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Antibiotic Susceptibility Profile of *Pseudomonas Aeruginosa* and Other Bacteria from Fish in Okwan Obolo Estuary, Nigeria

Offiong, Jacquiline Aniekan; Ekpai, Emediong Saviour; Archibong, Ekemini Anietie; D. I. Udoh

Department of Microbiology, University of Uyo, Nigeria

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ABSTRACT

This study was carried out to determine the antibiotics susceptibility profile of *Pseudomonas aeruginosa* and other bacteria from fish harvested in Okwan Obolo Estuary. Using an ice-cold pack, 1g of fish gills, muscles and intestine were used for the microbiological analysis. Antibiotic sensitivity tests were done on each isolates. The Total heterotrophic bacterial count was 1.02 x 10^6 CFU/g, 8.6 x 10^5 CFU/g and 3.0 x 10^5 CFU/g in gills, intestine and muscles respectively. *Pseudomonas aeruginosa* count in Intestine, Gills and Muscles were 5.4 x 10^5 CFU/g, 2.1 x 10^5 CFU/g and 1.1 x 10^5 CFU/g respectively. Total coliform count of 7.5 x 10^5 CFU/g was obtained from intestine, while 3.9 x 10^5CFU/g and 1.9 x 10^5CFU/g were recorded from gills and muscles respectively. The research further showed that other bacteria isolated from the fish were *Bacillus* spp., *Proteus* spp., *Shigella* spp., *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp. and *Escherichia coli*. The result shows that the most widely distributed bacterial isolates were *P. aeruginosa* and *Salmonella* spp. with 100% (3) occurrence, while the least distributed was *Proteus* spp. and *Klebsiella* spp. with 33.3% (1) occurrence. The most prevalent bacteria isolates were *Pseudomonas aeruginosa* and *Salmonella* spp. with 18.75% percentage prevalence, while the least prevalent bacteria isolates were *Proteus* spp. and *Klebsiella* spp with 6.25% prevalence. The antibiotics used were Gentamycin, Ciproflaxacin, Tetracycline, Erythromycin, Streptomycin and Neomycin. The isolates showed high resistant to Tetracycline, Neomycin and Erythromycin with 7mm zone of inhibition, and most isolates were sensitive to Gentamycin, Ciprofloxacin and Streptomycin with highest zone of inhibition of 21mm, 25mm and 24mm respectively. The isolation of multiple antibiotics resistant strains of *Pseudomonas aeruginosa* from fish in Okwan Obolo Estuary is an epidemiological concern, as the isolate is capable of causing infection in human.

Keywords: Antibiotic, Susceptibility, Profile, *Pseudomonas Aeruginosa,* Bacteria, Fish

INTRODUCTION

In a world where more than 70% of the planet is covered with water, aquatic may provide an essential component of the global food to improve nutrition, health and well-being of humans. Among several aquatic foods consumed by man, the most predominant is fish. Globally, fish production is about 154million tons per year with the consumption level of 18.5kg per capita per year. Projection also indicates that the aggregation of global fish supply will increase to 186million tons (2030) compared to 154million tons (Tacon and Metian, 2013). Similarly, globally fish consumption rate are growing faster than the global population growth because increase income and awareness of health benefits associated with consuming fish, as well as rising urbanization. In addition to directly providing high quality food, fisheries and aquacultures create economic value through the production, trade and marketing of wild and farmed fish (Cai *et al.*, 2019). Currently, more than 30% of Africa's population or roughly 200million people consume fish as the main animal protein source and micro-nutrition and the consumption rate increases yearly. Nigeria is not left out in fish consumption, occupying the second highest consumer of fish after Egypt in Africa (FAO, 2018).

Fish is an important source of animal protein especially for the poor who cannot afford to buy other animal protein. Its consumption has various health, environmental, social advantage and nutritional value that are essential to cognitive and physical development, especially in children, and are an important part of healthy diet over their terrestrial animal meat. It is an important source of energy and protein, comparable to or better

than many terrestrial types of meat (Pieniak *et al.,* 2011). The consumption of fish and seafoods, in general, are having several nutritive and health benefits. The fish meat contains several essential amino acids, highly unsaturated fatty acids, high level of iron, calcium and iodine and vitamin A. Furthermore, fish meat ha s been stated to improve intelligence, treat skin condition, improve brain development and decrease of heart disease, asthma, Alzheimer's disease, cancer, obesity, diabetes and others. Fish is a primary source of essential for pregnant mothers and children especially nutrients like, Omega-3 fatty acid that is crucial for early brain development (Lemma, 2017).

