Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria

[1]Adebayo-Tayo AC, [2]Odu NN, [3]Michael MU, [2]Okonko IO

[1]Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria
[2]Department of Microbiology, University of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria;
[3]Department of Microbiology, Faculty of Science, University of Uyo, Uyo, Akwa-Ibom State, Nigeria
mac2finney@yahoo.com;iheanyi.okonko@uniport.edu.ng; Tel.: +2348035380891

Abstract : This study reports on the incidence of multi-drug resistant (MDR) organisms isolated from sea foods in Uyo, Akwa-Ibom State, South-Southern Region of Nigeria. Studies were carried out microbiologically to isolate and identify MDR-organisms associated with different sea foods using standard methods. The microorganisms were subjected to disc diffusion techniques to test for their sensitivity to eleven different antibiotics. The microorganisms isolated from these sea foods were identified as *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli*, *Shigella* spp, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella* spp. It showed that *Staphylococcus aureus* was the most predominant organisms isolated from seafood samples with a prevalence value of 23.3%. This was followed by *Listeria monocytogenes* and *Shigella* spp. (16.7%), *Aeromonas hydrophila* (13.3%), *Escherichia coli*, *Salmonella* spp., and *Yersinia enterocolitica* (10.0%). The microbial load ranging from 1.9 x 10^6 to 3.6 x 10^6 Cfu/g for bacteria count and 2.0 x 10^6 to 5.7 x 10^6 Cfu/g for coliform count. Most of the isolates were highly susceptible to augmentin and ofloxicin (100.0%) and resistant to chloramphenicol and gentamicin (66.7%). Amoxicillin was found to be resisted by both gram positive bacteria (GPB) and gram negative bacteria (GNB). This study has shown that sea foods samples were grossly contaminated by multi-drug resistant organisms and thus constitute potential hazard to the public. Augmentin and Ofloxicin were drugs of choice for the treatment of any diseases caused by the organisms however; antibiotics sensitivity testing should always be carried out to determine the most effective antibiotics when infection by these isolates occurs. The occurrence of these MDR organisms could lead to epidemic if these sea foods are consumed. There is therefore a need for proper and adequate cooking of sea foods prior consumption.

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1. Introduction

Sea foods have traditionally being a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein. Today, even more people are turning to fish as a healthy alternative to real meat. The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids food in fatty pelagic fish species are extremely important aspect for health conscious people particularly in affluent countries where cardiovascular disease mortality is high. However, consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics (Brier, 1992). The term shellfish covers the bivalve molluscan shellfish (oysters, cockles, clams and mussels), the gastropods (periwinkles, sea snails) and the crustacean shellfish (crabs, lobsters, and shrimps). Seafood differs from other types of foods in a number of ways. Most seafood is still extracted from a wide population, and the fishermen are hunters with no influence on handling of their prey before it is caught. Thus, it is not possible to irritate the situation for slaughter animals, selecting only the most suitable specimen for slaughter and to rest and feed them well before killing (Hall, 1991).

When frozen sea frozen seafood product are consumed raw, there is the likelihood of endangering the health of the consumer especially when the micro-organism present include pathogenic ones. According to Higgins (2007), anyone who work in food safety sooner or later discover that one of the most valuable tools for prevention is simply reading about and understanding how past outbreak have occurred. Using major and frequently famous or at least newsworthy outbreaks, Phyllis (2007) illustrate critical factors come together to produce tragic and largely preventable results modern microbes often team up with old practice, short sighted decision or current consumer tend to produce an outbreak.

Bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish or shellfish (ICMSF, 1998). The microbiological flora in the intestines of sea foods such as finfish, shellfish and cephalopods is quite different being psychotrophic in nature and to some extent believes to be a reflection of general contamination in the aquatic environment. In filter feeding bivalve molluscan shellfish (oyster) and accumulation and concentration of bacteria and viruses from the environment is generally taking place (Taylor, 1988). However, some sea foods are processed in a modern fish industry which is technologically advanced and complicated industry in line with any other sea foods industry and with the same risk of products being contaminated with pathogenic microorganisms.

