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Antibiogram of Bacterial Flora of *Tilapia zilli* from Creeks Around Port Harcourt, Nigeria

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Abstract: The gills and intestines of *Tilapia zilli* collected from 4 different creeks around Port Harcourt, Nigeria were examined over a 7 month period for their bacterial flora. A total of 208 *Pseudomonas* sp., 129 *Aeromonas* sp., 90 *Klebsiella* sp. and 50 *Escherichia coli* were isolated and tested for their susceptibility to 10 commonly used antibiotics. Tests on 517 of the isolates showed that all of the *Pseudomonas* sp. were resistant to at least one of the antibiotics. The microflora of the fishes could only have been derived from their environment, i.e., water. The antibiotic susceptibility pattern of the isolates in the present study was compared with the susceptibility pattern of isolates from previous studies.

Key words: Antibiotics, bacterial flora, creeks, *Tilapia zilli*, Port-Harcourt

INTRODUCTION

Various studies have shown the possibility of transfer of antibiotic resistance from commensal enteric bacteria to pathogenic enteric bacteria vice versa (Richmond *et al*., 1979; Linton *et al*., 1981). Such organisms with multiple resistance to antibiotics have been isolated in appreciable numbers from rivers (Al-Jebouri and Al-Meshhadani, 1985), lakes (Jones *et al*., 1986) and other bodies of water. Whilst the use of drugs does not cause microbes to become resistant, widespread use does provide a selection pressure in favour of organisms possessing genes that code for resistance (Hinton *et al*., 1986). Transfer of genes for resistance by R-factors is known to occur widely among Gram-negative bacteria, especially the coliforms (Chatterjee and Starr, 1972; Linton *et al*., 1981).

Port Harcourt, the capital city of Rivers State Nigeria, is dominated by the River Niger, the third largest river in Africa, with one of the largest expanses of Delta in the world (Sokari *et al*., 1988). This area is usually very busy with activities of human settlement along the creeks, often resulting in pollution from domestic and industrial sources.

A number of industries such as the Port Harcourt Refinery Company (PHRC), Eleme Petrochemical Industries and a lot more are sited either on the bank or close to banks of the estuary. These industries discharge their partially treated and untreated effluents into the river causing contamination of water supplies from various industrial processes and disposal practices (Ogbonna *et al*., 2004; Otokunefor and Obiukwu, 2005). Industries which use large amounts of water in their processes (like steam production, as solvents, for washing purposes, as coolant, for rinsing, for waste disposal practices and for finishing operations etc.) discharge by way of sloughs that lead directly into the river. The run off from these industries negatively affects the water quality of the river, which affects the wildlife surrounding the river. Due to the inffectiveness of purification

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systems, wastewaters may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem (Beg *et al*., 2001, 2003).

Port Harcourt, the study area and many other towns are on the tributaries of the Niger and many of the recent industrial projects have been sited adjacent to, or within easy access of, the river (Anonymous, 1985). Apart from a recent report by Sokari *et al*. (1988), however, little is known about the incidence of antibiotic resistance bacteria in some selected creeks in Port Harcourt. The present study was therefore designed to determine the bacterial flora of *Tilapia zilli* along these creeks and their level of resistance and other organisms, from these sources to commonly used antimicrobial agents.

MATERIALS AND METHODS

Study Area

Fish specimens were collected from the Bonny River around Port Harcourt using local fishing methods/gears employed by the local fisherman from four stations namely Elechi, Dockyard, Nweja and Eagle Island along the Bonny River around Port Harcourt and along an arm of the same river that flows by various towns and fishing settlements. Appropriate common fishing gears employed were cast and seine nets. They were used in open and shallow creeks, respectively. The gears were chosen because they are adapted for sampling common fishes like *Tilapia* sp.

Cast nets of different mesh sizes (1-1.5 cm) with length of about 2-3.5 m were thrown randomly at four spots per sampling location while the seine nets of 5 mm measuring 8-10 m in length and 3 m deep was used three times per sampling location where cast nets were found to be difficult to be thrown. About 10 fishes were selected from about 100 fishes collected. These were transported to the laboratory for identification using appropriate keys (FAO, 1990).

Preparation of Fish Samples

The gills and intestines of *Tilapia zilli* were used in the preparation of samples for analysis. The parts were dissected and weighed. Five gram each of the gills and intestines were separately added to 45 mL of 0.1% peptone water diluent to give a 10^{-1} dilution. After thorough shaking, further serial 10-fold (v/v) dilutions were made by transferring 1 mL of the original solution to fresh peptone water diluent to a range of 10^{-2} dilution. Aliquots (0.1 mL) of various dilutions were transferred to plates of surface dried Nutrient Agar plates in duplicate and spread by means of flamed glass spreaders. Inoculated plates were incubated for 24-48 h at 37°C.

Bacterial Isolation and Identification Procedures

The presence of various species of bacteria in the fish samples collected from the four stations was evaluated using standard procedures (Cheesebrough, 1991; Anonymous, 1998; Hutton, 1983).

