include alpha and delta hemolysis.

Antibiotics susceptibility testing of the 225 *S. aureus* isolates obtained using the conventional biochemical method showed that all the isolates were resistant to one or more of the ten antibiotic agents screened. However, none was simultaneously resistant to all the antibiotic agents. All the isolates were resistant to ampicillin and penicillin (100%); 97.3% of the isolates resisted Oxacillin, and 88% were resistant to sulphamethoxazole/ Trimethoprim, respectively. Ninety per cent of the isolates were susceptible to ciprofloxacin and Gentamicin, and 82.7% to chloramphenicol. The resistance of these isolates to β -lactam antibiotics could result from indiscriminate use of these antibiotics both in human and veterinary medicine due to their availability and affordability. A total of 60 antibiotic

resistance patterns were obtained, with all the *S. aureus* isolates exhibiting multiple drug resistance.

The high resistance to ampicillin and penicillin obtained in this study agrees with the report obtained by Abudullahi (2005), where resistance to penicillin and ampicillin was 100%. The frequency of resistance to ampicillin and penicillin in this study is similar to the report of Kwaga and Adesiyun, (1984); Umoh, (1990); Abdullahi *et al.* (2005), who reported extensive spread resistance of *Staphylococci* to ampicillin and penicillin in their studies. A multidrug-resistant pathogen implies that it becomes more pathogenic compared to a non-multiple drug-resistant pathogen (Micheal *et al.*, 2018; WHO, 2020). They also make treatment difficult in individual infected patients (Adzitey *et al.*, 2012). There is an additional cost of treatment and extra days in the hospital stay (Yuasa *et al.*, 2019). Multidrug-resistant pathogens have a higher risk of producing death. For example, Methicillin-resistant *Staphylococcus aureus* (MRSA) infected individuals have been estimated to be 64% more likely to die than those infected with Methicillin susceptible *Staphylococcus aureus* (MSSA).

Conclusion

The study was able to find out that smoked dried catfish obtained from various markets in Kaduna do not meet international good bacteria quality. The dried catfish samples were highly contaminated with *Staphylococcus aureus* with an isolation rate of 92.0%. Over 90% of the isolates showed resistance to Oxacillin. Smoked dried catfish should be packaged in a transparent polyethene bag to avoid contamination by prospective buyers. Smoked dried catfish obtained from the market should be properly cooked before consumption. Cooking at a high temperature helps eliminate *Staphylococcus aureus* and other possible pathogens.

Conflict of Interests

The authors declare that they have no conflict of interest.

Authors' Contributions

The study was conceived and designed by MB and FEE. SAS, MB, FEE carried out the study, analysed the data and wrote the manuscript; MB oversaw the investigation. The final manuscript was read and approved by all authors.

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