It has been estimated that there are more than 80 million cases per annum of sea food borne illnesses on antibiotic resistance in the United States of America and that the cost of these illnesses is the order of many billions of dollars per year (Todd, 1989). The economic losses due to spoilage are rarely quantified but a report by the US National Research Council Committee (FND/NRC) estimated that one-fourth of the world food supply is lost through microbial activity alone (EEC, 1992). Thus, the need for control of quality of our sea foods to avoid high microbial contamination which may lead to antibiotics resistance is well documented and since the rate of seafood borne illnesses is increasing, there is also an urgent means of assuring quality of sea food.

Multi-drug resistant (MDR) gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphycolocci (MRCNS) have been a serious problem in the medical community (Okonko et al., 2009a,b). There has been serious concerns regarding increased prevalence of extended spectrum β-lactamases (ESBLs) in different parts of the world, although exact prevalence in not known (Amin et al., 2009), whereby antibiotic overuse, prescription of drugs with proper sensitivity test and over dosing may have created this problem in developing nations. The *K. pneumoniae* and *E. coli* contribute 10 to 40% of ESBLs (Amin et al., 2009).

The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment (Okonko et al., 2009a,b). In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing infections (Abubakar, 2009). The emergence of antibiotic resistance in the management of most infections are serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. Knowledge of etiological agents of infections and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies. The changing spectrum of microorganisms causing infections and the emerging resistance to many of the older and cheaper antibacterial agents require continuous monitoring (Abubakar, 2009).

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures, and preventing the spread of antimicrobial-resistant microorganisms (Okonko et al., 2009a,b). The worldwide escalation in both community- and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and new treatment alternatives (Chikere et al., 2008; Okonko et al., 2009a,b). In 2001, the World Health Organization (WHO) launched the first global strategy to counter this phenomenon, a key component of which is the development of surveillance programs to monitor trends in antimicrobial drug resistance and use (Hsu et al., 2007; Okonko et al., 2009a,b). Surveillance studies are valuable tool for assessing the changes in pattern of resistance of clinical isolates of antimicrobial agents. Trend towards increased antimicrobial resistance shown by many of the gram negative bacteria is worrying, and has developed as a consequence of widespread and inappropriate use of various agents (Amin et al., 2009). Therefore, this study was carried out to investigate the incidence of antibiotics resistance microorganisms in sea food. The aim of this study was to determine the microbial load of the seafoods' samples, isolate, characterize and identify pathogenic microorganisms associated with sea food and to determine the sensitivity and resistance patterns of the isolate to some antibiotics.

2. Materials and Methods

2.1. Sample collection

Samples of four different sea foods (fishes, crab, crayfish, and periwinkle) were purchased from Itu market in Akwa-Ibom State. The samples were collected in a sterile polythene bags and taken to the Bacteriology Laboratory, at the Department Microbiology, University of Uyo, Uyo, Akwa-Ibom State for microbiological analysis. The parts of the fishes used in this study include gills, intestine and the skin. One gram of each sample was weighed out

and soaked in 9ml of sterile distilled water and a ten fold dilutions of the homogenates were made using sterile pipettes as described by the methods of Oyeleke and Manga (2008).

2.2. Enumeration, Isolation, Characterization and Identification

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. Media used in this study included: Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were MacConkey agar (MCA), Mueller Hinton Agar (MHA), Mannitol Salt Agar (MSA) and Salmonella-Shigella Agar (SSA). All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 min. at 15lb pressure. From the 10-fold dilutions of the homogenates; 0.1ml of 10^{-5} dilution of the homogenate was plated in replicate on different media, using pour plate method as described by the methods of Oyeleke and Manga (2008). The plates were then incubated at 37°C for 24 - 48 h. MacConkey agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of *S. aureus*. Total viable aerobic bacteria count was performed on Nutrient Agar. At end of the incubation periods, colonies were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (Cfu/g). Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored at 4°C. Initially strains were identified based on the morphological behavior of the isolates on various differential media. The species level identification was then carried out by standard biochemical test (Bergey's Manual of Determinative Bacteriology ninth edition) and by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008).

