

Research Article

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase Negative *Staphylococci* (MRCoNS) Isolated from Fish and Fish Handlers in Maiduguri, Nigeria

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Abstract: Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Resistant Coagulase Negative *Staphylococci* (MRCoNS) are of increasing importance to animal and public health where it has been isolated in food production line. The presence of *Staphylococci* on fish is an indication of contamination or disease in fish. This study was conducted to isolate and phenotypically characterize MRSA and MRCoNS from fresh fish, fish handlers, utensils used for fish processing and the scavenging animals within two major markets supplying fresh fish in Maiduguri, Nigeria. A total isolation rate of 121 (21.1%), 38 (6.6%) and 39 (6.8%) were detected as *S. aureus*, MRSA and MRCoNS, respectively. MRSA was isolated from fresh fish (2.6%), fish handlers (15%), utensils (4.5%) and scavenging animals (15.9%). MRCoNS was isolated from fresh fish 9 (3.4%), fish handlers 8 (13.3%), utensils 9 (5.8%) and scavenging animals 13 (13.8%). MRSA isolates showed resistance to ceftiofur (100%), gentamicin (89.5%), ciprofloxacin (94.7%), oxacillin (76.3%) and tetracycline (68.4%). Similarly, MRCoNS isolates were resistant to ceftiofur (100%), oxacillin (79.4%), ceftazidime (66.6%) and tetracycline (64.1%). MRSA and MRCoNS were detected in most parts of fresh fish and the marketing environments supplying fish to consumers in Maiduguri. Further studies are needed to elucidate transmission routes of MRSA in relation to fresh fish and to provide tools for preventing the spread of MRSA.

Keywords: Fish handlers, fresh fish, Maiduguri, MRCoNS, MRSA, Nigeria

INTRODUCTION

Fish represents a significant proportion of protein in the diet of consumers globally (Feldhusen, 2000). In Nigeria, fish is eaten fresh, smoked or processed and forms a delicacy that cuts across socio-economic and educational barriers (Adebayo-Tayo *et al.*, 2008). Substantial evidence showed that fishes are on the list of foods associated with outbreaks of food-borne diseases (Huss and Valdimarsson, 1990). *Staphylococcal* species is one of the major bacterial agents causing food borne illnesses (EFSA, 2010). In most cases of food poisonings, the incidence of *staphylococcal* intoxication is usually under reported (Lawryniewicz-Paciorek *et al.*, 2007).

The wide spread use of antibiotic resulted in the development of resistance to β -lactam antibiotics through acquisition of the mobile cassette chromosome carrying the methicillin-resistant gene *mecA* (Wielders *et al.*, 2002) and *mecC* (Porrero *et al.*, 2014).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Resistant Coagulase-Negative *Staphylococci* (MR-CoNS) has been identified as multidrug-resistant zoonotic pathogens in humans and many animal species (Morgan, 2008; Barbier *et al.*, 2010). Even though, coagulase negative *Staphylococci* may also be a normal flora for skin and mucous membranes of human and animal species (Aklilu *et al.*, 2010). Therefore, the presence of *Staphylococci* on fish can be an indication of either post harvest contamination due to poor personnel hygiene or disease event in fish (Austin and Austin, 2007). In countries with endemic food-borne diseases, a significant number of outbreaks occurred due to consumption of contaminated fish (Hatha Mohamed and Lakshmanaperumalsamy, 1997). Contamination through hands and surfaces during evisceration of fish is a common route of pathogen spread (Buras, 1993). Epidemiological studies revealed a possibility of cross-infection between animals and humans with certain

strains (Seguin *et al.*, 1999; Weese *et al.*, 2006). Transmission between fresh fish and fish handlers can be mediated through unhygienic nature of environmental surfaces (Albuquerque *et al.*, 2007). Due possibly to limited evidence on isolation of MRSA from fish and fish handlers, the world Organization on animal health (OIE) recommended the continuous monitoring and surveillance of resistant microorganisms in aquatic animals (Smith *et al.*, 2013). The objective of the present study therefore was to determine the rate of isolation and phenotypically characterize MRSA and MRCoNS from fresh fish and fish handlers in two major fresh markets in Maiduguri, Nigeria.

