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Phenotypic and molecular detection of methicillin resistant *Staphylococcus aureus* (MRSA) Isolated from *Clarias gariepinus* (Burchel, 1822) and *Oreochromis niloticus* (Linneaus, 1758) IN Maiduguri

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Abstract
The phenotypic and molecular detection of methicillin resistant *Staphylococcus aureus* (MRSA) were carried out on fish and humans. A total of Nine hundred (900) swab samples were aseptically collected 350 each from Alau Lake and from commercial fish farms and one hundred (100) each from fish farmers and fishermen. For the phenotypic detection, the samples were inoculated on mannitol salt agar for *Staphylococcus aureus* isolation followed by oxacillin screening agar base (ORSAB) medium for MRSA isolation respectively. Genotype detection was carried out through the detection of *Nuc* gene for *S. aureus* and *mac*A gene for (MRSA) using Real-Time Polymerase chain Reaction (RT-PCR). The study also determined the prevalence and molecular detection of MRSA in *C. gariepinus*, *O. niloticus*. Fish Farmers and Fishermen in Maiduguri and its environs. The result presents 218 (31.14%), 97 (50.78%), 14 (14.43%) and 10 (10.30%) positive for *S. aureus*, MRSA, *nuc* gene and *mec*A gene respectively. The swap samples from *Oreochromis niloticus* and *Clarias gariepinus* examined presents *Clarias gariepinus* to have higher *S. aureus* prevalence in both phenotypic and genotypic detection method. The samples from fish farmers and fishermen present 111 (55.50%), 50 (25.00%), 6 (3.00%) and 3 (1.50%) positive values for *Staphylococcus aureus*, MRSA; *Nuc* genes and *Mac*A genes respectively. The phenotypic prevalence of MRSA was 97 (13.85%) and 50 (25.00%) for fish and fishermen respectively while the genotypic prevalence of MRSA was 14 (2.00%) and 3 (1.50%) for fish and fishermen. *S. aureus* isolates from fish subjected to antibiotic susceptibility testing shows all to be (100%) resistant to Vancomycin, Oxacillin, Ceftriaxone, Cephazolin and Cefoxitin, 23 (23.71%) to Clindamycin and 9.28% to Trimethoprim-sulfemethozole. All the isolates from fish were susceptible to Gentamicin and Ciprofloxacin. On the contrary all isolates from fish farmers display 100% resistance to Vancomycin, Oxacillin, Cefoxitin and Ceftrizone, but were susceptible to Gentamicin and Ciprofloxacin. Based on the findings of this study, it can be concluded that *S. aureus* and MRSA are present in fish from the wild, cultured, fish farmers and fishermen; Ciprofloxacin and Gentamicin are therefore recommended as drugs of choice for the treatment of *Staphylococcus aureus* and MRSA infection in fish in the study area.

Keywords: Phenotypic, molecular, methicillin, staphylococcus *aureus*, *Clairas gariepinus*, *Oreochromis niloticus*, fishermen, fish farmers, isolate, antibiotic

Introduction
Fish is a popular, highly nutritious aquatic vertebra and serves as a delicacy to most people of the sub-Saharan Africa, providing over 18% of total animal protein intake worldwide with a share as high as 40-60% in some West African states. According to FAO's statistics, fish consumption has declined by more than 2kg per person annually over the past few years from a per-output supply of 8.8kg in 1984 to 6.8kg in 1994. Fish could be in different form: fresh, dried, smoked, roasted and could also be sold dehydrated and pounded into flour. Clarias or Mud-fish belong to the family clariidae which is divided into two genera Clarias and heterobranchus each having three species. Clarias is divided into three species: *Clarias lazera*, C'. *anguillaris* and C. *gariepinus*. Clarias or Mud-fish as appropriately named has long bodies with dorsally flattened head enclosed by bony plates. They have large terminal mouth and four pairs of simple barbells, possess the crania backbone, gills and fins. In general terms it's considered to comprise the head, trunk and tail. These colors vary from species to species but are usually blackish on the back and whitish or slightly yellowish on the belly. Fish are