2.3. Antibiotics Susceptibility Testing

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique (Bauer *et al.,* 1996) and 0.5 MacFarland's 10^8/ml employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute (CLSI) (NCCLS, 2002; CLSI, 2006; Okonko et al., 2009a,b). Mueller-Hinton agar (Difco Laboratories, Michigan, USA) is the National Committee for Clinical Laboratory Standards (NCCLS) recommended medium for sensitivity analysis. It is an ideal medium for routine antimicrobial susceptibility tests since it shows good batch-to-batch uniformity and is low in tetracycline and sulfonamide inhibitors (Cheesbrough, 2006). Trypticase soy broth (BBL™ Trypticase™ Soy Broth, BIOTECH) was prepared. Five discrete colonies of the different identified isolates were inoculated into 5 ml of the broths and incubated at 35°C for 4 – 6 h. The inoculum for primary sensitivity testing was prepared from a broth that has been incubated for 4 – 6 h. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method using sterile cotton wool. The antibiotics used include; Augmentin, Amoxycillin, Chloramphenicol, Cloxacillin, Cotrimoxazole, Erythromycin, Gentamicin, and Tetracylcine and were tested against the isolates. The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates (Iroha *et al.,* 2009). The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37°C for 24 h. Interpretation of results was done using the zones of inhibition sizes (Cheesbrough, 2006; Okonko et al., 2009a,b).

3. Results Analysis

Table 1 shows the microbial loads of the seafood samples. It showed a high bacterial count ranging from 1.9 x 10^6 Cfu/ml to 3.6 x 10^6 Cfu/ml and total coliform count of 2.0 x 10^6 Cfu/ml – 5.7 x 10^6 Cfu/ml for the sea food samples.

Table 1: Microbial Loads of Seafood Samples

Samples	Total Bacterial counts	Total Coliform counts
Fish skin	2.1 x 10^6	2.0 x 10^6
Fish gills	3.6 x 10^6	5.7 x 10^6
Fish intestine	3.4 x 10^6	4.5 x 10^6
Crab	Nil	3.0 x 10^6
Crayfish	2.0 x 10^6	2.5 x 10^6
Periwinkle	1.9 x 10^6	2.3 x 10^6

Table 2 shows the frequency of occurrence of different bacterial species from seafood samples. It showed that *Staphylococcus aureus* was the most predominant organisms isolated from seafood samples with a prevalence value of 23.3%. This was followed by *Listeria monocytogenes* and *Shigella spp* with a prevalence value of 16.7% each, *Aeromonas hydrophila* with a value of 13.3%, *Escherichia coli*,

Salmonella spp, and *Yersinia enterocolitica* which occurred in the same frequency with a prevalence value of 10.0% each.

Table 2: Frequency of occurrence of different bacterial species from seafood samples

Isolates	No. (%)	Fish skin	Fish gills	Fish intestine	Crab	Crayfish	Periwinkle
Aeromonas hydrophila	4 (13.3)	4(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Escherichia coli	3(10.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)
Listeria monocytogenes	5(16.7)	0(0.0)	0(0.0)	0(0.0)	5(100.0)	0(0.0)	0(0.0)
Salmonella spp	3(10.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)
Shigella spp	5(16.7)	0(0.0)	0(0.0)	5(100.0)	0(0.0)	0(0.0)	0(0.0)
Staphylococcus aureus	7(23.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(100.0)
Yersinia enterocolitica	3(10.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)
Total	**30(100.0)**	**4(13.3)**	**0(0.0)**	**11(36.7)**	**5(16.7)**	**3(10.0)**	**7(23.3)**

Table 3 shows the antibiotics sensitivity profiles of different bacterial species from seafood samples. It showed that some of the isolates were highly sensitivity to some of the tested antibiotics. The percentage sensitivity of the isolates to the tested antibiotics ranged from 0.0% to 86.7%. Percentage sensitivity of the isolates to the tested antibiotics in decreasing order of magnitude were augmentin (86.7%), gentamicin (73.3%), cloxacillin (60.0%), tetracycline (56.7%), co- Trimoxazole (50.0%), erythromycin and chloramphenicol (46.7%), and amoxicillin (0.0%). From Table 3, it can be deduced that all strains of *E. coli*, *L. monocytogenes*, *Salmonella spp*, and *Shigella spp* were highly sensitive (100.0%) to augmentin and all strains of *E. coli*, *Salmonella spp*, *Shigella spp* and *Y. enterocolitica* were 100.0% sensitive to gentamicin as shown in Table 3. Six (85.7%) strains of *S. aureus* isolates were sensitive to augmentin. Three (75.0%) strains of *Aeromonas hydrophila* were sensitive to augmentin. Only one (33.3%) strain of *E. coli*, *Salmonella spp* and *Y. enterocolitica* was sensitive to chloramphenicol and tetracycline.

