

Multi-Drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria

¹Hafsat Ali Grema, ¹Yaqub Ahmed Geidam, ²Abubakar Suleiman, ¹Isa Adamu Gulani and
¹Roy Bitrus Birma

¹Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri,
P.M.B. 1069, Maiduguri, Borno State

²Department of Veterinary Microbiology, Faculty of Veterinary Medicine,
Ahmadu Bello University, Zaria, Nigeria

Abstract: Multi-drug resistant bacteria were isolated from fresh fish and fish handlers using conventional methods of bacterial isolation such as colonial morphology, gram staining and biochemical tests. The bacteria isolated include *Staphylococcus aureus*, *Streptococcus sp*, *E. coli*, *Klebsiella sp*, *Proteus sp*. and *Brucella sp*. These bacterial isolates were subjected to antibiotic susceptibility testing using disc diffusion technique against ten antimicrobial agents. *S. aureus* isolates showed resistance to gentamycin, tetracycline, oxacillin, ciprofloxacin and ceftiofur while *Streptococcus sp* were resistant to tetracycline, chloramphenicol and clindamycin. All the bacterial isolates were resistant to tetracycline while susceptible to ceftiofur, cephalosporin, erythromycin and clindamycin. The multi drug resistance pattern of *Staphylococcus aureus* isolates showed resistance to three and more antimicrobial agents while none was resistant to 10 antimicrobial agents. All other isolates were resistant to four and more different antimicrobial agents while no isolates was resistant to one and ten antimicrobial agents. Therefore the continuous monitoring and surveillance of multi-drug resistant bacteria in fish and fish handlers will not only reduce the risk of disease to the fishes but public health hazard to fish handlers and consumers in general.

Keywords: Bacteria, fish, fish handlers, Maiduguri, multi-drug resistance, Nigeria

INTRODUCTION

The microbial flora of freshly captured fish is usually a reflection of the environment in which it was harvested. Generally, ponds and rivers that harbor fish may be the source of the microorganisms due to indiscriminate dumping of human and animal excreta as well as other environmental wastes into natural water bodies or washing of excreta from land into water during the rainy season (Cabral, 2010). Free roaming animals especially dogs and birds contribute to fecal contamination of surface water and ponds (Green *et al.*, 2012, Mauffret *et al.*, 2012). These microorganisms from feces or environmental waste contain antibiotic resistant gene that may disseminate and contaminate aquatic environment. On the other hand, aquaculture represents one means that supplement wild fish due to the growing demand of fish protein in the population (Alday *et al.*, 2006). Therefore, fish farmers normally add antibiotics into feed as growth promoters, for therapeutic and prophylactic purposes to supplement this increasing demand (FAO, 2005).

Antibiotics used for animals either for therapy, prophylactic or growth promotion purposes at a sub therapeutic dose can result in transfer of resistant genes

from animals to humans and thereby establishing a reservoir of resistant microbes (Angulo *et al.*, 2004; Maripandi and Al-Salamah, 2010). Subsequently, fish contamination with antibiotic-resistant bacteria can be a major threat to public health, as it can be transferred to other bacteria of human clinical significance (O'Brien, 2002). The choices of antibiotic for the treatment of common infectious diseases in humans are becoming increasingly limited, expensive and ineffective due to the emergence of antibiotic resistant bacteria (Weese *et al.*, 2011).

Monitoring and surveillance of antibiotic resistant bacteria in animals intended for human consumption is important for the regulation of resistance both in animals and man as well as to detect trends and changes of the resistance pattern (WHO, 2001). The world health organization on animal health (OIE) recommended the continuous monitoring and surveillance of resistant microorganisms in aquatic animals (Smith *et al.*, 2013) thereby monitoring the trend and level of resistance in the aquatic environment. The multi drug resistance survey in food animals will help to develop guidelines for the prudent use of antimicrobials (Geidam *et al.*, 2012). In addition, many countries have records of bacterial resistance rate from

animals and their products, the public health impact of the isolates and the antimicrobial susceptibility testing of the isolates in an outbreak (CDC, 2012).

Therefore, the present study was aimed at determining the isolation rate, antimicrobial susceptibility test and multi-drug resistance pattern of bacteria isolated from fish and fish handlers 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°N and 13°N and longitude 12°E and 15°E. 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.

Sampling: Samples were collected using sterile moist cotton swab from the surface of the fish skin, viscera and over the gills during evisceration. 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 of fish handlers 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.