affected by various pests and diseases which has paused threat in the fish farming industries both for commercial and local purposes. In recent years, there have been rapid development in the knowledge of fish diseases and it is difficult for Scientists to appreciate all of the significant developments previous texts and publications have concentrated on the pathology of the principal diseases, but have not emphasized on the biology of the etiological agents. Some special chapters and previews have been published on fungi, viruses and parasite of fish but bacteria seem to have been comparatively neglected for sometimes [1]. *Staphylococcus* aureus (S. *aureus*) is a major causative 'bacterial pathogen of global importance. The organism causes significant epidemiologic and therapeutic problems in both humans and animals. It also causes a wide range of illnesses from minor skin infections, such as pimples, impetigo, to life-threatening diseases such as meningitis, endocarditis, mastitis and toxic shock syndrome [2, 3] The organism is considered to be the most resistant of all non-spore forming pathogens, with well-developed capacities to withstand high salt concentrations, extremes pH and high temperatures [3]. It is also known to be notorious in their acquisition of resistance to new drugs that continue to defy control measures with many strains carrying a wide variety of multi drug resistance genes on their plasmids [4]. Human isolates of S. *aureus*, unlike animal isolates, are frequently resistant to penicillins [5-7]. An organism exhibiting this type of resistance is referred to as methicillin (oxacillin) resistant S *aureus* (MRSA). Such organisms are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides macrolides, chloramphenicol, tetracycline and fluoroquinolones [8]. In addition, MRSA strains should be considered to be resistant to all cephalosporins, cephems and other β-lactams (such as ampicillin-sulbactam, amoxicillin-clavulanic acid, tircacillin-clavulanic acid, piperacillin-tarzobactam and the carbapenems) regardless of the *in-vitro* test results obtained [9]. Extensive data continued to be published on the epidemiological pattern, molecular characteristic and changing trend of the MRSA strains in human population [10, 11]. However, available data on MRSA in veterinary medicine is still emerging, for full epidemiological appreciation of clinical implication and public heath importance. In developed countries, prevalence levels of MRSA in different types of animals have been published [12-15]. The prevalence of MRSA in farm and domestic animals including sheep, goats, horses and cattle as well as in different pet or companion animals such as dogs and cats is showing an emerging trend and increasing prevalence, particularly among domesticated animals [16]. In horses, the prevalence level ranged between 0-2.7% [14], in cattle 0.18%-0.4% [16, 17] and in goat: 0-1.3% [18]. With these reported levels, and unique characteristic of MRSA, in term of rapid disseminability, the organism might be considered as a potential zoonotic pathogen of veterinary importance. The zoonotic perspective of MRSA has been evaluated in some studies, with particular reference to colonization and transmission to humans due to contact with domestic animals [19]. Similarly, zoonotic infections, with potential source of MRSA infection due to human population have been reported [20, 13, 14]. The possibility of concurrent infection of MRSA due to zoonotic source, posed serious public health problem to the entire community. Its transmission is solely from humans to animals but can also be from animals to humans (zoonotic) with Methicillin-Resistant *Staphylococcus aureus* (MRSA) infection occurring typically with contact between the hands of humans and anterior nares of animals. Due to increase in demand of fish, the intensification of culture system to increase production has caused a lot of fish mortality of which and the aetiology is not properly known. *Staphylococcus aureus* is one of the problematic micro-organism that caused disease in both human and fish. Therefore, the aim of this work was detect the presence of *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) in *C. gariepinus, O. niloticus*, fish farmers and fishermen in Lake Alau and commercial fish farms in Maiduguri and its environ, isolate and identify *S.aureus* and MRSA in *C. gariepinus* and *O. niloticus*, Fish Farmers and Fishermen in Lake Alau and commercial Fish farms Maiduguri and its environs, determine the antibiotic susceptibility patterns of the *S. aureus* and methicillin resistant *S. aureus* (MRSA) isolates from *C. gariepinus* and *O. niloticus*, fish farmers and fishermen in Lake Alau and commercial fish farms in Maiduguri and its environs and detect the presence of *S. aureus* and MRSA isolates using of real-time PCR

Materials and Methods

Study Area

This study was conducted at Lake Alau, Maiduguri and its environs in Borno State, situated between latitude 11º51ˈN and longitude 13º05ˈE [21].

Study Design

A cross sectional study was conducted in accordance with the method of Tristan *et al*. [22] to determine the presence and prevalence of *S. aureus* and methicillin resistant *S. aureus* (MRSA) in *C. gariepinus* and *O.niloticus* fish farmers and fishermen in Maiduguri and its environs. Sterile swap was used to collect samples from nostrils of (fish farmers and fishermen) and from gill, skin, oral cavity and crushed samples (*Clarias gariepinus* and *Oreochromis niloticus*) were aseptically collected using a multistage sampling technique. Sample size for the experiment was determined using statulator an online statistical calculator.

