ANTIBIOTICS SUSCEPTIBILITY PATTERNS OF BACTERIAL SPECIES ISOLATED FROM FRESH SEA FISHES SOLD IN MARKETS IN WARRI, DELTA STATE NIGERIA.

Owhe-Ureghe, U.B.
Department of Microbiology, Delta State University, Abraka

ABSTRACT

Five fresh sea fishes (*Tilapia sp, Chrysichthys nigrodigitatus, Citharinus sp, Tilapia guineensis, and Lates niloticus*) bought from the main market and makava market in Warri Delta State Nigeria were investigated for their bacterial flora using standard conventional plating techniques. The total aerobic viable counts ranges from 7.5×10^6 CFU/mL for *Lates niloticus* to 7.0×10^7 CFU/mL for *Citharinus sp*; while the mean coliform counts range from 3.6×10^5 CFU/mL for *Citharinus sp* to 4.4×10^6 CFU/mL for *Tilapia guineensis*. Eight bacterial species were isolated; these include; *Bacillus cereus, Vibrio cholerae, Vibrio parahaemolyticus, Aeromonas hydrophila, Escherichia coli, Salmonella sp, Pseudomonas aeruginosa* and *Shigella dysenteriae*. The sensitivity profile of the isolates using some antibiotics battery shows that *Vibrio cholerae* and *Aeromonas hydrophila* were sensitive to all the antibiotics, while other bacterial isolates elaborated varying degrees of sensitivity and resistance. The diameter of the zones of inhibition for sensitive isolates range from 16-27 mm, while that for the resistant isolates ranged from 0-13mm. The result also shows that *Pseudomonas aeruginosa* was resistant to 8 out of the 15 antibiotics used in this study, while the remaining bacterial isolates were resistant to 1-4 antibiotics. The occurrence of pathogenic bacterial species with multiple drug resistance in sea fishes called for the proper pre-treatment of human, animal and domestic wastes before being dumped into the sea and fresh water environment, in order to reduce the possible contamination of this essential food resource (fish) with the consequent health implications.

INTRODUCTION

In the world today, there is a reemergence of the consumption of fish due to the new awareness about its low cholesterol, fat and good quality of animal protein. Fish is an essential food item in the diet of many people in West Africa. It provides an average of 35% of the total animal protein intake (FAO, 1978). Among coastal and riverian people, fish consumption is higher and contributes more than 50% of the animal protein in their diet e.g. 70% in Sierra Leone, 80% in Ghana (Halliday, 1986). Nerquaye-Tetteh (1986) reported that much of the fish consumed in West Africa region consist of cheap sea species, such as Sardinella, Bonga, Mackerel, Horse Mackerels, Anchovies and Tilapia.

In Nigeria, the populace depend more on sea fishes as fresh water species are more expensive and are bought by the elites in the society. This is an important feature of the domestic demand for fish in the sub region where income is generally low. In many Nigerian communities, preference for meat type is influence by religious beliefs and taboos, contrary to fish which is generally accepted by all.

Fish as a food is indispensable to the maintenance of good healthy living because of its high protein content; however, it can also be responsible for ill health (Adams and Moss, 1999). There are intrinsic and extrinsic hazards associated with fish consumption, but the hazard of primary concern is that of microbial origin. Hazards of other origin can be controlled during the processing but that of microbial origin is most difficult to control due to poor personal hygienic practices of the fish handlers and ignorance of the sources of contamination. Diseases associated with the consumption of fish and fish products are not commonly reported in Nigeria due to lack of monitoring and non-attendance of patients to government hospitals as a result of lack of fund to pay for hospital bills.

Sea and fresh water fish business in the environs of Warri, Delta State Nigeria is

very

lucrative as many people patronize the sellers. Fish consumption is not affected by religion and is used as a special delicacy in many ceremonies such as marriages, burial and birthday parties. A proportion of the sea fishes and fresh water fishes sold in Warri and its environs are fresh (unfrozen) types in contrast to the frozen sea fishes sold in the hinterland.