MATERIALS AND METHODS

Study area: Maiduguri is the capital of Borno state which is located in the north-eastern part of Nigeria. It has an area of 69,435 km² and lies between latitude 10 and 13⁰N and longitude 12 and 15⁰E. Maiduguri consists of two major fresh fish markets that supply the state and neighbouring countries. One of the major occupations of the residents around Gamboru and Kachallari area of Maiduguri includes fishing and processing of fish. Rivers and lakes located in Maiduguri include Lake Alau, Lake Chad and river Benue. These are the major rivers that supply the artisan fisher men in the study area. For logistic reasons, two major fresh fish markets in Maiduguri were conveniently selected on the basis of volume of fish marketed. Fish handlers in the context of this study include individuals involved in selling and evisceration of fresh fish only while utensils are tools used in processing such as chopping board and knives for evisceration as well as water reservoirs.

Sample collection: A total of 572 swab samples were collected from the two major fresh fish markets selected for bacteriological examination. Sampling of domestic animals was incorporated into the study when we observed the presence of dogs, cats, chickens and ducks in the market environment that feed on fish remnant. Out of the 572, 263 were from fish (Tilapia = 90, cat fish = 98 and carp fish = 75), 60 from fish handlers (hand = 30, nostril = 30), 195 from dogs (skin = 27, nostril = 27, perineum = 27), cats (skin = 2, nostril = 1, perineum = 0), chickens (skin = 7), ducks (skin = 3) and utensils (knives = 55, watering containers = 50, chopping boards = 50). Samples were collected from the skin, nostril and perineum of dogs and cats as well as skin under the feather in chickens and ducks. Each moist cotton swab was rolled over the surface of the fish skin, viscera and over the gills during evisceration and transported in ice to the Diagnostic laboratory, University of Maiduguri within an hour.

Fish handlers were selected based on voluntary participation with no consideration to health status. All

samples were collected during market visit using materials described above. Nasal swabs were collected by inserting moist cotton swab with normal saline into the anterior nasal mucosa of both nostrils and rotating the swab stick against the wall of the mucosa while hand swab was collected by rolling the stick over the dorsal and palmar surfaces of the hands. The swabs were then immediately covered and transported in ice to the Diagnostic laboratory, University of Maiduguri.

In addition, 30 sample collection forms were administered to the fish handlers that participated in the study to enhance the chances of MRSA isolation when we observed scavenging animals roaming in the market that were owned by the fish handlers. The questions in the sample collection forms include age, gender, time spent in fish processing, hygiene such as hand washing with sanitizers, contact with scavenging animals, skin infections and the use of antibiotics.

Isolation and identification of MRSA: Skin, gills, viscera and nasal swabs were inoculated directly onto blood agar, mannitol salt agar (Oxoid, UK). Samples were incubated for 24 h at 37°C and viewed microscopically for Gram reaction and biochemical tests (coagulase, maltose, lactose, trehalose and Voges-Proskauer) conducted. *S. aureus* identification was conducted using latex agglutination test (Pastorex Staph-plus, BioRad, France). All staphylococcal strains tested negative for latex agglutination test were considered coagulase negative *Staphylococci*. Both *S. aureus* and CoNS were screened for methicillin resistance by streaking onto Oxacillin Resistant Screening Agar Base (ORSAB) media (Oxoid, UK) supplemented with 1 mg oxacillin and polymyxin B at 50,000 IU/L and then incubated aerobically at 37°C for 24-48 h for the detection of MRSA. Growth of intense blue colonies was considered MRSA-positive.

Antibiotic susceptibility testing: Isolates were selected for antimicrobial susceptibility testing according to Kirby-Bauer disc diffusion techniques on Mueller Hinton agar using the following antibiotic discs: gentamycin (CN) 10 µg, Tetracyclin (TE) 30 µg, Oxacillin (OX) 1 µg, cephazolin (KZ) 30 µg, Chloramphenicol (C) 30 µg, Sulphadiazine and Trimetoprim (SXT) 25 µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (E) 15 µg, Cefoxitin (FOX) 30 µg and Clindamycin (DA) 2 µg. The zone of inhibition was interpreted according to Clinical Laboratory Standard Institute (CLSI, 2010). Multidrug resistance was defined as resistance to ≥4 antimicrobials (Oteo *et al.*, 2005).