Collection of Fish Samples

Twenty five (25) samples of *Clarias gariepinus* and *Oreochromis niloticus* of three stages of growth (fingerlings, juvenile and adult) were used; the samples were collected from Lake Alau and various commercial fish farms in Maiduguri

Collection of Swap Sample from Fish and humans

Samples from juveniles and adults were collected from three sites (skin, oral cavity and gills) while fingerlings were crushed using pestle and mortar. The samples were collected using sterile cotton-tips swabs (Tyconpaey) by gently rotating it to be in contact with the three target area of (skin, oral cavity and gills), while the crushed fingerlings were made into homogeneous suspension and the swab sticks were inserted into the suspension to obtain the samples, the swab sticks were then placed into containers and then capped. For samples in humans, nasal swabs from 100 fish farmers and fishermen were aseptically collected, by gently inserting and retracting the sterile swab sticks and then rotating it so that it will be in contact with nasal mucosa of fish farmers and fishermen. Each sample collected were labeled according to site of sample collection, age, species and strain of the fish and stored in refrigerator before phenotypic characterization.

Laboratory Examination of Sample

Bacteriological culture media

All the media used in this study were obtained from Oxoid (Basingstoke,UK), which were of microbiological grades and these include; mannitol salt agar (CM2), nutrient agar (CM3) and Oxacillin Resistance Screening Agar Base (ORSAB) (CM1008).

Laboratory procedures

Bacterial Isolation and Identification of *Staphylococcus aureus*

Swab samples from *C. gariepinus* and *O. niloticus* and nasal swabs from fish farmers and fishermen for the isolation of *S. aureus* and MRSA were determined as described by [24, 25]. Primary Isolation of *S. aureus* was carried out by culturing the samples on mannitol salt agar prepared according to conventional technique. The cultured plates were incubated at 37ºC aerobically in an incubator for 24 hrs, thereafter examined for the presence of *Staphylococcus* like-colonies. The plates that showed no growth were discarded. Typical of *S. aureus* was further sub-cultured onto nutrient agar plates, from which subsequent growth was examined using biochemical tests; catalase and coagulase test for the presence of *S. aureus.*

Biochemical characterization

Identification of Methicillin Resistance *S. aureus* (MRSA)

This was performed using Oxacillin Resistance Screening Agar Base (ORSAB) which is a selective chromogenic medium for the detection and differentiation of MRSA. *Staphylococcus aureus* isolates were plated onto ORSAB and incubated for 24 hours at 37ºC thereafter, colonial morphology was examined.

Antimicrobial Sensitivity Testing

All identified *S. aureus* species were examined for antimicrobial susceptibility by disc diffusion method using nutrient agar in accordance with the guidelines of Clinical and Laboratory Standard Institute [23]. The results of sensitivity or resistance were interpreted according to [23]. Ten antimicrobials were tested to determine their break points for testing the *S. aureus* isolates (Table 1).

Table 1: Antimicrobial Discs used and their break point for Testing *S. aureus* isolates in Maiduguri and its environs.

Antimicrobial	Concentration (ug)	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Erythromycin	15	≤13	14-22	≥23
Vancomycin	30	NA	NA	≥17
Oxacillin	1	≤10	11-12	≥13
Ceftriaxone	30	≤13	14-20	≥21
Ciprofloxacin	5	≤15	16-20	≥21
Gentamycin	10	≤12	13-14	≥15
Cephazolin	30	≤14	15-17	≥18
Trimethoprim+sulfamethozole	23.75	≤10	11-15	≥16
Cefoxitin	30	≤21	NA	≥22
Clindamycin	2	≤14	15-20	≥21

NA-Not available, Source: [23].

Molecular Detection of *S. aureus* and MRSA

Real Time Polymerase Chain Reaction (RT-PCR)

All the phenotypic detected MRSA from *C. gariepinus* and *O. niloticus*, fish farmers and fishermen were subjected to RT-PCR for detection of *nuc* gene specific for *S. aureus*. All the *nuc* positive and *nuc* negative isolates were subjected to RT-PCR for detection of *mec*A gene encoding resistance as described by [26]. The nucleotide sequence of primers and probes used for real-time PCR were outlined on Table 2.