Isolation and identification of bacterial isolates: The examination was conducted to isolate, identify and confirm bacterial isolates. Conventional methods of bacterial isolation such as growth on selective media, gram staining and biochemical tests were used for the identification of the different bacterial isolates. One set

of the swab stick was soaked in nutrient broth and incubated aerobically at 37°C for 24 h for the growth of microorganisms. Each sample of the media growth was streaked on the nutrient agar plate and MacConkey agar plate for growing of non-fastidious organisms and to differentiate between lactose fermenters and non lactose fermenters, respectively. Then the streaked nutrient agar and MacConkey agar plates were incubated at 37°C for 24 h for the growth of microorganisms. The isolates were identified and confirmed based on colony and cell morphology, gram staining, positive catalase and coagulase tests and formation of yellow colonies on Mannitol salt agar for *S. aureus*. *E. coli* isolates were identified using colony and cell morphology, pinkish colonies on MacConkey agar, gram negative staining, indole and methyl red positive, Voges-Proskauer and citrate negative test. Streptococci were identified as gram positive, non-motile cocci, catalase and oxidase negative that showed no hemolysis on blood agar.

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 (Oxoid): gentamycin (CN) 10 µg, tetracyclin (TE) 30 µg, oxacillin (OX) 1 µg, cephalosporin (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

A total of 33 *S. aureus*, 26 *Streptococcus* sp, 26 *E. coli*, 12 *Klebsiella* sp. 35 *Proteus* sp. and 33 *Brucella* sp. were isolated from the fish (Table 1). According to sampling site in the fish, 12 (36.3%) *S. aureus* and 16 (45.7%) *Proteus* were isolated from the gills. For samples from the skin, 15 (45.4%) and 13 (39.3%) of *S. aureus* and *Brucella* sp were recovered. Highest number of *E. coli* 13 (50%) were isolated from the visceral samples while 16 (48.4%) of *Brucella* sp. were isolated (Table 2).

Table 3 present the distribution of bacteria isolated from the hand and nostril of fish handlers. Out of the 60

Table 1: Occurrence of bacteria isolated from three different fish types sampled in Maiduguri, Nigeria

Type of fish	Bacterial isolates (%)					
	<i>S. aureus</i>	<i>Streptococcus</i> sp.	<i>E coli</i>	<i>Klebsiella</i> sp.	<i>Proteus</i> sp.	<i>Brucella</i> sp.
Tilapia (n = 90)	12 (36.3)	9 (34.6)	4 (15.3)	6 (50.0)	16 (45.7)	4 (12.1)
Catfish (n = 98)	15 (45.4)	7 (26.9)	9 (34.6)	4 (33.3)	12 (34.2)	13 (39.3)
Carpfish (n = 75)	6 (18.1)	10 (38.4)	13 (50.0)	2 (16.6)	7 (20.0)	16 (48.4)
Total (n = 263)	33 (12.5)	26 (9.8)	26 (9.8)	12 (4.5)	35 (13.3)	33(12.5)

Table 2: Rate of bacterial isolates associated to gills, skin and viscera of fish sampled from Maiduguri, Nigeria

Bacterial isolates	Site of Occurrence (%)		
	Gills	Skin	Viscera
<i>S. aureus</i> (n = 33)	12 (36.3)	15 (45.4)	6 (18.1)
<i>Streptococcus</i> sp. (n = 26)	9 (34.6)	7 (26.9)	10 (38.4)
<i>E. coli</i> (n = 26)	4 (15.3)	9 (34.6)	13 (50.0)
<i>Klebsiella</i> sp. (n = 12)	6 (50.0)	4 (33.3)	2 (16.6)
<i>Proteus</i> sp. (n = 35)	16 (45.7)	12 (34.2)	7 (20.0)
<i>Brucella</i> sp. (n = 33)	4 (12.1)	13 (39.3)	16 (48.4)

Table 3: Distribution of bacterial isolates from fish handlers sampled in Maiduguri, Nigeria

Fish handlers/sites	Bacterial isolates (%)					
	<i>S. aureus</i>	<i>Streptococcus</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Proteus</i>	<i>Brucella</i>
Hand (n = 30)	16 (80.0)	9 (90.0)	7 (100)	11 (100)	15 (88.2)	4 (100)
Nostril (n = 30)	4 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	2 (11.7)	0 (0.0)
Total (n = 60)	20 (33.3)	10 (16.6)	7 (11.7)	11 (18.3)	17 (28.3)	4 (6.7)

Table 4: Antibiotic susceptibility pattern of bacteria isolated from fish and fish handlers in Maiduguri, Nigeria

