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Characterization of antibiotic-resistant *Staphylococcus aureus* from gills and gastro-intestinal tracts of catfish (*Clarias gariepinus*), and water samples from Jabi Lake, Abuja, Nigeria

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Abstract:

Background: The isolation of antibiotic resistant *Staphylococcus aureus* in freshwater fish poses a threat to public health because of the risk of human infections from consumption of such contaminated fish. Studies assessing antibiotic resistance of bacteria from body parts of fish and freshwater in Nigeria are sparse in the literature. This study therefore characterized *S. aureus* isolates from gills and gastrointestinal tract (GIT) of catfish (*Clarias gariepinus*), and water samples from Jabi Lake, Nigeria
Methodology: Over a period of three months (April to June 2018), gills and GIT samples of 30 fish, and water samples randomly collected from 6 sites of the Lake, were cultured on Mannitol Salt Agar (MSA) for the isolation of *S. aureus.* Standard biochemical tests were used for bacteria identification, and antibiogram of the isolates was determined by the disc diffusion method
Results: The bacterial colony count in the gills (54.6±1.41 x 10^5 CFU/ml) and GIT (54.3±1.31 x 10^5 CFU/ml) was significantly higher (p<0.05) than the count from water sample (27.7±2.85 x 10^5 CFU/mL). *S. aureus* was isolated from 53% (16 of 30) of the gills, 57% (17 of 30) of the GIT, and 33% (2 of 6) of the water samples (p<0.05). Ninety four point one percent of *S. aureus* recovered from gills were resistant to ampicillin while 53.3% from the GIT were resistant to levofloxacin. *S. aureus* from water samples were resistant (100%) to ciprofloxacin, norfloxacin, gentamycin, amoxicillin, rifampicin, erythromycin, ampicillin and levofloxacin, and 50% were resistant to streptomycin and chloramphenicol
Conclusion: The presence of antibiotic resistant *S. aureus* in this study may be the result of selective antimicrobial pressure from anthropogenic activities as a result of abuse and overuse of antimicrobials leading to residual antibiotics in the aquatic environment

Keywords: *Clarias gariepinus*; gill, gastrointestinal tract; antibiotic; Staphylococcus

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Caractérisation de *Staphylococcus aureus* résistant aux antibiotiques à partir de branchies et du tractus gastro-intestinal de poisson-chat (*Clarias gariepinus*) et d'échantillons d'eau du lac Jabi, Abuja, Nigéria

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Abstrait:

Contexte: L'isolement de *Staphylococcus aureus* résistant aux antibiotiques chez des poissons d'eau douce constitue une menace pour la santé publique en raison du risque d'infections humaines résultant de la consommation de ce poisson contaminé. Les études évaluant la résistance aux antibiotiques de bactéries provenant de parties du corps de poissons et d'eau douce au Nigéria sont rares dans la littérature. Cette étude a donc caractérisé les isolats de *S. aureus* provenant des branchies et du tractus gastro-intestinal de poisson-chat (*Clarias gariepinus*), ainsi que des échantillons d'eau de Jabi Lake, au Nigeria. Méthodologie: sur une période de trois mois (avril à juin 2018), échantillons de branchies et de GIT. de 30 poissons et d'échantillons d'eau prélevés au hasard sur 6 sites du lac ont été cultivés sur gélose au sel de mannitol (MSA) afin d'isoler *S. aureus*. Des tests biochimiques standard ont été utilisés pour l'identification des bactéries et l'antibiogramme des isolats a été déterminé par la méthode de diffusion sur disque.
Résultats: Le nombre de colonies bactériennes dans les branchies (54,6 ± 1,41 x 105 UFC/ml) et le GIT (54,3 ± 1,31 x 105 UFC/ml) étaient significativement plus élevés ($p < 0,05$) que le nombre issu de l'échantillon d'eau (27,7 ± 2,85 105 UFC/mL). *S. aureus* a été isolé chez 53% (16 sur 30) des branchies, 57% (17 sur 30) du GIT et 33% (2 sur 6) des échantillons d'eau ($p < 0,05$). Quatre-vingt-quatorze pour cent des *S. aureus* récupérés des branchies étaient résistants à l'ampicilline, tandis que 53,3% des GIT étaient résistants à la lévofloxacine. *S. aureus* à partir d'échantillons d'eau était résistant (100%) à la ciprofloxacine, la norfloxacine, la gentmycine, l'amoxicilline, la rifampicine, l'érythromycine, l'ampicilline et la lévofloxacine, et 50% étaient résistants à la streptomycine et au chloramphénicol
Conclusion: La présence de *S. aureus* résistant aux antibiotiques dans cette étude peut être le résultat d'une pression antimicrobienne sélective résultant d'activités anthropiques résultant d'un usage abusif et excessif d'antimicrobiens entraînant la présence d'antibiotiques résiduels dans le milieu aquatique.