RESULTS

In totality, 572 samples were collected from fish, fish handlers, domestic animals and the utensils used in fish processing. One hundred and twenty one isolates

Table 1: Distribution of MRSA and MRCoNS isolations (%) from swab samples collected from fish, fish handlers, domestic animals and utensils used in processing fish at Maiduguri fish markets, Nigeria

Isolates	Sampling units				Total (%)
	Fish (n = 263)	Fish handlers (n = 60)	Domestic animals (n = 94)	Utensils (n = 155)	
<i>S. aureus</i>	33 (12.5)	20 (33.3)	22 (23.4)	46 (29.7)	121 (21.1)
MRSA	7 (2.6)	9 (15.0)	15 (15.9)	7 (4.5)	38 (6.6)
MRCoNS	9 (3.4)	8 (13.3)	13 (13.8)	7 (5.8)	39 (6.8)

Table 2: Distribution of *S. aureus*, MRSA, MRCoNS isolated from fresh fish, fish handlers, domestic animals scavenging in the fish markets and the utensils used in fish processing

Source	Site	Number of samples	<i>S. aureus</i> positive (%)	MRSA positive (%)	MRCoNS positive (%)
Fish					
Tilapia	Skin	30	9 (30)	2 (6.6)	2 (6.6)
	Gills	30	4 (13.3)	0 (0)	0 (0)
	Viscera	30	5 (16.7)	1 (3.3)	0 (0)
Cat fish	Skin	30	6 (20)	3 (10.0)	3 (10.0)
	Gills	33	0 (0)	0 (0)	0 (0)
	Viscera	35	0 (0)	0 (0)	2 (5.7)
Carp fish	Skin	25	0(0)	0 (0)	0 (0)
	Gills	25	8 (32)	1 (12.5)	2 (8.0)
	Viscera	25	1 (4)	0 (0)	0 (0)
Sub total		263	33 (12.5)	7 (2.6)	9 (3.4)
Fish handlers	Hand	30	16 (53.3)	7 (23.3)	8 (26.6)
	Nostril	30	4 (13.3)	2 (6.6)	0 (0)
Sub total		60	20 (33.3)	9 (15.0)	8 (13.3)
Domestic animals					
Dogs	Skin	27	6 (22.2)	3 (11.1)	7 (25.9)
	Nostrils	27	8 (29.6)	6 (22.2)	0 (0)
	Perineum	27	5 (18.5)	3 (11.1)	3 (11.1)
Cats	Skin	2	0 (0)	0 (0)	2 (100)
	Nostrils	1	0 (0)	0 (0)	1 (100)
	Perineum	0	0 (0)	0 (0)	0 (0)
Chickens	Skin	7	2 (28.5)	2 (28.5)	0 (0)
Ducks	Skin	3	1 (33.3)	1 (33.3)	0 (0)
Sub total		94	22 (23.4)	15 (15.9)	13 (13.8)
Utensils	Knives	55	13 (23.6)	2 (3.6)	1 (1.8)
	Chopping boards	50	9 (18)	1 (2.0)	3 (6.0)
	Water reservoirs	50	24 (48)	4 (8.0)	5 (10)
Sub total		155	46 (29.7)	7 (4.5)	9 (5.8)
Main total		572	121 (21.1)	38 (6.6)	39 (6.8)

of *S. aureus* were recovered from all the samples out of which 38 (6.6) were MRSA, while 39 (6.8) were MRCoNS (Table 1).

Table 2 is a summary of the number of *S. aureus*, MRSA and MRCoNS isolated from all the samples. A total of 7 (2.6%), 9 (15.0%), 15 (15.9%) and 7 (4.5%) MRSA isolates were detected from fish, fish handlers, domestic animals and the utensils, respectively. A total of 9 (3.4%), 8 (13.3%), 13 (13.8%), 9 (5.8%) MRCoNS were isolated from fish, fish handlers, domestic animals and the utensils used in fish processing. Seven (43.8%) and two (50%) isolates of MRSA were recovered from the hands and nose of fish handlers, respectively, while two isolates of MRCoNS were recovered from hands of fish handlers.