Table 2: Nucleotide Sequence of the Primers and Probes used for the Real-Time PCR

Primer or probe name	Sequence (5′-3′)	5′ Reporter dye 3′ Quencher
Nuc nuc Forward nuc Reverse nuc Probe	CAA AGC ATC AAA AAG GTG TAG AGA TTC AAT TTT CTT TGC ATT TTC TAC CA TTT TCG TAA ATG CAC TTG CTT CAG GAC AC	Texas Red FAM
mec A mec A Forward mec A Reverse mec A Probe	GGC AAT ATT ACC GCA CCT CA GTC TGC CAC TTT CTC CTT GT AGA TCT TAT GCA AAC TTA ATT GGC AAA TCC	FAM TAMRA

Source: [26]

Extraction of DNA

Bacterial genomic DNA was extracted from both culture (*S. aureus* and MRSA) using the method described by [27].

Amplification using Real Time Polymerase Chain Reaction (qPCR)

Polymerase chain reaction (PCR) amplification of DNA template was carried out in a thermo cycler (7500 RT PCR SYSTEM). The Amplification was performed for both *Mec* A gene and *nuc* gene using the method of [28] (Table 3). The summary of the thermo-cycling program used is given on (Table 4).

Table 3: Reaction of RT-PCR Master Mix for Amplification of *nuc* and *mec*A genes of *S. aureus*

Components	Volume(µl)
PCR Buffer	10
PCR Nucleotide Mix, 10 Mm each	1
MgCl2 25 mM1 8 pl	8
Taq polymerase 1.25 pl	1.25
dNTP 200 pg l pl	1
Forward Primer 1 pl	1
Reversed Primer _ 1 pl	1
Fluorogenic probes 1 pl	1
DNA templates 1 pl	1
Nuclease free Water 24.75 pl	24.75
Total	50 µl

All this mixture was done on ice to preserve the DNA template

Table 4: Thermo-cycling Program of RT-PCR Amplification of *nuc* and *mec* A Gene of *S. aureus*

Step	Temperature(^{0}C)	Time	Number of cycles
Initial Denaturation	95	1min	1cycle
Denaturation	95	1min	35cycle
Annealing	65	1min	35cycle
Extension	60	1min	35cycle
Final Extension	60	1min	1cycle

Data analysis

The effect of variation of species, strain, stage of growth and location of sample collection, of the phenotypic and molecular detection of methicillin resistant *Staphylococcus aureus* isolated from the two species and fish handlers were subjected to Cross Tab at α0.05 using SPSS (version 16).

Results

Phenotypic Characteristics and Molecular Detection of *S. aureus* and MRSA in *C. Gariepinus* and *O. niloticus* Fish Farmers and Fishermen in Maiduguri and its environs

Table 5 presents, the result of phenotypic characteristics of *S. aureus* and MRSA in *C. gariepinus*, *O. niloticus*, fish farmers and fishermen in Maiduguri and its environs. Out of the seven hundred (700) samples collected from fish, 218 (31.14%) showed golden yellow colouration on mannitol salt agar. These were presumed to be staphylococci. Out of the 218 presumptive staphylococci, the biochemical characteristics revealed 191 (87.61%) positive for both catalase and coagulase test, arising from the isolates presumed to express the phenotypic characteristics of S*taphylococcus aureus* in this study. The *S. aureus* isolates were further sub-cultured on ORSAB media to identify MRSA, where 97 (50.78%) showed deep blue colouration on the media which is the phenotypic characteristics appearance of MRSA. Genotypically, 14 (14.43%) and 10 (10.30%) were positive for *Nuc* gene and *mec* A gene respectively. Out of the two hundred samples collected from fish farmers and fishermen, 111 (55.50%) showed golden yellow on mannitol salt agar. These were assumed to be staphylococci. On biochemical characteristics, 87 (78.37%) and 96 (86.48%) were positive for catalase and coagulase respectively. These isolates were presumed to be S*taphylococcus aureus* in this study. The *S. aureus* isolates were further sub cultured on ORSAB media to identify MRSA, where 50 (52.08%) showed deep blue colouration on the media which is the phenotypic characteristic appearance of MRSA. Genotypically, 6 (12.00%) and 3 (6.00%) were positive for *Nuc* gene and *Mec*A gene respectively.

