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Antibiotics Susceptibility Study of *Staphylococcus aureus* Isolates from Dry Catfish Sold in Some Open Markets in Zaria - Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author JOO designed the study, performed the statistical analysis, wrote the protocol, managed literature searches and wrote the first draft of the manuscript. Authors GOA and JAO managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to determine the antibiotics susceptibility of *Staphylococcus aureus* isolated from dry catfish sold in some open markets in Zaria.
Place and Duration of Study: Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, from December 2017 to February 2019.
Methodology: Two hundred dry catfish samples were collected aseptically, wrapped in foil paper, packaged in clean polyethylene bags from open markets in Zaria and analysed by standard microbiological methods. Antibiotics susceptibility of the isolates was tested using the disc diffusion method. Eight antibiotics belonging to eight classes were employed in the study. The β-lactamase test was done using the acidimetric method while the plasmid curing was done by exposing the multidrug resistant isolates to varying concentrations of acridine orange in Mueller Hinton broth.
Results: From the 200 samples of dry catfish, 138 presumptive staphylococcal isolates were

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obtained. Amongst the 138 staphylococcal isolates out of which 109 (79%) were confirmed as *Staphylococcus aureus* by Microgen Staph ID kits. The susceptibility test showed that 46.8% of the *S. aureus* isolates were multidrug resistant. The isolates were sensitive to Gentamicin (100.0%) and Vancomycin (100.0%). The phenotypic expression of resistance showed that 27.8% of isolates were resistant to Cefoxitin, Clindamycin and Linezolid. Eighteen (35.3%) out of the total resistant isolates were β-lactamase producers. From the study, 50% of isolates resistant to Cefoxitin and Clindamycin became sensitive to the drugs after exposure to sub-MIC concentration of acridine orange solution.

Conclusion: This study showed that some *S. aureus* isolates from dry catfish in this study were resistant to commonly used antibiotics in the study area whereas others were sensitive this may be because of the use of these drugs in animal feeds.

Keywords: Antibiotics susceptibility; β-Lactamase; fish; multidrug resistance.

1. INTRODUCTION

Staphylococcal species is one of the major bacterial agents causing food borne illnesses [1]. Outbreak investigations have suggested that improper handling of cooked or processed food is the main source of contamination. Lack of maintaining cold chain allows *S. aureus* to form staphylococcal enterotoxins. Although *S. aureus* can be eliminated by heat treatment and by competition with other flora in pasteurized and fermented foods, respectively, staphylococcal enterotoxins produced by *S. aureus* are still capable of causing staphylococcal foodborne disease because of their heat tolerance capacity. This fact should be considered in risk assessment and devising appropriate public health interventions [2].

The presence of Staphylococci in fish can be an indication of both post-harvest and processing contamination due to poor personal hygiene [3]. Improper packaging could also be a means of contamination. In open markets, fish are displayed unpackaged in most cases, exposed to wind-current which carries dust particles from points to points, from human droplets as well as from bins contaminated with microorganisms and deposit same on exposed fish. This is a hazardous practice since it can lead to outbreak of diseases caused by these organisms. It can also be a reasonable source for the transmission of antibiotics resistance genes to the clinical species and vice versa.

The control of these organisms by chemoprophylaxis and chemotherapy using antibiotics has resulted in the increased prevalence of resistance of these agents [4]. The rate of resistance may be affected by the type of organisms concerned, the volume and type of drug used and the method of application. In developed countries, stringent control of antibiotics use coupled with effective surveillance of antibiotics resistance pattern in the population has successfully reduced the prevalence of the antibiotics resistance [5]. The situation is different in developing countries like Nigeria. Information concerning the drug resistant pattern of prevailing pathogenic bacteria and the appearance of new resistant characteristics is of utmost value for a proper selection of antimicrobial agents for therapeutic purpose. *S. aureus* is a serious threat to hospitalized patients globally and it now represents a challenge for public health as community associated infections appear to be on the increase in both adults and children in various regions and countries [6,7]. Its genetic plasticity has facilitated the evolution of many virulent and drug-resistant strains, presenting a major and constantly changing clinical challenge [8].