Several gram positive (*Clostridium botulinum, Staphylococcus aureus, Bacillus cereus, Streptococcus sp*),and gram negative bacterial species (*Escherichia coli, Shigella sp, Salmonella sp, Pseudomonas aeruginosa, Vibrio sp, Aeromonas, Pasteurella multocida*) have been associated with fishes harvested from fresh water and marine environments (Lewis, 1975; Nair *et al*., 1975; Sarkar *et al*., 1985; Nizan and Hammerschida, 1993; Kori-Siakpere, and Owhe-Ureghe, 2001). Report by FAO fisheries department indicated that most bacterial species isolated as bacterial flora are implicated as aetiologic agents of fish diseases (Sakata and Hattori, 1988) a finding which may favour a concept of fish-borne human zoonotic infections.

It has been suggested that the bacterial floral of fishes is a reflection of the aquatic environment from which they were harvested (Showan and Hobbs, 1967). This has also been corroborated by Kori-Siakpere and Owhe-Ureghe (2001) who reported the bacterial flora of *Channa obscura* from Ilushi river, Edo State Nigeria.

The health implications of the consumption of unwholesome food and water are centered on the contamination of these materials with bacterial species and other microbes, (Adams and Moss, 1999). In the developed and developing countries, the consequences of food borne illness, like diarrhoea diseases are enormous, because it is a major cause of morbidity and mortality, particularly among children. An estimated billion (109) episodes occur each year and nearly 5 million children under age of 5 die as a result.

Krumperman (1983) proposed that antibiotic resistance patterns of E. coli can be used as "fingerprints" to determine the source of the faecal contamination and that organisms obtained from the environment shows varying degrees of susceptibility and resistance to antibiotics. The paucity of information on the bacterial flora of fishes and their antibiotic sensitivity patterns in this locality prompted me to determine the bacterial flora and antibiotic sensitivity patterns of these bacterial flora of five fresh sea fishes sold in some markets in Warri, Delta State, Nigeria; to some commonly used antibiotics; Compare the antibiotics susceptibility patterns of isolates from the different species of fishes investigated.

MATERIALS AND METHODS

Materials:

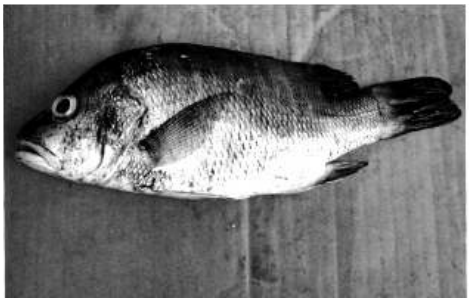
Materials used in this study includes, test tubes, conical flask, Pipettes, Glass slides, Petri-dishes, non-absorbent cotton wool, Bunsen burner, Metler balance, chemical balance, Incubator, Autoclave, Refrigerator, Dissecting board and set, Mortar and Pestle, Whatman no 1 fitter paper and Antibiotics sensitivity disks.

Reagents and Media

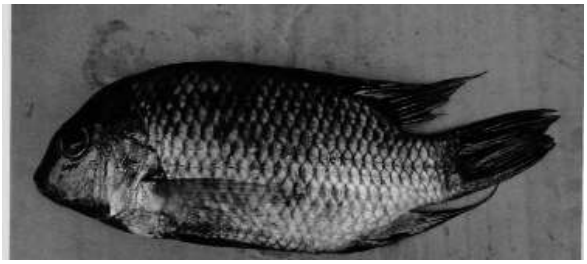
Grams reagents, Lead acetate paper, 70% alcohol, Methylated spirit, MacConkey agar, Nutrient agar, Nutrient broth, Urea agar, Citrate agar, Thiosulphate citrate bile salt sucrose agar, Oxidase strip reagent, hydrogen peroxide, indole reagents and 7 different sugars.