Mots-clés: *Clarias gariepinus*; branchies, tractus gastro-intestinal; antibiotique; Staphylocoque

Introduction:

Lakes and dams are reservoirs containing water of great importance for fishing, agricultural, industrial and domestic usage. The reservoirs serve as regions of biodiversity and are also used as tourists, recreational and conservation locations (1). However, concerns of public interest have been raised because reservoirs are most often exposed to environmental pollutants from anthropogenic activities that may make the water unsuitable for aquatic flora and fauna.

Jabi Lake, which was formerly a small water body, is natively one of the largest freshwater bodies in the Jabi district of Abuja, Nigeria, which residents depended on for their domestic water needs. This lake was expanded for commercial and fishing purposes in 1993 (2). However as at present, the lake is surrounded by a big shopping mall and residential houses, and is subjected to recreational activities. It is thus considered as a close basin for different types of drainages in the city of Abuja. The lake is also an important source of fishing activities in the district but subject to high levels of pollution from domestic drainage and raw domestic sewage.

The drainage of contaminated wastewater into the lake is a great health risk due to the possibility of large number of pathogens and antibiotic products being discharged into the water body daily. Recently, detection of *Staphylococcus aureus* from fish, *Oreochromis niloticus*, and wastewater samples from Qarun Lake, with a prevalence of 81.5%, suggests that this pathogen may be abundant in lakes (3). Many species of staphylococci are capable of releasing enterotoxin that can cause gastroenteritis in humans when foods contaminated by these species are consumed (4).

Water drainages containing antimicrobial agents, metals and biocides

are also diverse sources for antimicrobial resistance in water bodies (5). The presence of antibiotic resistant microorganisms in fish has been documented in several lakes around the world (6, 7). However, there are no documented studies on antibiotic resistance and detection of *S*. *aureus* in Jabi Lake. Hence, the objective of this study was to determine the prevalence of antibiotic resistant *S*. *aureus* in catfish (*Clarias gariepinus*) and water samples from Jabi Lake.

Materials and Methods:

Study location and condition

This study was conducted on Jabi Lake, Abuja, Nigeria. This lake is natural water located in Jabi district of Abuja with 9.0771°N and 7.4233°E coordinates. The lake experiences two weather conditions annually, a warm humid rainy season and a dry season (8). At the bank of the lake are a recreational park and a shopping mall, and fishing activities usually takes place inside the lake throughout the year.

Samples collection

Thirty cat fish (*C. gariepinus*) were randomly collected from Jabi Lake in order to harvest their gills and intestine, while water samples were randomly collected from 6 locations in the lake between April and June 2018. The gills of the fish were selected because of their perennial contact with water while the intestines were selected because of their ability to absorb food and the tendency to harbor microorganisms. The fishes were killed by destruction of the brain with a needle, and the microbial load on the skin was reduced by washing the fish with 70% ethanol (9). After dissection with sterile scissors, the gills and the intestine were separated for analysis.