Table 3: Antibiotics sensitivity profiles of different bacterial species from seafood samples

Isolates	Tested Antibiotics (%)								
	No. Tested	AUG	GEN	CHL	AMX	TET	ERY	CXO	COT
A. hydrophila	4	3(75.0)	3(75.0)	3(75.0)	0(0.0)	3(75.0)	2(50.0)	2(50.0)	2(50.0)
E. coli	3	3(100.0)	3(100.0)	1(33.3)	0(0.0)	1(33.3)	1(33.3)	2(66.7)	1(33.3)
L. monocytogenes	5	5(100.0)	1(20.0)	2(40.0)	0(0.0)	4(80.0)	4(80.0)	4(80.0)	4(80.0)
Salmonella spp	3	3(100.0)	3(100.0)	1(33.3)	0(0.0)	1(33.3)	2(66.7)	2(66.7)	1(33.3)
Shigella spp	5	5(100.0)	5(100.0	3(60.0)	0(0.0)	4(80.0)	2(40.0)	3(60.0)	2(40.0)
S. aureus	7	6(85.7)	4(57.1)	3(42.9)	0(0.0)	3(42.9)	2(28.6)	3(42.9)	4(57.1)
Y. enterocolitica	3	1(33.3)	3(100.0)	1(33.3)	0(0.0)	1(33.3)	1(33.3)	2(66.7)	1(33.3)
Total (%)	**30**	**26(86.7)**	**22(73.3)**	**14(46.7)**	**0(0.0)**	**17(56.7)**	**14(46.7)**	**18(60.0)**	**15(50.0)**

Keys: AUG= Augmentin, GEN= Gentamicin, CHL = Chloramphenicol, AMX= Amoxicillin, TET= Tetracycline, ERY = Erythromycin, CXO= Cloxacillin, COT= Co-Trimoxazole

Table 4 shows the antibiotics resistance pattern of different bacterial species from seafood samples. It showed that the percentage resistance ranged from 13.3% to 100.0%. Percentage resistance of the isolates to the tested antibiotics in decreasing order of magnitude were amoxicillin (100.0%), erythromycin and chloramphenicol (53.3%), co-Trimoxazole (50.0%), tetracycline (43.3%), cloxacillin (40.0%), gentamicin (26.7%), and augmentin (13.3%). From Table 4, it can be deduced that all strains (100.0%) of the isolates were highly resistant to amoxicillin. None of the strains of *E. coli*, *L. monocytogenes*, *Salmonella spp* and *Shigella spp* isolates were resistant to augmentin and none of the strains of *E. coli*, *Salmonella spp* and *Shigella spp* and *Y. enterocolitica* isolates were resistant to gentamicin. However, 2(66.7%) strains of *E. coli*, *L. monocytogenes* and *Salmonella spp* isolates were resistant to 4 (50.0%) of the 8 tested antibiotics (chloramphenicol, tetracycline, erythromycin and co-Trimoxazole) and only 1 (33.3%) strain of them to cloxacillin. One (33.3%) strains of *E. coli*, *Salmonella spp*, and *Shigella spp* isolates were resistant to gentamicin while 4(80.0%) strains of *L. monocytogenes* isolates were resistant to gentamicin (Table 4).