According to the World Health Organization [9], monitoring and surveillance of antibiotics resistant bacteria in animals intended for human consumption is important for the regulation of antibiotics resistance, to detect trends and changes of their resistance patterns. Therefore, this present study aimed at isolating *S. aureus* from dry catfish samples being the species widely consumed in various forms by the locales in the study area; analyse antibiotics susceptibility with the view to determining the multidrug resistance pattern of the isolates.

2. MATERIALS AND METHODS

2.1 Samples Collection, Preparation and Culture

Samples of dry catfish were randomly collected aseptically, wrapped in foil paper, packaged in clean polyethylene bags from open markets in

Zaria, and transported to the Pharmaceutical Microbiology Laboratory, Ahmadu Bello University Zaria, for analysis and isolation of *S. aureus*. Each of the fish sample was crushed using a sterile electronic grinder to form fine powder. Mannitol salt agar was used for the isolation of *S. aureus* following standard microbiological procedure [10]. The homogeny of the grind fish was soaked in distilled deionized water and then sieved. The filtrate was adjusted to 0.5 McFarland scale from which 0.1 mL was cultured.

2.2 Isolation and Identification of *S. aureus*

Growths from primary culture plates on mannitol salt agar showing discrete golden yellow colonies were presumptively identified as *S. aureus*. These were sub-cultured onto slants of Mueller Hinton agar and incubated at 37ºC for 24 hours. Representative surface colonies of mannitol fermenters were isolated and subjected to Gram reaction and standard biochemical such as catalase and coagulase production based on standard procedures [10,11]. MicrgenTM Staph-ID System was employed to confirm the identity of the isolated *S. aureus.* The identified *S. aureus* isolates were stored in the refrigerator at 4ºC for further analysis.

2.3 Determination of Antibiotics Susceptibility of *S. aureus* Isolates

The agar disc diffusion method was used to determine the antibiotics susceptibility of *S. aureus* isolates. Pure isolates of S. aureus were emulsified in five millilitre of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standard (approximately a cell density of 1.5×10^8 cfu/mL) [12]. The standardized suspension was inoculated on Mueller Hinton agar using sterile swab sticks to ensure even distribution and confluent growth. The sensitivity disc of the various antibiotics was aseptically and gently placed using a sterile forceps on the dried inoculated agar surface and incubated at 37°C for 18 hours. After incubation, the plates were examined, the zones of inhibition were measured, and results were interpreted according to CLSI [13] (Table 1). Selected antibiotics used include Clindamycin (2 µg), Gentamicin (10 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Trimethoprim-sulfamethoxazole (1.25 µg + 23.75 µg), Tetracycline (30 µg), Linezolid (30 µg) and Erythromycin (15 µg). An inhibition zone of 19 mm or less around cefoxitin disc indicated MRSA [14].

2.4 Test for β-lactamase Production

Isolates that were multidrug resistant were subjected to β-lactamase test using the acidimetric method. Benzylpenicillin-phenol red reagent was prepared in-house by diluting two millilitre of 0.5% solution of phenol red with 16.6 mL of distilled water and added to a vial of 20×10^6 units of benzylpenicillin. One mol/litre NaOH was added drop wise until the solution just turns purple (pH 8.5). The reagent was dispensed in aliquots of 0.1 mL into sterile tubes and sufficient colony of the isolates was added to the tubes and observed within 30-60 minutes of incubation at room temperature. A colour change from purple to yellow was indicative of a positive result [15].