Samples Collection

The five sea fishes, *Tilapia sp, Chrysichthys nigrodigitatus, Citharinus sp, Tilapia guineensis*, and *Lates niloticus* weighting 50 -120g, were bought from Warri main market and Makava market in Warri, Delta State, Nigeria. These were transported to the microbiology laboratory, Delta State University, Abraka in ice packed container for analysis, within 1hr. The photographs of the fishes are presented in Fig. 1.



Tilapia sp.



Tilapia guineensis

Citharinus sp.

Chrysichthys nigrodigitatus

Lates niloticus

Fig. 1: *The five fresh sea fishes*

Microbiological Analysis: The fishes were weighed with a chemical balance and disinfected externally with 70% ethanol. Each was then cut opened from the anus to the mouth region and the gut removed under aseptic condition. Approximately 1.0 g of the gut was homogenized in 9 mL of normal saline using a sterile Mortar and Pestle. The homogenate was then transferred into a sterile Pyrex glass boiling tube and further serially diluted tenfold in normal saline. The diluted samples were then pour plated into freshly prepared sterile molten Nutrient agar, MacConkey agar, and Thiosulphate citrate bile salt sucrose agar to determine the total aerobic viable, coliform, and vibrio counts respectively as described by Harrigan and McChance (1982). The arithmetic mean of the counts at a chosen dilution was used to calculate the microbial population in the original samples.

Typical coliform, vibrio and viable colonies were randomly sub cultured onto sterile media and tested for their Gram reaction, motility, H_2S production, formation of spores, citrate utilization, carbohydrate fermentation, ability to produce indole, catalase, oxidase and urease enzymes. The various isolates were subsequently identified with the scheme of Cowan and Steel (1985) and recorded in Tables 2 and 3.

Antibiotics Sensitivity Testing

About 1 mL of overnight cultures of the isolates in nutrient broth was flooded on sterile nutrient agar plates. Antibiotic sensitivity discs of commonly used antibiotics were then placed on the surface of the seeded agar plates. These were allowed to stabilized and then incubated of $37^{O}C$ for 24hr. The zones of inhibition of the different antibiotics were measured and recorded in Table 4.

RESULTS

The result of bacterial burden of the five sea fishes recorded in Table 1 shows that the mean total aerobic viable counts ranged from 7.5×10^6 CFU/mL for *Lates niloticus* to 7.0×10^7 CFU/mL for *Tilapia guineensis*. The mean coliform counts obtained ranged from 3.6×10^5 CFU/mL for *Chrysichthys nigrodigitatus* to 4.4×10^6 CFU/mL for *Tilapia guineensis*; while the mean Vibrio counts of

the fishes ranged from 2.2×10^2 CFU/mL for *Citharinus sp* to 2.5×10^2 CFU/mL for *Tilapia guineensis*.

In all, 8 bacterial species were isolated (Tables 2) from the 5 types of fresh five sea fishes investigated. They include *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp, Shigella dysentariae, Vibrio cholerae, Vibrio parahaemolyticus* and *Aeromonas hydrophilia*. Tables 3 show the comprehensive list of all the bacteria species isolated. *Salmonella sp, Aeromonas hydrophila* and *Pseudonomas aeruginosa* were obtained, from all the five fresh sea fishes while *Bacillus cereus* was isolated from *Tilapia sp*, *Chrysichthys nigrodigitatus, Tilapia guineensis* and *Lates niloticus*. *Vibrio parahaemolyticus* was obtained from all the fishes except *Tilapia sp*, while *Shigella dysentariae* was isolated from 3 fishes except *Tilapia sp* and *Tilapia guineensis*.