Two (50.0%) stains of *A. hydrophila* isolates were resistant to 3 (37.5%) of the tested antibiotics (erythromycin; cloxacillin and co-Trimoxazole) and one (25.0%) strain were resistant to the 4 (50.0%) of the antibiotics (augmentin, gentamicin, chloramphenicol, and tetracycline). As for *S. aureus* isolates, 5(71.4%) strains were resistance to erythromycin, 4(57.1%) strains to 3(37.5%) of the 8 tested antibiotics (chloramphenicol, tetracycline and cloxacillin); 3(42.9%) strains to 2(25.0%) antibiotics

(gentamicin and co-Trimoxazole) and only 1 (14.3%) to augmentin. In the same vein, 3(60.0%) strains of *Shigella spp* were resistant to 2(25.0%) of the tested antibiotics (erythromycin and co-Trimoxazole); 2(40.0%) to 2(25.0%) antibiotics (chloramphenicol and cloxacillin) while 1(20.0%) was resistant to tetracycline (Table 4).

The results also showed a multi-drug resistant (MDR) pattern of these isolates to the 8 tested antibiotics in which some of their strains were resistant to 6 - 8 of the tested antibiotics. Some strains of the *A. hydrophila* and *S. aureus* isolates were resistant to all the 8(100.0%) tested antibiotics. Also, some strains of *L. monocytogenes* and *Y. enterocolitica* isolates were resistant to 7(87.5%) of the 8 tested antibiotics while those of *E. coli*, *Salmonella spp* and *Shigella spp* isolates were resistant to 6(75.0%) of the 8 tested antibiotics (Table 4).

Table 4: Antibiotics resistance pattern of different bacterial species from seafood samples

Isolates	No. of isolates tested	Tested Antibiotics (%)								MDR pattern
		AUG	GEN	CHL	AMX	TET	ERY	CXO	COT	No. of antibiotics (%)
A. hydrophila	4	1(25.0)	1(25.0)	1(25.0)	4(100.0	1(25.0)	2(50.0)	2(50.0)	2(50.0)	8(100.0)
E. coli	3	0(0.0)	0(0.0)	2(66.7)	3(100.0)	2(66.7)	2(66.7)	1(33.3)	2(66.7)	6(75.0)
L. monocytogenes	5	0(0.0)	4(80.0)	3(60.0)	5(100.0	1(20.0)	1(20.0)	1(20.0)	1(20.0)	7(87.5)
Salmonella spp	3	0(0.0)	0(0.0)	2(66.7)	3(100.0)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	6(75.0)
Shigella spp	5	0(0.0)	0(0.0)	2(40.0)	5(100.0	1(20.0)	3(60.0)	2(40.0)	3(60.0)	6(75.0)
S. aureus	7	1(14.3)	3(42.9)	4(57.1)	7(100.0	4(57.1)	5(71.4)	4(57.1)	3(42.9)	8(100.0)
Y. enterocolitica	3	2(66.7)	0(0.0)	2(66.7)	3(100.0	2(66.7)	2(66.7)	1(33.3)	2(66.7)	7(87.5)
Total (%)	**30**	**4(13.3)**	**8(26.7)**	**16(53.3)**	**30(100.0)**	**13(43.3)**	**16(53.3)**	**12(40.0)**	**15(50.0)**	

Keys: AUG= Augmentin, GEN= Gentamicin, CHL = Chloramphenicol, AMX= Amoxicillin, TET= Tetracycline, ERY = Erythromycin, CXO= Cloxacillin, COT= Co-Trimoxazole, MDR = Multi-drug resistance

4. Discussion

The study revealed that though seafood are cheap source of protein, they habour a lot of pathogenic microorganisms that constitute health hazard to man. A high microbial load was recorded in this study. The high bacterial count of 3.6 x 10^6 Cfu/g and 5.7 x 10^6 Cfu/g of the seafood samples when compared with the International Commission on Microbiological Specification for Food (ICMSF, 1998) and the United States Food Drugs and Administration (USFDA) suggestions of a microbial count of not greater than 10^5 and coliform count of greater than 10^2 of shellfish for consumer's safety indicated that raw seafoods are not safe for human consumption. This may be due to the retaining ability of the seafoods in that it absorbed organisms from its environments into their soft tissue. The potential of water to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Okonko et al., 2009c). Water-related diseases continue to be one of the major health problems globally. One of the strategies for tackling this problem is the provision of protected sources such as boreholes, standpipes, protected wells and springs. However, such facilities are located some distances requiring transportation to homes (Taulo et al., 2008; Okonko et al., 2009c). During transportation, water gets contaminated with bacteria which grow and proliferate during storage in the homes. This contamination may lessen the health benefits of water source improvements (Okonko et al., 2009c).