2.5 Plasmid Curing of Isolates

Mueller Hinton agar broth containing acridine orange of concentration ranging from 75 µg to 150 µg was prepared in sterile capped test tubes. 0.2 mL of standardized culture of each organism resistant to two or more antibiotics tested was added to a series of tubes containing different concentrations of acridine orange. The tubes were incubated at 37ºC for 24 hours and were observed for visible turbidity as a sign of

Table 1. Antibiotic susceptibility interpretative chart

Antibiotics	Class	Conc.(µg)	Susceptible	Intermediate	Resistant
Ciprofloxacin (CIP)	Fluoroquinolones	5	≥21	16-20	≤15
Clindamycin (DA)	Lincosamides	2	≥21	15-20	≤14
Cefoxitin (FOX)	Penicillins	30	≥22	-	≤21
Sulphamethoxazole/ Trimethoprim (SXT)	Folate antagonist	25	≥16	11-15	≤10
Gentamicin (CN)	Aminoglycosides	10	≥15	13-14	≤12
Tetracycline (TE)	Tetracyclines	30	≥19	15-18	≤14
Linezolid (LZD)	Oxazolidinones	30	≥21	-	≤20
Erythromycin (E)	Macrolides	15	≥23	14-22	≤13

bacterial growth. The highest dilution without visible sign of bacterial growth was recorded as the minimum inhibitory concentration (MIC) of the acridine orange for the organism. The dilution just below the MIC was recorded as the sub-MIC of acridine orange. The organisms from the sub-MIC tubes were inoculated on fresh plates of Mueller Hinton agar and discs of antibiotics were placed. The plates were incubated at 37ºC for 24 hours. The diameter of the zone of inhibition were measured and compared with initial values before curing.

3. RESULTS

3.1 Prevalence of *Staphylococcus aureus* Isolates in Dry Catfish Samples

Out of the 200 catfish samples collected from open markets in Zaria, presumptive staphylococcal isolates were obtained from 138 samples. Amongst the 138 staphylococcal isolates, 94% were coagulase positive and 79% of the total isolates were confirmed *Staphylococcus aureus* by Microgen Staph ID kits (Table 2). This implies that the isolation rate of *S. aureus* from the dry catfish samples was 54.5% (109/200).

3.2 Antibiotics Susceptibility Profile of *Staphylococcus aureus* Strains Isolated

Eight antibiotics belonging to eight classes were used in this study to classify the 109 *Staphylococcus aureus* isolates into susceptible, intermediate or resistant strains. This classification was done in comparison with the CSLI interpretative chart (Table 1). The results of the susceptibility test showed that 46.8% isolates were resistant to one or more antibiotics whereas 6.4% were classified as multidrug resistant (MDR) strains having resisted the activity of one or more antibiotics from three classes. Gentamicin (100.0%) was highly effective against the isolates followed closely by Trimethoprim-sulfamethoxazole, Tetracycline, Erythromycin, Linezolid and Ciprofloxacin with susceptibility rate of 97.3%, 94.4%, 93.5%, 89.9% 8 and 87.2% respectively. Cefoxitin (28.4%) and Clindamycin (17.4%) were the most resisted antibiotics followed by Linezolid (10.1%) while Clindamycin had a higher intermediate susceptibility profile of 31.2% followed by Ciprofloxacin with intermediate susceptibility profile 7.3% (Table 3).

3.3 Multiple Drug Resistance (MDR) Index analyses of *S. aureus* Isolates

The multiple antibiotics resistance index of *Staphylococcus aureus* isolated showed that 16.5% of the isolates had MDR of ≥0.22. 53.2% of isolates were susceptible to all antibiotics tested while 30.3% were resistant to only one of the antibiotics studied recording MDR of 0.0 and 0.11 respectively (Table 4).

3.4 Resistance Phenotypes of Antibiotics Resistant Isolates

The phenotypic resistance profile of the isolates showed that, out of the 51 isolates that were resistant to at least one antibiotics, 13.7% were multidrug resistant, 21.6% were resistant to two antibiotics whereas 64.7% of the isolates were resistant to one antibiotics (Table 5).

3.5 β-lactamase Production by Antibiotics Resistant Isolates

The β-lactamase test results show that, out of the 51 isolates that were resistant to one or more antibiotics, 18(35.3%) were capable of producing β-lactamase enzyme whereas 33(64.7%) did not produce the enzyme (Fig. 1).

3.6 Plasmid Curing of Antibiotics Resistant Isolates of *S. isolates.*

The results of the plasmid curing of isolates indicated that all isolates except 50% of those resistant to Cefoxitin (FOX) and Clindamycin (DA) were cured of resistance factors by becoming sensitive to the antibiotics to which they showed phenotypic resistance previously before curing (Table 6).