The diameter of the zones of inhibition (Tables 4) of the sensitive isolates ranged from 16 - 24mm, while that for the resistant isolates ranged from 0-13mm. The result (Tables 5) also shows that *Pseudomonas aeruginosa* was resistant to 8 out of the 15 antibiotics used in this study, while the remaining bacterial isolates were resistant to 1 - 4 antibiotics

Table 1: Bacterial load of five fresh sea fishes (CFU/mL)

	TS	CN	CS	TG	LN
Mean total viable counts	8.1×10^6	8.3×10^6	7.0×10^7	8.0×10^6	7.5×10^6
Mean coliform counts	4.5×10^5	5.1×10^5	3.6×10^5	4.4×10^6	5.0×10^5
Mean vibrio counts	3.1×10^2	2.5×10^2	2.2×10^2	2.5×10^3	3.3×10^2

Key: TS = *Tilapia sp*, CN = *Chrysichthys nigrodigitatus*, CS = *Citharinus sp*
TG = *Tilapia guineensis*, LN = *Lates niloticus*

Table 2: Biochemical Characterization of the Isolates

Gram stain	Shape	Motility	Catalase	Oxidase	Urease	Indole	Spore formation	H_2S production	Citrate utilisation	Glucose	Lactose	Mannitol	Sucrose	Sorbitol	Raffinose	Arabinose	Suspected Isolates
+	R	+	+	NT	NT	-	+	NT	+	+	-	+	+	+	+	+	*Bacillus cereus*
-	R	+	+	-	-	+	NT	-	+	+	+	+	+	+	+	+	*Escherichia coli*
-	R	+	+	+	+	-	NT	-	+	+	+	-	+	+	+	+	*Pseudomonas aeruginosa*
-	R	+	+	-	-	-	NT	-	-	+	-	-	-	+	-	+	*Salmonella species*
-	R	-	+	-	-	+	NT	-	-	+	-	+	-	+	-	+	*Shigella dysentariae*
-	CR	+	-	+	-	+	NT	-	-	+	-	+	+	-	-	-	*Vibrio cholerae*
-	CR	+	-	+	-	+	NT	-	-	+	-	+	-	-	-	-	*Vibrio parahaemolyticus*
-	R	+	-	+	-	+	NT	-	+	+	+	-	+	-	-	-	*Aeromonas hydrophila*

R = Rod shaped, CR = Curve Rod, + = Positive, - = Negative, NT = Not Tested

Table 3: Bacterial isolates obtained from five fresh sea fishes

	Fish type	Bacteria Obtained
1	*Tilapia sp*	*Escherichia coli, Vibrio cholerae, Bacillus cereus, Salmonella sp, Aeromonas hydrophila, Peudomonas aeruginosa.*
2	*Chrysichthys nigrodigitatus*	*Bacillus cereus, Vibrio cholerae, Vibrio parahaemolyticus, Aeromonas hydrophila, Escherichia coli, Salmonella sp, Pseudomonas aeruginosa, Shigella dysentoriae*
3	*Citharinus sp*	*Vibrio cholerae, Shigella dysentariae, Escherichia coli, Salmonella sp, Aeromonas hydrophila, Pseudomonas aeruginosa, Vibrio parahaemolyticus*
4	*Tilapia guineensis*	*Vibrio cholerae, Vibrio parahaemolyticus, Bacillus cereus, Aeromonos hydrophila, Escherichia coli, Salmonella sp, Pseudomonas aeruginosa*
5	*Lates niloticus*	*Escherichia coli, Shigella dysentariae, Vibrio cholerae Salmonella sp, Bacillus cereus, Vibrio parahaemohyticus, Aeromonas hydrophila, Pseudomonas aeruginosa*

