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, Mackeral, Horse Mackeral, 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 Nige-
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 flora 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, Crysichthys 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.
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, H2S 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°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 $7.5 \times 10^6$ CFU/mL for *Lates niloticus* to $7.0\times10^7$ CFU/mL for *Tilapia guineensis*. The mean coliform counts obtained ranged from $3.6\times10^5$ CFU/mL for *Chrysichthys nigrodigitatus* to $4.4 \times 10^6$ CFU/mL for *Tilapia guineensis*; while the mean Vibrio counts of

Fig. 1: The five fresh sea fishes
the fishes ranged from $2.2 \times 10^2$ CFU/mL for *Citharinus sp* to $2.5 \times 10^2$ 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 dysenteriae*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Aeromonas hydrophila*. Tables 3 show the comprehensive list of all the bacteria species isolated. *Salmonella sp*, *Aeromonas hydrophila* and *Pseudomonas 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 fish species except *Tilapia sp* while *Shigella dysenteriae* 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)**

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Bacteria Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tilapia sp</em></td>
<td><em>Escherichia coli</em>, <em>Vibrio cholerae</em>, <em>Bacillus cereus</em>, <em>Salmonella sp</em>, <em>Aeromonas hydrophila</em>, <em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td><em>Chrysichthys nigrodigitatus</em></td>
<td><em>Bacillus cereus</em>, <em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Escherichia coli</em>, <em>Salmonella sp</em>, <em>Pseudomonas aeruginosa</em>, <em>Shigella dysenteriae</em></td>
</tr>
<tr>
<td><em>Tilapia guineensis</em></td>
<td><em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Escherichia coli</em>, <em>Salmonella sp</em>, <em>Pseudomonas aeruginosa</em>, <em>Vibrio parahaemolyticus</em></td>
</tr>
<tr>
<td><em>Lates niloticus</em></td>
<td><em>Escherichia coli</em>, <em>Shigella dysenteriae</em>, <em>Vibrio cholerae</em>, <em>Salmonella sp</em>, <em>Bacillus cereus</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Pseudomonas aeruginosa</em></td>
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</tbody>
</table>

**Table 2: Biochemical Characterization of the Isolates**

<table>
<thead>
<tr>
<th>Bacteria Obtained</th>
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<th>LN</th>
<th>LN</th>
<th>LN</th>
<th>LN</th>
<th>LN</th>
<th>LN</th>
<th>LN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td><em>Salmonella sp</em></td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td><em>Bacillus species</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td><em>Shigella species</em></td>
<td>S</td>
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<tr>
<td><em>Vibrio cholerae</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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**KEY:** R = Rod shaped, CR = Curve Rod, + = Positive, - = Negative, NT = Not Tested

**Table 3: Bacterial isolates obtained from five fresh sea fishes**

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Bacteria Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tilapia sp</em></td>
<td><em>Escherichia coli</em>, <em>Vibrio cholerae</em>, <em>Bacillus cereus</em>, <em>Salmonella sp</em>, <em>Aeromonas hydrophila</em>, <em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td><em>Chrysichthys nigrodigitatus</em></td>
<td><em>Bacillus cereus</em>, <em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Escherichia coli</em>, <em>Salmonella sp</em>, <em>Pseudomonas aeruginosa</em>, <em>Shigella dysenteriae</em></td>
</tr>
<tr>
<td><em>Tilapia guineensis</em></td>
<td><em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Escherichia coli</em>, <em>Salmonella sp</em>, <em>Pseudomonas aeruginosa</em>, <em>Vibrio parahaemolyticus</em></td>
</tr>
<tr>
<td><em>Lates niloticus</em></td>
<td><em>Escherichia coli</em>, <em>Shigella dysenteriae</em>, <em>Vibrio cholerae</em>, <em>Salmonella sp</em>, <em>Bacillus cereus</em>, <em>Vibrio parahaemolyticus</em>, <em>Aeromonas hydrophila</em>, <em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

**Table 4: Antibiotics Susceptibility pattern of the various bacterial isolates**

**Table 5: The antibiogram of the bacterial isolates**
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, Amoxycillin, Ofloxacine and Ciprofloxicine, 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