Table 5: Phenotypic Characteristics and Molecular Detection of *S. aureus* and MRSA in *C. Gariepinus* and *O. niloticus*, Fish farmers and Fishermen in Maiduguri and its environs

Test	Fish (*O. niloticus* and *C. gariepinus*)		Fish farmers and Fishermen	
	No. of Samples Tested	No. (%) Positive	No. of Samples Tested	No. (%) Positive
Mannitol Salt Agar	700	218 (31.14)	200	111 (55.50)
Catalase	218	191 (87.61)	111	87 (78.37)
Coagulase	218	191 (87.61)	111	96 (86.48)
ORSAB	191	97 (50.78)	96	50 (52.08)
Nuc Gene-PCR	97	14 (14.43)	50	6 (12.00)
*Mac*A Gene-PCR	97	10 (10.30)	50	3 (6.00)

KEY: No. number%-percentage

Prevalence of MRSA in *C. Gariepinus* and *O. niloticus*, Fish farmers and Fishermen in Maiduguri and its environs

Table 6 shows the Prevalence of MRSA in *C. Gariepinus* and *O. niloticus* fish farmers and fishermen in Maiduguri and its environs. Out of the 700 samples analysed from fish, 218 (31.14%), 97 (50.78%), 14 (14.43%) and 10 (10.30%) were positive for *Staphylococcus aureus*, MRSA; *nuc* gene and *mec*A gene respectively. Out of 200 samples analysed from fish farmers and fishermen, 111 (55.50%); 50 (52.08%); 6 (12.00%) and 3 (6.00%) were positive for *S. aureus*, MRSA *nuc* gene and *mec* A gene respectively. The phenotypic prevalence of MRSA was 97 (50.78%) for *C. gariepinus* and *O. niloticus* fish and 50 (52.08%) for fish farmers and fishermen. The genotypic prevalence of MRSA was 10 (10.30%) for *C. gariepinus* and *O. niloticus* fish and 3 (6.00%) for fish farmers and fishermen

Table 6: Prevalence of MRSA in *C. Gariepinus* and *O. niloticus*, Fish farmers and Fishermen in Maiduguri and its environs

Samples	No. tested	*S. aureus*	MRSA	*Nuc* Gene	*mac*A Gene
		No.(%) Positive	No. (%) Positive	No.(%) Positive	No.(%)Positive
C. gariepinus and *O. niloticus*	700	218 (31.14)	97 (50.78)	14 (14.43)	10 (10.30)
Fish farmers and Fishermen	200	111 (55.50)	50 (52.08)	6 (12.00)	3 (6.00)

KEY: No. number%-percentage

Specific Prevalence of *S. aureus* in *C. gariepinus* and *O. niloticus* in Maiduguri and its environs based on Two Strains (Cultured and Wild)

Table 7 presents the specific prevalence of *S. aureus* in *C. gariepinus* and *O. niloticus* in Maiduguri and its environment based on two strains, cultured and wild. Out of the 350 swab samples examined from wild fish, 116 (33.14%) were phenotypically positive for *S. aureus*, while 102 (19.14%) was phenotypically positive for 350 fish swab samples from cultured fish. There was no significant difference between the strains of the fish using phenotypic detection method ($p > 0.289$). Of the phenotypically positive *S. aureus* isolates from the wild and cultured fish samples, 7 (6.03%) and 7 (6.86%) were genotypically positive respectively. There was no significant difference between the strains of the fish using genotypically

Table 7: Specific prevalence of *S. aureus* in *C. gariepinus* and *O. niloticus* in Maiduguri and its environs based on Two Strains (Cultured and Wild)

Methods	Species	No. tested	*S. aureus*	P-value	Odds Ratio	95% CI	
			No. (%) positive			Lower	Upper
Phenotypic detection	Wild	350	116(33.14)	0.289^{ns}	1.205	0.875	1.661
	Cultured	350	102(29.14)				
Genotypic detection	Wild	116	7(6.03)				
	Cultured	102	7 (6.86)	1.000^{ns}	1.000	0.347	1.021

ns = Not Significant at α 0.05

Species-Specific Prevalence of MRSA in *C. gariepinus* and *O. niloticus* in Maiduguri and its environs based on species