The organisms isolated in this study were *Staphylococcus aureus*, *Listeria* monocytogenes, *Shigella spp*, *Aeromonas hydrophila*, *Escherichia coli*, *Salmonella spp*, and *Yersinia enterocolitica*. All these pathogen isolated in this study are of food and public health implication and hence, hazardous and injurious to human health if consumed. The isolation of *Salmonella* spp. and *S. aureus* in this study is of practical impact. It shows that most of the seafood products might have been contaminated from source. It is an evidence of poor sanitary conditions. *E. coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence et al., 2009). Pathogenic bacteria such as *Salmonella spp* and *S. aureus* can lead to outbreak of diseases such as typhoid, food poisoning if the seafoods from the rivers were not properly cooked. The presence of *Salmonella* spp in the seafood samples could also lead to gastroenteritis or salmonella food poisoning. The finding of this study is in accordance with the report of Cipriano (2003). The presence of *Salmonella* sp. was also

reported in a previous study on ready-to-eat seafood by Okonko et al. (2008a,b, c).

The presence of the most frequently isolated index of food and water quality and indicators of faecal contamination such as *E. coli* reported in this study is an indication of faecal contamination of the seafoods products used for this study unhygienic handling of the fishes right from the source or contamination of the fishes itself during harvesting, handling and storage and this might have adverse effect on the health of the consumers (Okonko et al., 2008a,b,c, 2009c,d). The presence of *E. coli* reported in this study is also in agreement with the findings of Adesokan et al. (2005) and Okonko et al. (2009c,d) who reported the presence of *E. coli* among other organisms. The presence of indicator organisms revealed that water was polluted with faecal matter; however, presence of *E. coli* indicates recent faecal pollution. *E. coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence et al., 2009). Members of the gram negative bacteria e.g. *E. coli* are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread (Clarence et al., 2009). Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence et al., 2009). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag et al., 2008).

Antibiotics treatment of bacterial diseases in fish culture has been applied for many years. The antimicrobial sensitivity profiles showed that 73.3% and 86.7% of the isolates were highly sensitive to both gentamicin and augmentin respectively. They also showed varying sensitivity to cloxacillin (60.0%), tetracycline (56.7%), and co- Trimoxazole (50.0%). Sensitivity to erythromycin and chloramphenicol were low with 46.7% percentage sensitivity. Amoxicillin was highly resisted by both gram positive and gram negative bacteria in this study. Varying percentage resistance was also reported in this study with erythromycin and chloramphenicol 53.3%, co- Trimoxazole (50.0%), tetracycline (43.3%), cloxacillin (40.0%). Relatively low resistance was reported for gentamicin (26.7%) and augmentin (13.3%).

Aminoglycosides have good activity against clinically important gram negative bacilli (Ullah et al., 2009). The aminoglycoside antibiotics include gentamcin, kanamycin, amikacin etc. These act by inhibiting bacterial protein synthesis. Among the non-β-lactams, gentamicin showed good activity with 48% isolates found susceptible in a study by Ullah et al. (2009), which is more than 29% recorded in Israel and 36% recorded in India (Colodner et al., 2007). This may be due to increased use of gentamicin in India and Israel as compared to Pakistan. Gentamycin is routinely used synergistically with a beta-lactam antibiotic or vancomycin for empirical therapy in infective endocarditis (Nwadioha et al., 2010). Gentamycin, a relatively cheap and an easily available antibiotic, is effective against the gram-negative bacilli and the grampositive cocci in this study. The 73.3% percentage sensitivity to gentamicin by these isolates in this study is deviation to what was previously reported by Chikere et al. (2008) and Nkang et al. (2009b) who reported 100.0% sensitivities of gram negative isolates. Our finding is also similar to a study done in Calabar claiming 80% effectiveness (Martins et al., 2005) and a study done in Kano claiming 70.7% and 76.7% effectiveness against gram negative bacilli and gram negative cocci respectively (Nwadioha et al., 2010). Chikere et al. (2008) reported the sensitivities of gram negative bacilli to gentamycin to be 100% and gram positive cocci to be 93.3%. This is also comparable to our findings. According to Ullah et al. (2009), pattern of resistance to aminoglycosides is affected by selective pressure in different regions. Ako-Nai et al. (2005) presented a report in which 1.8% of staphylococcal isolates were resistant to gentamycin in a study Ibadan, Nigeria.