Table 4: Antibiotics Susceptibility pattern of the various bacterial isolates

Isolates	Zones of inhibition of Antibiotics (mm)														
	Chloramphenicol 25µg	Cefuriazone 10 µg	Nitrofuration 15 µg	Gentamicin 10 µg	Ampicillin 10 µg	Augumentin 10 µg	Nalidxic acid 5 µg	Amoxylcillin 15 µg	Peflacine 25 µg	Tetracycline 10 µg	Ofloxacine 15 µg	Cotrimozazole 30 µg	Ciprofloxacine 30 µg	Erythromycin 10 µg	Ceporex 5 µg
Escherichia coli	23	13	25	26	11	26	27	25	23	23	25	25	23	25	23
Pseudomonas aeruginosa	0	0	10	0	9	21	20	20	11	0	24	24	20	19	11
Salmonella species	9	22	25	27	22	28	25	23	24	9	23	26	22	9	10
Bacillus species	17	15	9	20	8	9	20	16	17	16	10	18	16	19	17
Shigella species	20	21	24	22	8	9	22	26	20	22	21	10	22	23	21
Vibrio cholera	25	22	21	20	23	26	24	25	21	20	24	20	22	21	23
Vibrio parahaernolyticus	20	23	21	24	12	23	25	24	26	24	20	21	25	20	20
Aeromonas hydrophila	19	18	20	19	21	22	18	20	21	21	20	19	20	20	20

Table 5: The antibiogram of the bacterial isolates

Isolates	Zones of inhibition of Antibiotics (mm)														
	Chloramphenicol 25µg	Cefuriazone 10 µg	Nitrofuration 15 µg	Gentamicin 10 µg	Ampicillin 10 µg	Augumentin 10 µg	Nalidxic acid 5 µg	Amoxylcillin 15 µg	Peflacine 25 µg	Tetracycline 10 µg	Ofloxacine 15 µg	Cotrimozazole 30 µg	Ciprofloxacine 30 µg	Erythromycin 10 µg	Ceporex 5 µg
Escherichia coli	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S
Pseudomonas aeruginosa	R	R	R	R	R	S	S	S	R	R	S	S	S	S	R
Salmonella species	R	S	S	S	S	S	S	S	S	R	S	S	S	R	R
Bacillus species	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S
Shigella species	S	S	S	S	R	R	S	S	S	S	S	R	S	S	S
Vibrio cholera	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Vibrio parahaernolyticus	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
Aeromonas hydrophila	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

KEY: R = Resistant, S = Sensitive

DISCUSSION

Seafood has the potential to pose a wide spectrum of public health problems from harmful bacteria species through contamination during distribution from the point of harvest to final preparation. The result of this study (Tables 2 and 3) shows 6 - 8 Gram positive and Gram negative bacteria species associated with the gastrointestinal tract of the five sea fishes, these are related to earlier report by Sarkar *et al*.(1985) from similar marine environment. Many of these organisms could have found their way into the water body through contamination from untreated domestic, animal and human wastes that are usually dumped into the marine water of Warri. This is supported by the report of Sarkar *et al*. (1985) and Kori-Siakpere and Owhe-Ureghe (2001), that the microbial load of sea and fresh water fishes is a reflection of the aquatic environment, the diets of the fish and the physio- chemical parameters of the environment from which they were harvested. One obvious implication of this school of thought is that the microbiological quality of the waters affects mobile or migratory species as well as sedentary shell fish. Contamination by enteric bacteria in polluted harvest area is sporadic and difficult to control as the result of this study tend to suggests. Apart from typical marine bacterial species like, *Vibrio cholerae, V. parahaemolyticus* and *Aeromonas hydrophila*, other isolates were enteric related.

The antibiotics susceptibility studies on the bacterial isolates obtained in this investigation shows that (Table 4 and 5), all were sensitive to Nalidixic acid, Amoxylcillin, Ofloxacine and Ciprofloxacine, while they show varying degrees of sensitivities and resistance to other antibiotics used in this investigation. Apart from *Pseudomonas aeruginosa* that was resistant to 8 out of the 15 antibiotics used in this study. Out of the 8 bacterial species isolated, 6 (75%) were resistant to 1 - 4 antibiotics, while only 2 (25%) (*Vibrio cholerae and Aeromonas hydrophila*) were sensitive to all antibiotic used in this study. Multiple drug resistance phenomenon observed in this study due to *P. aeruginosa* and more than 50% of the bacterial isolates have also been reported in this Nigerian environment (Owhe-Ureghe *et al*., 2000) and other parts of the world (Watkins and Cabelli, 1985). The result further showed that the *Vibrio sp* and *Aeromonas hydrophila* which are typical marine organisms were either 100% sensitive to all the antibiotics or resistant only to one antibiotic.