Out of the 27 swabs collected from dogs, 12 MRSA were isolated. Although no MRSA was isolated from the cats sampled, 3 MRCoNS isolates were recovered. For utensils used for fish processing, varying level of MRSA and MRCoNS contamination has been

recorded. Four isolates of MRSA and 5 isolates of MRCoNS were recovered from the water reservoir used by fish handlers while 1 MRSA isolate was recovered from chopping boards (Table 2).

The results of antimicrobial susceptibility testing of MRSA isolates is presented in Table 3. Isolates of MRSA were highly resistant to cefoxitin (100%), gentamicin 34/38 (89.5%), ciprofloxacin 36/38 (94.7%), tetracycline 26/38 (68.4%) and oxacillin 29/38 (76.3%). Susceptibility of MRSA isolates were recorded against cephalosporin 36/38 (94.7%), sulphadiazine/trimetoprim 35/38 (92.1%) and chloramphenicol 34/38 (89.5%).

Table 4 presents the antimicrobial susceptibility testing results of MRCoNS isolates. MRCoNS isolates were resistant to cefoxitin (100%), oxacillin (79.4%), cephalosporin (66.6%) and tetracycline (64.1) while susceptible to gentamicin (53.8%), clindamycin (64.1%) and erythromycin (76.9%).

Table 3: *In vitro* antimicrobial susceptibility testing of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from fish, fish handlers, utensils used in fish processing and domestic animals scavenging in Maiduguri fish markets, Nigeria

Antibiotics	Drug concentration (µg)	Zone diameter break points nearest to whole number (mm)		
		S	I	R
Gentamycin	10	≥15	13-14	≤12
Tetracycline	30	≥19	15-18	≤14
Oxacillin	1	≥13	11-12	≤10
Cephazolin	30	≥18	15-17	≤14
Sulphadiazine and trimetoprim	25	≥16	11-15	≤10
Ciprofloxacin	5	≥21	16-20	≤15
Erythromycin	15	≥23	14-22	≤13
Cefoxitin	30	≥25	-	≤24
Clindamycin	2	≥21	15-20	≤14
Chloramphenicol	30	≥18	13-17	≤12
Antibiotics	Antimicrobial susceptibility (%)	Intermediate (%)	Antimicrobial resistance (%)	
Gentamycin	0 (0.00)	0 (0.00)	34/38 (89.5)	
Tetracycline	6/38 (15.7)	6/38 (15.7)	26/38 (68.4)	
Oxacillin	9/38 (23.7)	0 (0.00)	29/38 (76.3)	
Cephazolin	36/38 (94.7)	0 (0.00)	2/38 (5.3)	
Sulphadiazine and trimetoprim	35/38 (92.1)	0 (0.00)	3/38 (7.9)	
Ciprofloxacin	2/38 (5.3)	0 (0.00)	36/38 (94.7)	
Erythromycin	26/38 (68.4)	3/38 (7.9)	9/38 (23.7)	
Cefoxitin	0 (0.00)	-	38/38 (100)	
Clindamycin	18/38 (47.4)	7/38 (18.4)	13/38 (34.2)	
Chloramphenicol	34/38 (89.5)	1/38 (2.6)	3/38 (7.9)	

Table 4: *In vitro* susceptibility testing of Methicillin Resistant Coagulase Negative Staphylococci (MRCoNS) by disk diffusion method

Antibiotics	Drug conc. (µg)	Zone diameter break points nearest to whole number (mm)		
		S	I	R
Gentamycin	10	≥15	13-14	≤12
Tetracycline	30	≥19	15-18	≤14
Oxacillin	1	≥13	11-12	≤10
Cephazolin	30	≥18	15-17	≤14
Sulphadiazine and trimetoprim	25	≥16	11-15	≤10
Ciprofloxacin	5	≥21	16-20	≤15
Erythromycin	15	≥23	14-22	≤13
Cefoxitin	30	≥25	-	≤24
Clindamycin	2	≥21	15-20	≤14
Chloramphenicol	30	≥18	13-17	≤12
Antibiotics	Antimicrobial susceptibility (%)	Intermediate susceptibility (%)	Antimicrobial resistance (%)	
Gentamycin	21/39 (53.8)	0/39 (0.0)	18/39 (46.1)	
Tetracycline	6/39 (15.3)	8/39 (20.5)	25/39 (64.1)	
Oxacillin	3/39 (7.6)	5/39 (12.8)	31/39 (79.4)	
Cephazolin	10/39 (25.6)	3/39 (7.6)	26/39 (66.6)	
Sulphadiazine and trimetoprim	19/39 (48.7)	7/39 (17.9)	13/39 (33.3)	
Ciprofloxacin	10/39 (25.6)	5/39 (12.8)	24/39 (61.5)	
Erythromycin	30/39 (76.9)	0/39 (0.00)	9/39 (23.0)	
Cefoxitin	0/39 (0.0)	-	39/39 (100)	
Clindamycin	25/39 (64.1)	5/39 (12.8)	9/39 (23.0)	
Chloramphenicol	11/39 (28.2)	4/39 (10.2)	24/39 (61.5)	