Water samples from the lake were collected into sterile glass bottles (500 mL) at a depth of 20 cm below the water surface from randomly selected locations (middle, east and western) of the lake. The container was immediately labeled and kept in ice chest box, and conveyed to the laboratory of the Department of Biological Sciences, Baze University, Abuja, Nigeria, within 6 hours for culture and isolation of *S. aureus.*

Isolation, identification and character-ization of *S. aureus*

One milliliter of water sample (collected with a pipette) was serially (5 folds) diluted with 9 ml sterile distilled water. One milliliter of each serial dilution was added to 20 ml of sterile Mannitol Salt Agar (MSA) plate using pour plate method, and this was thoroughly mixed and allowed to set. The plates were incubated at 37 ºC but briefly opened for 30 seconds to remove condensed water on the agar surface. The plates were then covered, inverted and incubated at 37ºC for 48 hours. Suspected colonies of *S. aureus* based on their size, shape, color, pigmentation, consistency, and Gram reaction, were sub-cultured on nutrient agar plates to obtain pure cultures (10).

One gram of the gills and intestine were cut out, transferred and crushed with a sterile pestle in a sterile mortar containing 9 ml of sterile distilled water. A serial dilution (5 folds) of each homogenate was prepared prior to inoculation. A 1 mL aliquot of the serially diluted homogenate from the gills and intestine was incubated at 37^{o}C for 24 hour on MSA plates by pipetting 0.1 ml of 10^{-4} dilution aseptically unto the surface of the agar using a sterile bent glass rod to spray the homogenate. After incubation, the colonies were counted using a colony counter (Brunswick Scientific C-110). Yellow colonies presumptively identified as *S. aureus* were then sub-cultured onto nutrient agar slants, following which they were Gram stained and tested for catalase, coagulase and deoxyribonuclease (11).

Antibiotic susceptibility testing

Antibiotic sensitivity test was performed on each *S. aureus* isolate by the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) on Mueller-Hinton (MH) agar (12) using the following antibiotic

discs; ciprofloxacin (10 µg), norfloxacin (10 µg), gentamycin (10 µg), amoxicillin (20 µg), streptomycin (30 µg), rifampicin (20 µg), erythromycin (30 µg), chloramphenicol (30 µg), ampicillin (20 µg) and levofloxacin (20 µg). The MH plates were incubated at 37°C for 24 hours, and the zone of inhibition around the disc was interpreted according to CLSI guideline (12).

Statistical analysis

One way analysis of variance (ANOVA) and Chi square test were performed using Origin 8.1 Lab pro software for windows.

Results:

Colony count

Fig 1 shows the bacterial colony count in the gills and GIT of fish, and water samples. The mean count was highest in the gills with 54.64 x 10^5 CFU/ml followed by the GIT with 54.38 x 10^5 CFU/ml. The water sample had the lowest colony count of 27.78 x 10^5 CFU/ml (p<0.05)

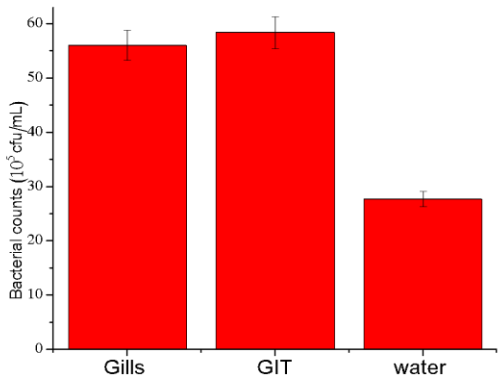

Fig.1: Bacterial count in the gills and gastrointestinal tracts of fish, and water samples (F=120.56, p=0.0013)

Isolation of *S. aureus*

Fig 2 shows percentage of *S. aureus* isolated from the gills and GIT of fish, and water samples. *S. aureus* was isolated from 53% (16 of 30) of the gills, 57% (17 of 30) of the GIT, and 33% (2 of 6) of the water samples (p <0.05).

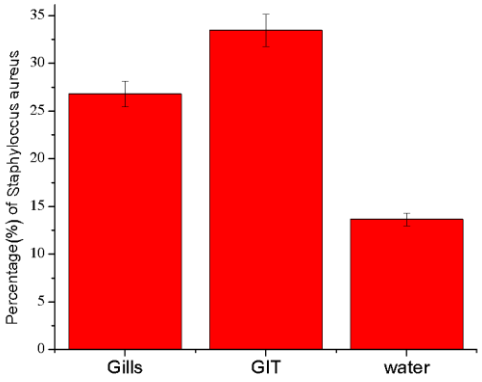

Fig. 2: Percentage isolates of *S. aureus* in the gills and GIT of fish, and water samples (X^2 = 13.26, p = 0.0013)