Bacterial isolates	No. of antibiotic resistance isolates (%)									
	CN	TE	OX	KZ	C	SXT	CIP	E	FOX	DA
<i>S. aureus</i> (n = 53)	34 (64.1)	29 (54.7)	29 (54.7)	8 (15.0)	11 (20.7)	7 (13.2)	22 (41.5)	4 (7.5)	41 (77.4)	9 (16.9)
<i>Streptococcus</i> (n = 36)	NA	18 (50.0)	NA	NA	21 (58.3)	12 (33.3)	NA	5 (13.8)	NA	15 (41.7)
<i>E. coli</i> (n = 33)	11 (33.3)	14 (42.2)	NA	NA	5 (15.1)	2 (6.1)	3 (9.1)	NA	0	NA
<i>Klebsiella</i> sp (n = 23)*	NA	13 (56.5)	2 (8.6)	0	4 (17.3)	0	1 (4.3)	3 (13.0)	0	7 (30.4)
<i>Proteus</i> sp (n = 42)*	16 (38.1)	13 (30.9)	4 (9.5)	1 (2.3)	3 (7.1)	4 (9.5)	9 (21.4)	1 (2.3)	0	0
<i>Brucella</i> sp (n = 37)*	2 (5.4)	17 (45.9)	4 (10.8)	8 (21.6)	0	5 (13.5)	3 (8.1)	13 (35.1)	0	0

NA: Not Applicable in CLSI (2010); *: Interpretation based on presence or absence of zone of inhibition

Table 5: Multidrug Resistance pattern of bacteria isolated from fish and fish handlers in Maiduguri, Nigeria

Bacterial isolates	Resistance of bacterial isolates to no. antibiotics (%)									
	1	2	3	4	5	6	7	8	9	10
<i>S. aureus</i> (n = 53)	0 (0)	1 (1.8)	37 (69.8)	10 (18.8)	4 (7.5)	0 (0)	4 (7.5)	16 (30.1)	2 (3.7)	0 (0)
<i>Streptococcus</i> (n = 36)	0 (0)	3 (8.3)	0 (0)	6 (16.7)	11 (30.5)	2 (5.5)	0 (0)	0 (0)	0 (0)	0 (0)
<i>E. coli</i> (n = 33)	0 (0)	0 (0)	6 (18.1)	25 (75.7)	0 (0)	14 (42.2)	7 (21.2)	5 (15.1)	13 (39.3)	0 (0)
<i>Klebsiella</i> sp (n = 23)	0 (0)	0 (0)	0 (0)	9 (39.1)	3 (13.0)	1 (4.3)	0 (0)	0 (0)	2 (8.6)	0 (0)
<i>Proteus</i> sp (n = 42)	0 (0)	2 (4.7)	2 (4.7)	3 (7.1)	10 (23.8)	1 (2.3)	6 (14.2)	9 (21.4)	0 (0)	0 (0)
<i>Brucella</i> sp (n = 37)	0 (0)	3 (8.1)	0 (0)	8 (21.6)	4 (10.8)	0 (0)	3 (8.1)	4 (10.8)	0 (0)	0 (0)

samples collected, 20 isolates of *S. aureus* were detected from the nostril and hands of fish handlers while 100% of *E. coli*, *Klebsiella* and *Brucella* sps, were recovered from the hand of fish handlers only whereas no isolate of *E. coli*, *Klebsiella* or *Brucella* sp. was recovered from the nostril of the fish handlers.

The result of the antibiotic sensitivity tests for the bacterial isolates is presented in Table 4. *S. aureus* isolates showed resistance to gentamycin, tetracycline, oxacillin, ciprofloxacin and ceftiofloxacin while *Streptococcus* sp. was resistant to tetracycline, chloramphenicol and clindamycin. Forty two percent of *E. coli* and 50% of *Streptococcus* sp. isolates were resistant to tetracycline while on the other hand 33.3% of the isolates were resistant to gentamycin. Due to the absence of breakpoint for zone of inhibition for *Klebsiella*, *proteus* and *Brucella* sp. isolates in CLSI (2010), isolates with no zone of inhibition were considered resistant. All the bacterial isolates in this study showed a moderate resistance (42-56%) to tetracycline while susceptible to ceftiofloxacin and clindamycin.

Table 5 presents the multi drug resistance pattern of the bacterial isolates to a number of antibiotics used in the study. Highest numbers of *Staphylococcus aureus* isolates (69.8%) were resistant to 3 antibiotic agents while none of the isolates was resistant to 10 antibiotic agents. For *E. coli*, highest numbers of isolates (75.7%) were resistant to 4 and more different antibiotic agents while no isolates was resistant to 1, 2 and 10 antibiotic agents. For *Klebsiella*, *Proteus* and *Brucella* sp., most isolates were resistant to 4, 5, 7 and 8 antibiotic agents. All the bacterial isolates showed no resistance to 1 and 10 antibiotics.