Table 5: Multiple drug resistance patterns of MRSA and MRCoNS isolated from fish, fish handlers, domestic animals scavenging and utensils used in fish processing in Maiduguri, Nigeria

Resistance pattern	MRSA (%)	MRCoNS (%)
≥4 antibiotics	19 (50)	22 (56.4)
3 antibiotics	5 (13.2)	8 (20.5)
2 antibiotics	6 (15.8)	4 (10.2)
1 antibiotic	8 (21.1)	4 (10.2)
No resistance	0 (0.00)	1 (2.5)
Total	38 (100)	39 (100)

Table 5 presents the multidrug resistance pattern of MRSA and MRCoNS isolates tested against the

antibiotics. Multi drug resistance is defined as isolate resistant to ≥4 antibiotics (Oteo *et al.*, 2005). Multi drug resistance to ≥4 was observed in 19 (50%) of MRSA isolates and 22 (56.4%) of MRCoNS isolates.

Table 6 is the response of fish handlers to questions in the sample collection forms.

The male: female ratio of the fish handlers was uneven (27, 90% = males). Majority of the fish handlers' age was between 21-30 years. Eighteen (60%) of the fish handlers perform only evisceration while 12 (40%) perform both selling and evisceration of fish.

Table 6: Response to questionnaire survey for fish handlers in Maiduguri fish markets, Nigeria

Survey questions	Respondent (%)
Gender	
Male	27 (90)
Female	3 (10)
Age	
<20	7 (23.3)
21-30	15 (50)
>31	8 (26.6)
Job title/type of work in the fish market	
Fish seller/processor	12 (40)
Fish processor	18 (60)
How long have you been working in this fish market?	
<5 years	9 (30)
6-10 years	17 (56.6)
>10 years	4 (13.3)
Time spent in fish processing per day	
<5 h	11 (36.6)
6-10 h	19 (63.3)
Do you wash your hands before and/or after fish processing?	
Yes	15 (50)
No	9 (30)
How often?	
Once	3 (20)
Twice	6 (40)
>thrice	4 (26.6)
Always	2 (13.3)
Do you use any hand sanitizer?	
Yes	2 (13.3)
No	13 (86.6)
Do you have any pet in the fish market?	
Yes	19 (63.3)
No	11 (36.6)
In 6 months, do you have direct contact with any of the animals listed below?	
Chicken	9 (30)
Cattle	0
Horse	0
Goat	5 (16.6)
Dog	7 (23.3)
Cat	4 (13.3)
None	5 (16.6)
In 6 months, do you experience any skin infection?	
Yes	11 (36.6)
No	19 (63.3)
If yes, do you use un-prescribe antibiotic?	
Yes	8 (26.6)
No	3 (10)

Seventeen (56.6%) worked in the fish market for around 6-10 years. For personnel hygiene such as hand washing, 15 (50%) washed their hands before and/or after fish handling while only 2 (13.3%) uses hand sanitizer during hand washing. Nineteen (63.3%) fish handlers kept pets in the fish markets and some of the fish handlers have direct contact with domestic/pet animals in the last six months. Eleven (11, 36.6%) fish handlers experienced skin disease in the last six month prior to the study and 8 (26.6%) used un-prescribed antibiotic to treat the skin infection.