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types onto freshly prepared NA plates. Discrete colonies, which developed on the plates were transferred into 10% sterile glycerin solution and preserved for further analysis. This served as pure stock culture for subsequent characterization tests. Various tests were carried out on the bacterial isolates for possible identification. One milliliter of broth culture of each isolate was used for all the tests except otherwise stated. Bacterial isolates were identified in accordance with the schemes of Baron and Sydney (1990) and the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of 517 strains isolated were determined on Diagnostic Sensitivity Test (DST) agar (OxoidCM 261). Both single disks and multodisks (Oxoid U4) were used according t o the techniques described by Johnston *et al*. (1983) and Al-Jebouri and Al-Meshhadani (1985). The multodisk contained (μg): ampicillin, 30; gentamycin, 100; streptomycin, 30; cotrimoxazole, 30; tetracycline, 50; colistinsulphate, 10; carbenicillin, 100; cefioro-xime, 10 and taroid, 10.

RESULTS AND DISCUSSION

Characterization of Microorganisms

The dominant bacteria from the different bodies of water were identified as *Pseudomonas*, *Micrococcus*, *Bacillus*, *Staphylococcus*, *Proteus*, *E. coli*, *Klebsiella*, *Aeromonas*, *Corynebacteria*, *Vibrio* and *Alcaligenes* species (Table 1). Apart from the fact that microorganisms are described to be ubiquitous, Coleman *et al*. (1974) and Ogbonna *et al*. (2004) also affirmed that their presence especially in an aquatic environment depends upon the nature of materials being added during natural storm water run offs as well as soil erosion and discharge from sewage effluents. In addition to this observation, the study areas were described as very busy with activities of human settlement along the creeks resulting in pollution from domestic and industrial sources which brought about increase in the amount of organic matter as well as nutrients for the microorganisms (Ogbonna *et al*., 2006; Otokunefor and Obiukwu, 2005). This indicates that most of the major contributors to the pollution of the rivers are the households that throw their wastes materials into the river and also whose wastewater eventually end up in the rivers.

Antibiotic Resistance among Bacteria from the Different Bodies of Water

The resistance patterns of the strains from the different bodies of water (Table 2) and on a cumulative basis are presented in Table 3. In this study, Gentamicin was the only antibiotic to which most of the organisms tested, irrespective of the source, were susceptible. Except for gentamicin, there was no antibiotic used in the test to which at least one estuarine strain was not resistant (Table 2). For the microorganisms, *Pseudomonas* sp. were more widely resistant to antibiotics than any of the other organisms like *Proteus* and *Aeromonas* species. All 208 *Pseudomonas* strains were resistant to at least one of the 10 antibiotics tested.

The level of susceptibility to gentamincin observed among the isolates tested in the present study (%) is comparable to the results of previous investigators (Cooke, 1976; Johnston *et al*., 1983; Wray *et al*., 1986; Sokari *et al*., 1988; Ibiebele and Sokari, 1989). *Pseudomonas* sp. are known to be highly resistant to antimicrobial agents (Al-Jebouri, 1985), it may also be because the drug is not used as frequently as other chemotherapentic agents on account of its nephrotoxic side effects (Baker and Breach, 1980) the level of resistance among *E. coli* and other strains to the antibiotics tested in the present study is similar to that observed by other workers. Al-Jebouri (1985) and Antai and Anozie (1987), have variously reported a high level of resistance among *E. coli.* Strains from raw sewage and teaching hospital and pediatric clinics in Port Harcourt, respectively. Some factors may also account for the level of resistance, particularly as many of the strains tested were multiply resistant. It is known that *E. coli* strains are rarely found in soil, vegetation or water in the absence of excremental contamination (Anonymous, 1983; Erah *et al*., 2002). It could be said therefore, that *E. coli* strain tested in the present study was of faecal origin. With the uncontrolled use of antibiotics and common practice of self medication typical of the Nigerian setting (Antai and Anozie, 1987; Sokari *et al*., 1988) there would be a selection pressure in favour of organisms possessing genes that code for resistance

Table 1: Morphological and biochemical characteristics of bacteria isolated from the creeks