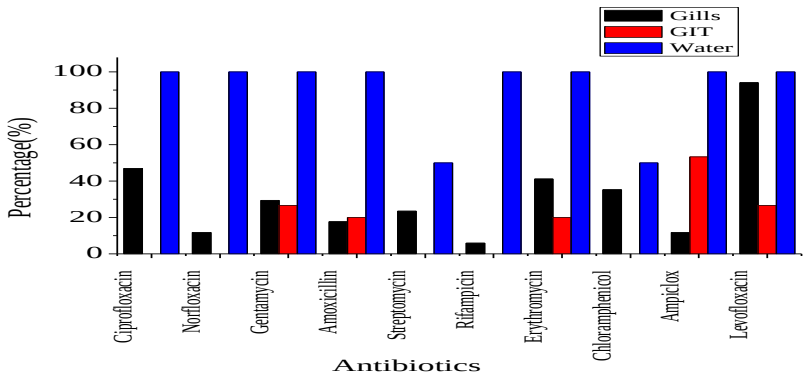

Fig 3: Antimicrobial susceptibility of *Staphylococcus aureus* from gills and gastrointestinal tracts of fish, and water samples

Antimicrobial susceptibility of *S. aureus*

Ninety four point one percent (94.1%) of *S. aureus* recovered from gills were resistant to ampicillin while 53.3% of those recovered from the GIT were resistant to levofloxacin. *S. aureus* recovered from water samples were resistant (100%) to ciprofloxacin, norfloxacin, gentamycin, amoxicillin, rifampicin, erythromycin, ampicillin and levofloxacin, and 50% were resistant to streptomycin and chloramphenicol (Fig 3)

Discussion:

The findings of this study indicated that the gills and GIT of the fish, and the water samples from Jabi Lake were contaminated with *S. aureus*. This may be due to the introduction of biological pollutants containing pathogenic micro-organisms such as *S. aureus,* through human activities around the lake. The presence of *S. aureus* in the lake in both the fish and water, make this a reservoir for this pathogenic bacterium. This agrees with the findings of Gono et al., in Zimbabwe who isolated *S. aureus* from edible freshwater fish, *Tilapia rendalii* and *Oreochromis niloticus* (13).

Our study also showed that the counts of *S. aureus* in the gills and GIT of the fish were higher than from the water itself. This may sometimes be due to the large accumulation of bacteria in the gut of fish from the raw open sewage that is discharged directly into the lake. The potential danger of human infection by these pathogenic microorganisms from consumption of improperly cooked contaminated fish remains very considerable. The highest colony (54.68 x 10^5 CFU/ml) of bacteria in this study was found in the gills, a count that is close to 10^6 bacteria count per gram, a level reported to be unsuitable for human consumption (14).

Antibiotics are important for treatment of infectious diseases of man however, microorganisms are capable of developing resistance especially following misuse of antimicrobials, making it possible for microorganisms that have not been exposed to antibiotic acquire resistance. In this study, a high prevalence of *S. aureus* resistant to ampicillin, ciprofloxacin and erythromycin was observed in the gills of fish, compared to the GIT, which may due to genetic mutations as reported by Toroglu et al. (15). The recovery of *S. aureus* resistant to multiple antibiotics in this study may be attributed to the effect of polluted run-off water generated by anthropogenic activities, and discharged into the lake, with high tendency for transfer of antimicrobial resistant determinants to the aquatic environment and the fish in the lake (15).

Conclusion:

The results from this study revealed that *C. gariepinus* from Jabi Lake harbored high number of *S. aureus* resistant to multiple antibiotics in the gills and GIT of fish, as well as high prevalence in the lake water samples. Therefore water from this lake must be tested to determine bacterial load and antibiotic resistance in contaminating microorganisms. In order to reduce lake pollution, wastewater discharged into the lake must be treated before discharge. For safe consumption, fish from this lake must be properly cooked to prevent development of infections from *S. aureus* or other pathogenic microorganisms that might have contaminated the lake.

Conflict of interest:

Authors declare no conflict of interest

Acknowledgements:

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