Table 8 shows the specific prevalence of MRSA in *C. gariepinus* and *O. niloticus* in Maiduguri and its environs based on species. Out of the 700 swabs phenotypically examined MRSA using ORSAB, 79 (22.58%) and 18 (5.14%) were MRSA positive for *C. gariepinus* and *O.niloticus* respectively. There was significant difference between the species and positive phenotypic examination method ($p< 0.001$). Of the phenotypically positive MRSA isolates from *C. gariepinus* and *O.niloticus* examined, 10 (12.65%) and 0 (0.00%) respectively were genotypically positive. There was significant difference between the species and the positive genotypic reaction ($p< 0.002$)

Table 8: Species-Specific prevalence of MRSA in *C. gariepinus* and *O. niloticus* Maiduguri and its environs

Methods	Species	No. Tested	MRSA	P- value	Odds Ratio	95% CI	
			No.(%) positive			Lower	Upper
Phenotypic detection	*C. gariepinus*	350	79 (22.58)	0.001*	0.186	0.109	0.318
	O. niloticus	350	18(5.14)				
Genotypic detection	*C. gariepinus*	79	10(12.65)				
	O. niloticus	18	0 (0.00)	0.002*	0.971	0.954	0.989

* = Significant at α 0.05

Specific prevalence of MRSA among strains of cultured and wild in Maiduguri and its environs

The specific prevalence of MRSA among strains of cultured and wild *C. gariepinus* and *O. niloticus* in Maiduguri and its environs indicated that of 350 wild fish swab samples examined, 49 (14.00%) were phenotypically positive for MRSA, while 48 (13.71%) of 350 fish swab samples from cultured fish were positive for MRSA phenotypically. There was no significant difference between the strains of the fish and phenotypic analysis ($p > 1.000$). Of the phenotypically positive MRSA isolates from the wild and cultured fish samples, 4 (8.16%) and 6 (12.50%) were genotypically positive respectively. There was no significant difference between the strains of the fish in the genotypically positive isolates ($p > 0.752$) (Table 9)

Table 9: Specific prevalence of MRSA among Two Strains of Cultured and Wild in Maiduguri and its environs

Methods	Species	No. tested	MRSA	P-value	Odds Ratio	95% CI	
			No. (%) positive			Lower	Upper
Phenotypic detection	Wild	350	49 (14.00)	1.000^{ns}	1.024	0.667	1.573
	Cultured	350	48 (13.71)				
Genotypic detection	Wild	49	4 (8.16)				
	Cultured	48	6 (12.50)	0.546^{ns}	0.663	0.185	2.369

ns = Not Significant at α 0.05

Specific prevalence of MRSA in Fish Farmers and Fishermen in Maiduguri and its environs: The specific prevalence of MRSA in fish farmers and fishermen in the Maiduguri and it's environ are shown in Table 10. Out of 100 samples from fish farmers examined, 46 (46.00%) were positive for *S. aureus*, 25 (54.34%) and 2 (8.00%) were positive for ORSAB and *Nuc* gene respectively and none was positive for *mec*A gene and MRSA. Of the 100 fishermen samples examined, 65 (65.00%), 25 (38.46%), 4 (16.00%) and 3 (12.00%) were positive for *S. aureus*, ORSAB, *Nuc* gene, *mec*A gene respectively.

Table 10: Specific prevalence of MRSA in Fish Farmers and Fishermen in Maiduguri and its environs

Samples	No. Tested	*S. aureus*	ORSAB	*Nuc* Gene	*mec*A Gene
		No. Positive (%)	No. Positive (%)	No. Positive (%)	No. Positive (%)
Fish farmers	100	46 (46.00)	25 (54.34)	2 (8.00)	0 (0.00)
Fishermen	100	65 (65.00)	25 (38.46)	4 (16.00)	3 (12.00)

Antimicrobial resistance profile of *S. aureus* isolates from *C. gariepinus* and *O. niloticus* in Maiduguri and its environs

The antimicrobial resistance profile of *S. aureu i*solates in fish indicated that all the *S. aureus* isolated from fish were resistant to Vancomycin, Oxacillin, Ceftriaxone, Cephazolin and Cefoxitine. Additionally, 23.71% of the isolates were resistant to Clindamycin, 9.28% to Trimethoprim-sulfemethozole and 2.06% to Erythromycin. The result also showed that all the isolates were susceptible to Gentamycin and Ciprofloxacin, while 87.63% of *S. aureus* showed intermediate response to Trimethoprim-sulfemethozole, 74.22% to Erythromycin and Clindamycin, 3.09% to Ciprofloxacin and Cephazolin (Table 11)