However, it can then be deduced that gentamicin and augmentin are the antibiotics of choice for the treatment of any infection caused by these organisms isolated in seafood products. Though contrary to the findings of Okonko et al. (2009a) who reported that *E. coli* from clinical isolates were resistant to the gentamicin. Our present finding is in agreement with previous studies by other investigators. Uzeh *et al.* (2006) and Ajao and Atere (2009) reported that *E. coli* was sensitive to gentamicin. In a similar study by Kebira et al. (2009), 80% of the isolates were susceptible to gentamicin. Adedeji and Abdulkadir (2009) reported the isolates were generally highly susceptible to gentamicin (89%). Okonko et al. (2009a) reported 100% resistance to gentamicin and tetracycline by *E. coli* and *S. aureus* in a similar study.

Resistant to amoxicillin reported in this study is in agreement with previous reports by other investigators in a similar study (Adel-Rauf, 2004). Percentage resistance in the range of 20.0-66.7% reported for Co-Trimoxazole is in contrary to the zero percent resistance to septrin (cotrimoxazole)

reported in Calabar by Nkang et al. (2010). Co-Trimoxazole resistance remained stable, approximately 20.0% to 66.7%, this is in agreement with what was reported by Oteo et al. (2005) and other previous investigators. Okonko et al. (2009a) reported that all the gram negative isolates were resistant to co-Trimoxazole. Aiyegoro et al. (2007) reported that 66.7% of the pathogens were resistance to septrin (co-Trimoxazole) in their study. Also, 53.3% resistance to chloramphenicol was recorded in this study. This compares favourably with previous studies. Strains of *Salmonella species* resistant to chloramphenicol and other recommended antibiotics have been identified in several parts of Latin America, Asia and Africa (Benoit et al., 2003).

Tetracycline is an antibiotic that inhibits bacterial growth. They are bacteristatic and widely used as a broad- spectrum antibiotic with activity against Gram- positive and Gram- negative bacteria. Resistance to tetracycline is common and this is further confirmed from the results obtained in a study by Adedeji and Abdulkadir (2009). Resistance to tetracycline has developed because it is readily available in the country and has been widely misused (Adedeji and Abdulkadir, 2009). Resistance to tetracycline has been reported by Nkang et al. (2009a,b). Abubakar (2009) reported high rate of resistance to tetracycline and gentamicin in their study. Tetracycline-resistant *Salmonella enterica* Serovar Typhimurium and Eenteritidis was reported by Halawani and Shohayeb (2008). Recently, an epidemic multi-drug have emerged, presumably due to the extensive use of resistant strain serovar Typhimurium phage type 104 antimicrobial agents both in humans and animals (Halawani and Shohayeb, 2008). Ako-Nai et al. (2005) presented a report in which 70.2% of staphylococcal isolates were resistant to tetracycline in a study Ibadan, Nigeria. Similar resistance profiles were presented among *S. epidermidis* in some hospitals in Turkey to be resistant to tetracycline (Abubakar, 2009)

One of the species of *Aeromonas* has been reported as the causative agent of furunculosis, one of the destructive bacterial diseases of farmed salmonid fish. As yet, a suitable vaccine against the disease is not available, and control of furunculosis is still dependent upon the use of antibiotics. Currently, conventional therapy for furunculosis consists of oxytetraclycline, potential sulphonamide, or more usually, oxolinic acid; application of oxolinic acid as a chemotherapeutic agent against infectious diseases in fish. However, oxolinic acid therapy in fish farming has been compromised by the development of resistant strains. Recent work has suggested that some of the new fluorinated 4-quinolones, including enrofloxacin which is already licensed for veterinary use may be more effective in the treatment of *Aeromonas* infections in fish than the current regimen of oxolinic acid.

Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Okonko et al., 2009a). Bacteria of the Klebsiella genus may cause numerous infections in human, which are often treated with beta-lactam antibiotics (Amin et al., 2009). The fundamental mechanism of *Klebsiella* resistance to penicillin or cephalosporin involves the production of enzymes called extended spectrum β-lactamases (ESBLs), because of resistance of many *Klebsiella spp.* strains to β-lactamases; alternative antibiotic therapy can make use of aminoglycosides and quinolones (Amin et al., 2009; Okonko et al., 2009a). However, *for S. aureus* an increase of resistance has been reported. The underlying mechanisms seem to be unchanged (Okonko et al., 2009a).

Multi-drug resistance (MDR) pattern was also reported in this study, been resistant to more than 6 of the 8 tested antibiotics. This is comparable to the findings in previous studies by other investigators elsewhere in Nigeria. Nkang et al. (2009a) reported 69.2% resistance to test antibiotics by *Salmonella spp*. Over the past decade, particularly in developing countries, the increase in resistance of animal origin non-typhoid *Salmonellae* to broad-spectrum antibiotics such as cephalosporins, tetracycline, and quinolones has been extremely worrisome (Streit et al., 2003).

The problems of multi-drug resistance (MDR) have been the driving force for the development of newer quinolones (Amin et al., 2009). MDR and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge. Also, the rising prevalence of drug resistance such as penicillin-resistant pneumococci worldwide mandates selective susceptibility testing and epidemiological investigations during outbreaks (Okonko et al., 2008d). In line with the assertions of Abubakar (2009), even though susceptibility pattern shown in this study emphasizes the need for in-vitro sensitivity reports before initiation of antibiotic therapy, it must not be forgotten that in-vitro antimicrobial sensitivity reports serve only as guide and that conditions in-vivo may be quite different. The data presented in this and in previous studies may be of immense value for use to determine trend in antimicrobial sensitivities, to formulate local antibiotic policies to compare local with national and international data and above all, to assist clinicians in the rational choice of antibiotic therapy and to prevent misuse, or over use of antibiotics. The data obtained in this

study shows that the bacteria causing most community and nosocomial infections are still susceptible to antimicrobial agents routinely used in the hospital though this is changing. Although the disc diffusion method was used to assess sensitivity and resistance and can be correlated clinically, further investigations employing the minimum inhibitory concentrations (MIC) method will be needed to obtain more reliable results (Abubakar, 2009).

5. Conclusion

In conclusion, this study has shown that the seafood samples used for this study were contaminated with pathogenic microorganisms and multi-drug resistant organisms. Microbial drug resistance is a wide spread phenomenon which constitutes an ever present health hazard to the successful eradication of infectious diseases and a good number of bacteria possess different mechanism of developing resistance top antimicrobial drug. The dose administered should be adequate to control microbial population without destroying the host's tissues. The quality of our sea foods and the resistance of antibiotics on microorganisms associated with seafoods are of a major concern to man and public health authority. The data presented in this investigation are similar to those obtained in other cities in Nigeria and have shown the changing pattern in the types of organisms causing infections and their resistance to many of the commonly available antibiotics, thus leading to the use of newer and more costly agents (Ako-Nai et al., 2005; Nwanze et al., 2007; Kolawale et al., 2009; Okesola and Oni, 2009; Abubakar, 2009; Okonko et al., 2009a,b; Nkang et al., 2009a,b). The findings of this study helps to bring to knowledge that improper handling of seafoods result in contamination and measures should be taken so as to avoid environmental hazards and contamination. Public enlightenment campaign teams should be recognized to educate people on the measures which antibiotic should be taken, thus measures include avoidance of indiscriminate use and adding antibiotics to the feed of fish should be avoided.

CORRESPONDENCE TO:
Iheanyi O. Okonko
Department of Microbiology,
University of Port Harcourt,
East/West Road, P.M.B 5323, Choba,
Port Harcourt, River State, Nigeria;
E-Mail: mac2finney@yahoo.com;
iheanyi.okonko@uniport.edu.ng;
Tel.: +234-080-3538-0891

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