DISCUSSION

The result of this study revealed the presence of *S. aureus* and MRSA in fish and fish handlers which was similar to the findings of Mohammed *et al.* (2009) in a study of wild catfish in Maiduguri, Nigeria. MRSA

has been isolated in fish sampled in Korea (Rhee and Woo, 2010) and Malaysia (Atyah *et al.*, 2010). The source of contamination of fish may be from the source of water through contamination of rivers with waste water (Tolba *et al.*, 2007; Porrero *et al.*, 2014) or poor hygienic nature and sanitary practices of the fish handlers (Emikpe *et al.*, 2011). Fish from different water bodies are contaminated with microorganisms such as *Escherichia coli*, Proteus, Klebsiella, Streptococcus species and particularly *Staphylococcus aureus* (Nwabueze, 2011). Foods such as meats and fish can be contaminated with *S. aureus* by apparently healthy individuals (Bockserman, 2000; Hammad *et al.*, 2012) as the normal flora of fresh fish or shell fish is predominantly gram negative rods (Nair, 2000; Nooruddin *et al.*, 2013). Food handlers have been incriminated with MRSA colonization most exclusively from the nose and hand (Shimamura and Murata, 2008; Kasturwar and Shafee, 2011). The main reservoir of MRSA in humans is the nose while contamination of processed product occurs through skin contact (Lee, 2003; Asoh *et al.*, 2005) or aerosolization (Kucers and Bennett, 1987). Most of the MRSA were recorded in the skin surfaces of fresh tilapia fish 2 (6.6%) and the hand of fish handlers 7 (23.3). Therefore, the presence of MRSA on surfaces of fish and fish handlers suggests environmental contamination and possible transmission from fish to the handlers or vice versa (Shimamura and Murata, 2008; Vanderlinde *et al.*, 1999; de boer *et al.*, 2009). This increases the chances of colonization/infection of fish handlers which poses health hazard to the workers. According to Sugimoto *et al.* (2013), MRSA has been incriminated in a patient with foot infection after a cosmetic procedure known as fish pedicure showing increased risk of human transmission of MRSA from fresh water fish (Gurra rufa). In addition, fish handlers sampled in this study were implicated as nasal carriers and transmission to fish may occur transiently through colonization of the hand (Wertheim *et al.*, 2005) or indirectly through contamination of environmental surfaces (Mulligan *et al.*, 1993). Several studies have investigated the prevalence of MRSA in occupationally exposed people such as veterinarians, farmers and meat handlers with varying results (Loeffler *et al.*, 2005; Voss and Doebbeling, 2005; Nnachi *et al.*, 2014).

Environmental surfaces such as water reservoirs 4 (8.0%) and knives 2 (3.6%) were contaminated with MRSA in this study. This result is in agreement with the findings of Nnachi *et al.* (2014) who isolated MRSA from tables and knives of butchers which explains the reason why butchers and fish handlers may be colonized with MRSA. Several environmental surfaces have been implicated in the spread of resistant strains of *S. aureus* (Gaze *et al.*, 2008). A study conducted on isolation of MRSA from mobile phones of fish handlers found detectable MRSA at a level of 60% (Roy *et al.*, 2013).

The current study detected MRSA in pet animals such as dogs, cats, chickens and ducks kept in the fish market by the fish handlers. In dogs, 3 (11.1%), 6 (22.2%) and 3 (11.1%) of MRSA were isolated from the skin, nostril and perineum, respectively. The presence of MRSA in dogs has been reported in previous studies (Pak *et al.*, 1999; Leonard *et al.*, 2006; Aklilu *et al.*, 2010). All samples collected from chickens and ducks in the study were MRSA positive while no MRSA was isolated from cat samples. Although livestock were incriminated in contamination of meat and meat products in a slaughter house (EFSA (European Food Safety Authority), 2009), companion animals like dogs and cats kept by meat handlers may serve as source of MRSA contamination of the environment. MRSA have been isolated in cats in several studies (Cefai *et al.*, 1994) and serve as reservoir for human infection (Sing *et al.*, 2008), however no MRSA was detected in this study except MRCoNS 3 (100%). The presence of MRCoNS in cat may be due to the fact that most common Staphylococcal infection is a combination of *S. intermedius* and *S. felis* (Patel *et al.*, 1999).