Table 2. Distribution of staphylococcal isolates in the studied samples

Parameters	Occurrences	% occurrences
Total staphylococcal isolates	138	69
Coagulase positive isolates	129	94
Non-*Staphylococcus aureus* isolates	29	21
Confirmed *S. aureus* isolates (Microgen Staph ID kits)	109	79

Total number of samples (n) = 200

Table 3. Antibiotics susceptibility profile of isolates

Antibiotics	Class	Susceptibility profile n (%)		
		Sensitivity	Intermediate	Resistant
Ciprofloxacin (5 µg)	Fluoroquinolones	95(87.2)	8(7.3)	6(5.5)
Clindamycin (2 µg)	Lincosamides	56(51.4)	34(31.2)	19(17.4)
Cefoxitin (30 µg)	Penicillins	78(71.6)	0(0.0)	31(28.4)
Trimethoprim-sulfamethoxazole (1.25 µg + 23.75 µg)	Folate antagonist	106(97.3)	0(0.0)	3(2.7)
Gentamicin (10 µg)	Aminoglycosides	109(100.0)	0(0.0)	0(0.0)
Tetracycline (30 µg)	Tetracyclines	103(94.4)	3(2.8)	3(2.8)
Linezolide (30 µg)	Oxazolidinones	98(89.9)	0(0.0)	11(10.1)
Erythromycin (15 µg)	Macrolides	102(93.5)	4(3.7)	3(2.8)

Table 4. Multiple drug resistance (MDR) index of isolates

No. of antibiotics	MARI	Frequency	% frequency
0	0.00	58	53.2
1	0.11	33	30.3
2	0.22	11	10.1
3	0.33	07	6.4

Table 5. Resistance phenotypes of antibiotics resistant isolates

Resistance phenotype	No. of isolates	Resistance (%)
FOX, DA, LZD	04	07.8
LZD, E, DA	03	05.9
FOX, DA	08	15.7
CIP, SXT	03	05.9
CIP	03	05.9
DA	04	07.8
FOX	20	39.2
LZD	04	07.8
TE	02	03.9
Total	51	100.0

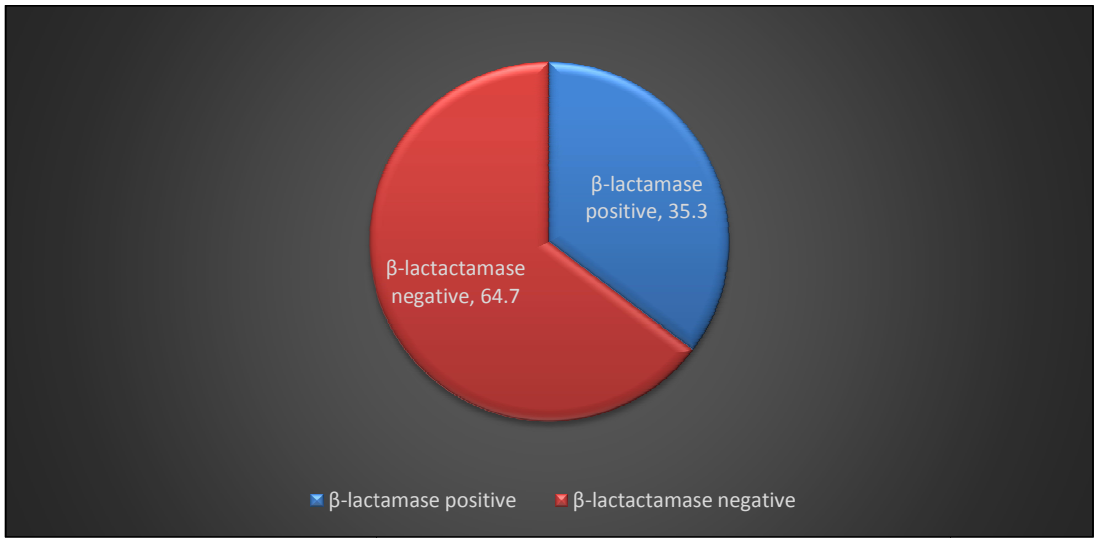

Fig. 1. β-lactamase production by antibiotics resistant isolates

Table 6. Resistance phenotypes of antibiotic resistant isolates before plasmid curing