Table 11: Antimicrobial Resistance Profile of *S. aureus* isolates in *C. gariepinus* and *O. niloticus* Maiduguri and its environs

Antimicrobial	No. Tested	Resistance No. Positive (%)	Intermediate No. Positive (%)	Susceptible No. Positive (%)
Erythromycin	97	2 (2.06)	72 (74.22)	2(23.71)
Vancomycin	97	97 (100)	0 (0.00)	0 (0.00)
Oxacillin	97	97 (100)	0 (0.00)	0 (0.00)
Ceftriaxone	97	97 (100)	0 (0.00)	0 (0.00)
Ciprofloxacin	97	0 (0.00)	3 (3.09)	94 (96.91)
Gentamycin	97	0 (0.00)	0 (0.00)	97 (100)
Cephazolin	97	94 (96.90)	3 (3.09)	0 (0.00)
T. sulfemethizole	97	9 (9.28)	85 (87.63)	3 (3.09)
Cefoxitin	97	97 (100)	0 (0.00)	0 (0.00)
Clindamycin	97	23 (23.71)	72 (74.22)	2 (2.06)

Key: No. = Number, % = percentage

Antimicrobial resistance profile of *S. aureus* isolates in Fish farmers and Fishermen in Maiduguri and its environs

The antimicrobial resistance profile of *S. aureus* isolates from fish farmers and fishermen in Maiduguri and it is environ showed 100% resistance to Vancomycin, Oxacillin, Cefoxitin and Ceftriaxone. Resistance from 98.00% of the isolates was recorded against Cephazolin. The results also showed that 100% of the isolates were susceptible to Gentamycin, while 96.00%, 14.00%, 4.00% and 2.00% susceptibility was also recorded against Ciprofloxacin, Erythromycin, Trimethoprim-sulfemethozole and Clindamycin respectively. Furthermore 96.00% of the *S. aureus* isolates showed intermediate response against Trimethoprim-sulfemethozole and Clindamycin, 86.00%, 4.00% and 2.00% to Erythromycin, Ciprofloxacin and Cephazolin respectively (Table 12).

Table 12: Antimicrobial resistance profile of *S. aureus* isolates in Fish Farmer and Fishers in Maiduguri and its environs

Antimicrobial Conc. (ug)	Number Tested	Resistance No. Positive (%)	Intermediate No. Positive (%)	Susceptible No. (%) Positive
Erythromycin 15	50	0 (0.00)	43 (86.00)	7(14.00)
Vancomycin 30	50	50 (100)	0 (0.00)	0(0.00)
Oxacillin 1	50	50 (100)	0 (0.00)	0(0.00)
Ceftriaxone 30	50	50 (100)	0 (0.00)	0(0.00)
Ciprofloxacin 5	50	0 (0.00)	2 (4.00)	48(96.00)
Gentamycin 10	50	0 (0.00)	0 (0.00)	50(100)
Cephazolin 30	50	49 (98.00)	1 (2.00)	0(0.00)
Trimethoprim-sulf. 23.75	50	0 (0.00)	48 (96.00)	2(4.00)
Cefoxitin 30	50	50 (100)	0 (0.00)	0(0.00)
Clindamycin 2	50	1 (2.00)	48 (96.00)	1(2.00)

Key: No. = Number, % = percentage

Discussion

In this study, the prevalence of MRSA as confirmed by PCR detection of *nuc* gene and *mec*A genes was 10.30% and 6.00% in *C. Gariepinus* and *O. niloticus* fish farmers and fishermen respectively. Although, prevalence confirmed by phenotypic detection using ORSAB was 50.78% and 52.08% for fish and humans respectively. The phenotypic appearance of MRSA result in this study appeared higher compared with the 21.20% and 45.00% reported by Hafsat *et al.* [29] in fish and fish handler in Maiduguri, Nigeria. The variation might be connected with difference in the method of study or the sources of fish used in the study. The former was a market based study while this present one was centred on cultured and wild sourced fish. The study recorded proportionate 25% phenotypic prevalence in both fish farmers and fishermen which might be due to pressure emanating from the usage of antibiotics by both fishermen and fish farmers. Fish is of great public concern because it may sustain and reserve MRSA, and become a source of infection and re-infection for humans and other animals. Accurate detection of bacterial pathogen is of paramount important in saving lives and wealth/cost. The diversity observed between the phenotypic and genotypic prevalence remained to be determined. Although, Lewis and Dyke. [30], Brown *et al.* [31] referred to non expression of *mecA* gene by phenotypic MRSA as pre-MRSA. The molecular detection agreed with the finding of Khakpoor *et al.* [32] who reported that PCR have very high specificity and sensitivity giving lower detection rate rather than phenotypic (culture method). This finding differs from the report of Suleiman *et al.* [33] which shows excellent relationship between PCR detection of *mec*A gene and phenotypic identification of MRSA on ORSAB medium. This study also identified *C. gareipinus* 12.65% to have higher prevalence than *O. niloticus*, This may be due to inherent characteristics of *O. niloticus* such as scales which make *O. niloticus* to resist colonization by MRSA. This observation agreed with the findings of other researchers who reported older age as risk factor of MRSA (Washid *et al.*, [34]; Graffunder and Venezia,