DISCUSSION

The microbial isolates identified in this study include *S. aureus*, *E. coli*, *Streptococcus*, *Klebsiella*, *Proteus* and *Brucella* sps. *S. aureus*, *Streptococcus* sp. and *E. coli* appeared to be the most prevalent bacterial species isolated. *S. aureus* is known to be easily carried on skin, skin glands and mucus membrane of human and animals as normal microflora (Moosavi and Lofti,

2009) causing infections such as rashes, inflammations of bones and the meninges as well as septicaemia (Aklilu *et al.*, 2010). *E. coli*, the predominant species of the faecal coliforms, has been found in the intestinal tract of fish (Del Rio-Rodriguez *et al.*, 1997; Dang and Dalsgaard, 2012), on the gills and the skin (Akinyemi and Buoro, 2011). Presence of *E. coli* in food indicates the possible cause of many gastro-intestinal diseases (Nataro and Kaper, 1998) and may constitute potential danger of antibiotic resistance transfer from aquatic bacteria to human. Microorganisms from human origin such as *E. coli*, *S. aureus* and *Klebsiella* have been found to survive and multiply in the gut and tissues of fish which render fish a potential source of human disease over long periods (Udeze *et al.*, 2012). All these pathogens have been identified to be present in the skin, gills and viscera of fish that were sampled in this study. The study showed that the skin and gills harbored more microorganisms than the viscera, confirming the observation of Olojo *et al.* (2010). The difference in the dominating microflora between sites of colonisation has been reported by previous investigation (Olojo *et al.*, 2010). *Bacillus* was present in virtually all fish samples tested in the study which is in agreement with the findings of Shinkafi and Ukwaja (2010). The organism is considered medically significant particularly *B. anthracis* which causes anthrax and *B. coagulase* which causes food spoilage. Diseases caused by *Bacillus* organism include self limited food poisoning, deep seated soft tissue infections and systemic infections (Tena *et al.*, 2007). *Klebsiella sp* is a gram negative non motile, facultative, anerobic rod that causes a wide range of community associated diseases. The classical clinical presentation ranges from localized abscesses to pneumonia and more generalized infections (Botelho-Nevers *et al.*, 2007; Elemam *et al.*, 2009; Seki *et al.*, 2013). While for *Proteus sp.*, urinary tract infections, endocarditis and septicemias has been reported (Endimiani *et al.*, 2005; Cohen-Nahum *et al.*, 2010; Kalra *et al.*, 2011).

The presence of *S. aureus*, *Streptococcus*, *E. coli*, *Klebsiella*, *Proteus* and *Brucella* in fish may result from indiscriminate deposition of human and animal excreta as well as other environmental wastes into ponds and rivers that harbor fish or through washing of land surfaces into water bodies during the rainy season (Cabral, 2010). Free roaming animals especially dogs and birds contribute to fecal contamination of surface water and ponds (Green *et al.*, 2012, Mauffret *et al.*, 2012). These microorganisms in water may carry gene of multi drug resistance that are transferable between human, animals and the environment (da costa *et al.*, 2013).

Multi drug resistance to strains is defined as being resistant to four or more antimicrobial agents (Oteo *et al.*, 2005) but sometimes as low as two antibiotics from different classes (Gibbs *et al.*, 2006). The result of this study revealed the presence of multidrug resistant

bacteria from fishes and fish handlers. All isolates showed high resistance to tetracycline. The result of this study recommended cephalosporin, erythromycin and clindamycin as good choice antibiotics for treatment of infection in the study area. Also all *E. coli* isolated in this study were found to be resistant to 4 or more antibacterial agents tested, a finding which is supported by earlier reports of Overdevest *et al.* (2011) that antibiotic resistance in Enterobacteriaceae has increased dramatically during the past decade. In addition, these results provide evidence that there is an increased emergence of antibiotic resistance from bacterial isolates of fish and fish handlers, a finding which is in agreement with the reports of Albuquerque *et al.* (2007) who found increasing emergence of antibiotic resistance in bacterial isolates originating from fish and fish handlers.

Although the use of antibiotics in human medicine has influenced the emergence of resistant bacteria, the use of antibiotic in animals has contributed to the problem of resistance and complicates the choice of treatment in human diseases (Novotny *et al.*, 2004). Especially the fact that transfer of resistant bacteria between aquatic animals and humans through food production line has been documented and can pose a threat to public health (Grema *et al.*, 2015). Although there is records of antimicrobial resistance 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 (da costa *et al.*, 2013). The findings in this study emphasize the importance of studying multiple genera of bacteria from different animals as sources of human exposure to antibiotic resistance strains (Geidam *et al.*, 2012). Therefore presence of multiple bacteria from fish and fish handlers poses not only risk of disease to the fishes but public health hazard to fish handlers and consumers in general.

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