Therefore professionals with frequent animal contact particularly >4 h/day are at greater risk of MRSA colonization (Paterson *et al.*, 2012). As part of personnel hygiene, 50% of the fish handlers washed their hands three times before and after fish processing. Apparently, this practice may be associated to the stinking foul-smelling odour of fresh fish rather than for protective purpose as only 2 (13.3%) out of the 15 fish handlers washed their hands with a hand sanitizer. Nineteen (63.3%) of the fish handlers keep pets in the fish markets. Several studies revealed the presence of similar clones of MRSA in animals and humans exposed to animals (Loeffler *et al.*, 2005; Voss and Doebbeling, 2005). Major sources of MRSA in abattoirs and food processing units are the animals coming into the abattoir (Gilbert *et al.*, 2012) thereby contaminating the environment through contact with processing equipment or workers (Soonthornchaikul *et al.*, 2006). Eleven (36.6%) of the fish handlers experienced skin infection in the last 6 months prior to the study, while 8 (26.6%) of them used unprescribed antibiotics to treat the infection. Majority of the signs associated with MRSA infection in humans is skin and soft tissue lesions (Umaru *et al.*, 2011). In addition, indiscriminate use of antibiotics increases chances of infection and spread of MRSA (Anupurba *et al.*, 2003; Arora *et al.*, 2010).

The antibiogram of MRSA isolated in the study indicates resistance to oxacillin (76.3%), gentamycin (89.5%), Ciprofloxacin (94.7%) and cefoxitin (100%). MRSA (68.4%) and MRCoNS (64.1%) isolates showed resistance to tetracycline. This is in conformity to the studies of Gelfand and Cleveland (2004) and Chah *et al.* (2014) who found 90% of MRSA and 81.3% of

MRCoNS resistant to tetracycline, respectively. High resistance of the isolates to tetracycline may be connected to with its broad spectrum of activity and being the most prescribed drug in many countries (Moodley *et al.*, 2011). Such profiles of antibiotic resistance occur most frequently in MRSA isolates from Nigeria (Olowe *et al.*, 2007; Maureen *et al.*, 2014). The overall resistance of these isolates to the antibiotics may have arisen as a consequence of antibiotic usage in aquaculture, terrestrial contamination of water bodies or contamination from fish processing units (Smith *et al.*, 2013). The findings of this study revealed sulphonamide and trimetropin, erythromycin and chloramphenicol as possible drugs of choice for the treatment of infection due to MRSA in the study area. All MRSA and MRCoNS isolates tested against cefoxitin showed 100% resistance while remarkable resistance was observed in isolates tested against oxacillin. Antimicrobial susceptibility testing using cefoxitin disc is the "gold standard" for phenotypic detection of MRSA (Velasco *et al.*, 2005) and low level oxacillin resistant MRSA (Witte *et al.*, 2007). Cefoxitin test is the preferred phenotypic method for testing *mecA* resistant gene (CLSI, 2010).

Furthermore, the result of this study revealed a high level of multidrug resistance in MRSA and MRCoNS, a finding which is in agreement with the report of Albuquerque *et al.*, (2007) who found increase emergence of antibiotic resistant *S. aureus* isolated from fish and fish handlers. Therefore, fish can serve as reservoir of resistant microorganisms in aquatic environment that can be a potential risk to human health. Although there is limited data on antimicrobial resistance of MRSA from fish and fish handlers, the world Organization for animal health (OIE), aquatic animal health code recommends the continuous monitoring and surveillance of antimicrobial resistance in microorganism associated with aquatic animals (Smith *et al.*, 2013). This is important due to the fact that transfer of resistant bacteria between aquatic animals and humans through consumption or handling has been documented and can pose a serious hazard to human health (Wegener, 2012).

CONCLUSION

Although the study was not designed to assess the transmission pattern of MRSA between fish and the fish handlers, a reasonable isolation rate of MRSA has been detected from both the fish and fish handlers. Therefore, it is clear that MRSA and MRCoNS do exist in aquatic animals and not only a risk to the fish but also the fish handlers. The presence of these isolates in fish poses public health hazard for consumers and fish handlers. Fish handlers should be properly educated on personal hygiene, ways of handling/processing fish as well as discouraging them from keeping animals in the fish markets.

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