CONCLUSION

The occurrence of pathogenic bacterial species with multiple drug resistance in sea fishes called for the proper pre-treatment of human, animal, and domestic waste before being dumped into the marine (salty water) and fresh water environment, in order to reduce the possible contamination of this essential food resource (fish) with the consequent health implication. Furthermore, it is advisable to subject fish and fish products to adequate cooking or adequate heat exposure during processing so that these products could be hygienically safe for human consumption.

REFERENCE

Adams, M.R. and Mass, M.O. (1999). *Food Microbiology.* The Royal Society of Chemistry, Cambridge.

Cowan S.T. and Steel K.J. (1985). *Manual for the identification of medical bacteria (2nd edn).* Cambridge University Press, New York.

FAO (1978). *Comparative studies on freshwater fisheries.* Technical Report Paper of FAO. Food and Agriculture Organisation **198**: 46.

Halliday, D. (1986). Protection of food commodities with insecticides In: fish processing in Africa, FAO Fish Report. *Food and Agriculture Organisation* **329**: 369-394.

Harrigan, W.F. and McChance, M.E. (1982). *Laboratory Methods in Food and Dairy Microbiology* 2nd ed. Academic Press Inc., London.

Kori-Siakpere, O. and Owhe-Ureghe, U.B. (2001). Bacterial flora of the gut of the African snakehead *Channa obsura* (Pisces: Channidae). *Bioscience Research communication,* **13(6):** 693-699.

Krumperman, P.H. (1983). Multiple antibiotics resistance indexing of Escherichia coli to identify high risk sources of faecal contamination of foods. *Applied Environmental Microbiology*, **46**: 165-170.

Lewis, D.H. (1975). Retention of *Salmonella typhimurium* by certain species of fishes and shrimp. *Journal of American Veterinary Association* **167**: 551-557.

Nair, N.V., Sengupia, D.N. and Ghosh, S. (1975). Halophilic Vibrio from fish and meat in Cacutta. *Indian Journal of Medical Research* **63**: 558-564

Nerquaye-Tetteh, G.A.(1986). Trigger fish (*Balistes sp*) Processing industry at Elmina. Fish processing in Africa. *FAO Fish Report*. 329-384.

Nizan, S. and Hammerschiag, E., (1993). First report of Pasteurellosis in freshwater hybrid tilapia (*Oreochromis aureus O. niloticus*) in Israel. *Bulletin European Association of Fish Pathology*, **13**:179-180.

Owhe-Ureghe, U.B., Onyesom, I. and Igumbor, O.E. (2000). *Pseudomonas aeruginosa*: Antibiotics susceptibility pattern of clinical isolates obtained from Ekpoma, Edo State, Nigeria. *Nigeria Journal of Science & Environment*. **2**: 101-103.

Sakata, T. and Hattori, M. (1988). Characteristics of *Vibrio vulnificus* isolated from diseased tilapia. *Fish Pathology*, **23**:33-40.

Sarkar, B.L., Nair, G.B., Bannerjel, A.K. and Pal, S.C. (1985). Seasonal distribution of *Vibrio parahaemolyticus* in fresh water environs and in association with fresh water fishes in Calcutta. *Applied Environmental Microbiology*, **49**:132 – 136.

Showan, J.M. and Hobbs, G. (1967). The bacteriology of fish spoilage and preservation. In: Progress in industrial microbiology (Hackenhull, D.J.D. Ed), Hiffer Books, London.