Probable identity	Test: Gram rxn	Mortality	Methyl red	Vogues pros	Oxidase	H_2S product	Indole	Coagulaze	Catalase	Citrate utilize	Urease	Nitrate reduct	Sugar fermentation: Glucose	Sucrose	Lactose	Maltac	Galactose	Mnnitoe	Arabinose	Morphology colony and cell characteristics
Pseudo monas sp.	-	+	+	-	+	-	-	-	-	+	-	+	A	A	-	A	A	-	-	Large, flat milky colonies, Entire edge and smooth surface, slightly curved rods, gram negative rods.
Micrococcus sp.	+	-	+	-	+	-	-	-	+	+	-	+	A	A	-	A	-	-	-	Yellow colonies, gram positive in cocci clusters and in chains.
Bacillus sp.	+	+	-	-	-	+	-	-	-	+	-	-	-	-	A	-	A	A	AG	Large grey or milky white colonies, straight rods. In chains give large rods.
Staphylo coccus	+	-	-	-	-	-	-	+	+	+	-	+	A	A	-	A	A	-	-	Large creamy white, smooth, opaque colonies, cocci in clusters and singles gram positive cocci.
Proteus	-	+	+	-	-	-	-	-	-	+	-	-	AG	AG	-	AG	AG	W	W	Grey swarming colonies straight rods g-rods.
Klebsiella	-	+	+	-	-	-	+	-	-	-	-	-	AG	AG	AG	AG	AG	AG	W	Yellow, large mucoid, colonies, straight rods in chains or singles gram rods.
Aeromonas	-	+	+	-	+	-	-	-	-	+	-	+	AG	AG	-	AG	AG	W	-	Gram negative rods.
Coryne bacterium sp.	+	-	+	-	-	-	-	-	-	+	-	+	A	-	-	-	A	-	-	Gram positive short rods.
Vibrio sp.	-	+	+	-	+	-	+	-	-	-	-	+	A	A	-	AG	AG	W	W	Gram negative rods.
Akaligenes	-	+	-	-	+	-	-	-	+	W	-	-	Produces alkaline						-	Gram negative rods.

+: Positive, -: Negative, AG: Acid and Gas, A: Acid, G: Gas, W: Weak, sp: Species

Table 2: Resistance pattern of bacteria from different bodies of water various antibiotics

Site/	No. of strain resistant to									
Organisms	Strain	AMP	GEN	STR	CXM	COT	OFX	TET	COL	CEF	CAR
ELECHI											
Pseudomonas	55	28	-	5	10	16	13	32	6	14	23
Aeromonas	30	16	-	3	6	9	8	17	2	8	14
E. coli	12	7	4	2	4	5	6	9	1	3	6
Staphylococcus	5	4	-	2	3	4	4	4	3	4	12
Klebsiella	23	18	2	10	5	3	12	8	3	4	12
Proteus	18	10	-	6	3	2	5	4	2	2	6
Dockyard											
Pseudomonas	53	24	-	4	9	14	10	24	4	12	20
Aeromonas	20	12	-	2	4	7	6	14	2	6	12
E. coli	10	5	3	2	3	2	4	5	2	4	5
Staphylococcus	22	16	-	2	2	3	3	4	1	2	4
Klebsiella	8	3	2	3	1	2	2	3	3	4	2
Proteus	6	4	-	2	4	2	1	1	2	2	1
Nweja											
Pseudomonas	58	34	4	6	14	18	15	35	8	10	25
Aeromonas	35	18	2	4	20	12	16	25	4	10	18
E. coli	18	7	5	4	6	3	6	7	3	6	8
Staphylococcus	25	3	2	4	2	11	3	14	2	3	5
Klebsiella	4	3	2	4	2	1	3	4	2	2	3
Proteus	2	2	1	1	1	1	2	1	2	1	2
Eagle island											
Pseudomonas	44	20	-	3	7	11	8	20	3	10	18
Aeromonas	18	10	-	1	3	6	5	12	1	4	16
E. coli	10	4	-	1	2	4	3	4	-	1	3
Klebsiella	20	14	-	1	1	2	2	3	1	2	3
Proteus	2	1	-	-	-	1	1	1	1	-	1

AMP: Ampiccilin, 300 µg: GEN; Gentamycin, 100 µg; STR: Streptomycin 30 µg; COT: Cotrimoxazole, 30 µg; TET: Tetracyclin, 50 µg; COL: Colistin Sulphate, 10 µg; CAR: Carbenicillin 100 µg; CEF: Cefioroxime, 10 µg; OFX: Tarioid 10 µg

Table 3: Cumulative resistance pattern of some frequently occurring bacteria from all fish parts

Organisms	Total No. of isolates	Strains (%)	AMP	GEN	STR	CXM	COT	OFX	TET	COL	CEF	CAR
Pseudomonas	208	40.2	106	4	18	40	59	46	111	21	46	86
Aeromonas	129	25.0	56	4	10	23	34	35	68	9	28	60
E-coli	50	9.7	23	11	9	15	14	19	25	7	16	23
Klebsiella	90	17.4	51	4	10	10	19	22	29	7	11	24
Other organisms isolated												
Proteus	18	3.5										
Corynebacterium	4	1.8										
Micrococcus	8	1.5										
Bacillus	6	1.2										
Alcaligenes	2	0.4										
Vibro sp.	2	0.4										
All	517											

(Hinton *et al*., 1986). Many faecal organisms of human origin in the environment studied could therefore be resistant to the antibiotics abused. Observation by other investigators (Langlois *et al*., 1983; Hinton *et al*., 1986; Sokari *et al*., 1988; Ibiebele and Sokari, 1989) indicate that resistance to antimicrobials may persist for a considerable number of years after antibiotic usage has been discontinued.

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