[35]). Additionally, the total detection between the wild and cultured *O. niloticus* and *C. gariepinus* indicated that cultured *O. niloticus* and *C. gariepinus* have higher prevalence than their wild counterpart which had prevalence of 12.50%. This was lower than 52.2% obtained by Mohammed *et al.* [36] in a study of wild catfish caught in Alau Lake in Maiduguri, Nigeria. Several studies have reported epidemiological and microbial scientific evidence that human beings in close/direct contact with fish are at risk of being infected with MRSA (Lewis *et al*., [37]; Voss *et al.,* [38]). Herdsmen, fish farmers, pet owners, veterinarian have higher prevalence of colonization compared to unexposed individuals This assertion is pinpointing that being in close contact with animals is a predisposing factor for acquiring MRSA. The laboratory testing to ascertain the antimicrobial resistance pattern of bacterial pathogens is an important guide for both empirical and pathogen specific therapy. The antimicrobial susceptibility testing in this study showed that the *S. aureus* isolates were highly susceptible to Ciprofloxacin and Gentamicin. This result is in agreement with the report of Hafsat *et al.* [29] and Ibrahim *et al.* [39] indicating that the two antibiotics are drugs of choice for the treatment of *S. aureus* and MRSA infections in Maiduguri. In order to discourage the emergence of Gentamicin and Ciprofloxacin resistant strains, monotherapy with the two should be discouraged. Ciprofloxacin, a newer drug with mode of action on DNA inhibition are relatively expensive and less available for abuse. Also, antimicrobial susceptibility pattern of *S. aureus* isolates showed high level of resistance (100%) to Vancomycin, Oxacillin, Ceftriaxone, Cephazoline. This agrees with the work of Vanden Eede *et al.* [40] who monitored the susceptibility trend of Erythromycin, Clindamycine and recorded resistance which vary accordingly. The drugs are relatively inexpensive and available from numerous outlets or medicine stores were they are sold even without prescription by experts in Nigeria. This study also showed that 86.0% and 96% of the isolates display intermediate resistance to Erythromycin and Trimethoprim-sulfemethoxole, meaning that at high doses, they will be susceptible. This antibiotic susceptibility profile also showed that all the isolates from both fish and fish handler were multi drug resistant. In most communities in Maiduguri, antibiotic are prescribed without laboratory investigation and there is indiscriminate abuse and purchase of over-the-counter drugs, these will inevitably cause antibiotic pressure with resultant antibiotic resistance that could have a consequential effect of transfer from animal and animal product to humans via consumption of these animal products.

Conclusion

In conclusion, *Staphylococcus aureus* and MRSA are present in both wild, cultured, fish farmers and fishermen. The prevalence was higher in *C. gariepinus* and cultured fish with the following prevalence 12.65%, and 12.50% respectively. It is noticed that almost all isolates were resistant to Vancomycin, Oxacillin, Ceftriaxone and Cephazoline. Ciprofloxacin and Gentamicin had best antimicrobial activity against MRSA isolates and may be drug of choice for treatment of infection due to *S. aureus* in Maiduguri and its environs.

Recommendations

Fish handlers should be encouraged on personal hygiene and hand sanitation as bio security measure. Fish farmers should be encouraged on confirmation of disease before medication. Molecular diagnostic techniques should be employed in the diagnosis of fish diseases or infections. Gentamicin and Ciprofloxacin are recommended for the treatment of *S. aureus* and MRSA infection in fish and fish handlers. Antibiotics prescription, policies should be employ by appropriate authorities to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens. Further study detection and surveillance of presence of MRSA in ready to eat fishes are recommended.

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