Resistance phenotype	No. of resistant isolates before curing	% Resistance	No. of resistant isolates after curing	% Cured
FOX, DA, LZD	4	22.2	0	100
LZD, E, DA	3	16.7	0	100
FOX, DA	8	44.4	4	50
CIP, SXT	3	16.7	0	100

4. DISCUSSION

S. aureus was detected in dry catfish displayed for retailing in open markets. The isolation rate of *S. aureus* in the retail dry catfish samples in this study (54.5%, 109/200) was relatively higher compared with previous reports, which had rates ranging from 18.1% - 23% [16,17,18,19,20]. This may be due to the differences in the recovery methods. Most of the researchers deployed swab method in the collection of fish samples for culture, which could have limited the recovery of the bacteria. However, in this study, samples were homogenised and inocula were made. This could be the reason for the high isolation rate of the bacteria. The market environment also may have played a vital role in the distribution of the bacteria that may also contribute to the observed disparity.

The impact on human health of *Staphylococcus aureus* infections in community and hospital settings has led to intensive investigation of this organism over the years [8]. The number of effective antibiotics has been reduced by the emergence of resistance to penicillin, methicillin and vancomycin [21], a problem that has been compounded by the emergence of methicillin-resistant *S. aureus* (MRSA) carriage and disease in the community [22]. In this current study, it is reported that 46.8% of the *S. aureus* isolates were resistant to at least one antibiotics whereas 6.4% are multidrug resistant. This trend may have been because of possible transfer of resistant strains from humans and droplets onto fish during processing, distribution, and marketing as well as environmentally by wind current since adequate packaging were lacking in the open markets surveyed. It could also be possible that the resistant isolates were from antibiotics laden environment or they may have been exposed to sub-MIC concentrations of these drugs in feeds over time leading to the acquisition of tolerance to these drugs. This is evident by the fact that 16.5% of the isolates had the MAR index of ≥0.22 (Table 4).

Retrospective and prospective studies have shown that after the introduction of an antibiotic not only the level of resistance of pathogenic bacteria, but also of commensal bacteria, increases [23]. Commensal bacteria constitute a reservoir of resistance genes for (potentially) pathogenic bacteria. Their level of resistance is considered a good indicator for selection pressure by antibiotics use and for resistance problems to be expected in pathogens. It is reported in this study that Gentamicin was highly effective (100%) against the *S. aureus* isolated from dry catfish. IThis however, contradicts the reports of Grema et al. [17,18] who reported gentamicin resistance of 89.5% and 64.1 respectively in Maiduguri, Nigeria. The resistance level observed in their study may be because 38% of the *S. aureus* were from human fish handlers who may have contaminated the fish with human *S. aureus* species that may have been exposed to antimicrobials at one time or the other. It was observed in our study that the isolates were slightly resistant to cefoxitin (28.4%), clindamycin (17.4) and linezolide (10.1). This may be due to increase use of these drugs as growth enhancers in animal feeds especially in the study area. Generally, all the antibiotics used in this study were very effective *in vitro* as no drug has its efficacy below 50% except for clindamycin tending towards ineffective by showing high level of intermediate susceptibility of 31.2% (Table 3).

5. CONCLUSION

From the results obtained in this study, most resistant isolates were able to resist the activity of mostly penicillins, lincosamides and oxazolidinones with evidence of multidrug resistance (MDR). This may be due to intrinsic resistance of *S. aureus* to antibiotics or acquired resistance due to acquisition of resistance plasmids. The results of this study show that majority of the isolates were susceptible to most of the antibiotics used in this work. The aminoglycoside was highly effective against the isolates followed by folate antagonist, macrolide and the tetracycline. These classes of drugs may be efficient in the treatment of staphylococcal infections due to the isolates encountered in this study location.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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