Recently, there has been an increase in factors that threatens fish production, factors ranging from excessive harvesting of fishes, (especially young fishes) to the threat caused by bacteria and other pathogenic microorganisms. The threat of fish caused by bacteria affects not only the fish but causes fish related food pathogens, (Tacon and Meitan, 2013). Heterotrophic bacteria derive energy from organic compound; they are widely distributed and most abundant forms. They are aerobic and anaerobic (Bhatiani and Chandna, 2015). Pseudomonas *aeruginosa* is facultattively aerobic bacterium, a gram-negative rod, a part of non-glucose fermenting normal microbial flora and widely distributed in natural environment such as water and soil. (Fazeli *et al.,* 2012). According to the Centre for Food Safety and Applied Nutrition in Washington (2010), most food related food borne illness are traced to *pseudomonas aeruginosa, Salmonella, Staphylococcus spp, E. coli, Vibro spp. And Clostridium spp.* According to Ayulo *et al., (*2024) and Hastein *et al.,* (2006), there have currently been trends of increase in antibiotic resistant species of bacterial isolates from fish samples. The emergency of antimicrobial resistance among fish pathogenic bacteria has become a major concern for several countries during the past decades as fish are consumed by humans and these bacteria causes several infections to humans. It is therefore important to understand the profile of antibiotic resistance in fish harvested in this part of the globe.

Heterotrophic bacteria derive energy from organic compound; they are widely distributed and most abundant forms. They are aerobic and anaerobic (Bhatiani and Chandna, 2015). Pseudomonas *aeruginosa* is facultattively aerobic bacterium, a gram-negative rod, a part of non-glucose fermenting normal microbial flora and widely distributed in natural environment such as water and soil. (Fazeli *et al.,* 2012). However, the aim of the research is to determine antibiotic susceptibility profile of *Pseudomonas aeuginaosa* and other bacteria from fish in Okwan Obolo Estuary.

MATERIALS AND METHODS

Sample Collection

Fish samples were gotten from Okwan Estuary located in Eastern OboloLocal Government Area, located between latitudes 4°28'and 4°33' North and longitudes 7°30' and 7°50' East. The collected fish samples were aseptically and immediately transported (using a thermal bag) to the University of Uyo Microbiology Laboratory.

Preparation of Sample

The scales of the fish were carefully removed using a clean knife, and the muscles, gills and intestine of the fish samples were removed under aseptic conditions. The Fish muscles, gills and intestine were microbiologically analyzed to isolation *Pseudomonas aeruginosa*. The muscles, gills and intestine of fish samples were pieces separately with a knife under aseptic condition.

MICROBIOLOGICAL ANALYSIS OF SAMPLES

Culturing and Bacterial Enumeration

The muscles, gills and intestine of fish samples were pieced separately with a knife under aseptic condition. One gram from the muscles, gills and intestine of the fish were measured separately with a standard weighing scale. A 10-fold serial dilution was done to a 10^{-4} dilution factor.

Determination of *Pseudomonas aeruginosa Count*

Standardly prepared centrimide agar (selective media for isolation of *Pseudomonas aeruginosa*) was gently poured into the different petri dishes and stirred well for proper mixing. The petri dishes containing the agar were allowed to solidify before being rapped with a foil paper and incubated in an inverted position at 37ºC for 24hrs. After which viable cells were enumerated and expressed as number of Colony Forming Unit/gram (CFU/g).

Determination of Total Heterotrophic Bacterial Count (THBC)

Using a pour plate method, 1ml of the dilution was inoculated into labeled petri dishes using nutrient agar. The plates were incubated at 37oC for 24hrs. after inoculation, total viable bacteria were expressed as a number of Colony Forming Unit/gram (CFU/g) with strict aseptic procedures were followed in every step of analysis.

Determination of Total Coliform Count (TCC)

Using a pour plate method, 1ml of the dilution was inoculated into labeled petri dishes using MacConkey agar. The plates were incubated at 37oC for 24hrs. After inoculation, total viable bacteria were enumerated and expressed as number of Colony Forming Unit/g.

Isolation And Identification of *Pseudomonas Aeruginosa*

After 24 hours of incubation at 37^0C, the petri dishes were analyzed for the presence of *Pseudomonas aeruginosa*. The presence of the microorganism (*Pseudomonas aeruginosa*) was indicated by the appearance of *bright green* colony. Various biochemical tests such as Gram staining, oxidase test, motility test, catalase test, indole test, and sugar fermatation test were carried out according to Cheesbrough (2005), to determine and confirm the presence of the microorganism (*P. aeruginosa).*

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility Test of *Pseudomonas aeruginosa* isolated from the fish samples from okwan estuary was done using the simple disk diffusion according to the Kirby-Bauer Test (disc diffusion technique). The Mueller-Hinton agar was used for this purpose.

Antibiotic disc used were: Amoxicillin(10µg), ofloxacin (5µg), Ampicillin (10µg), ceftriaxone (30µg), cefuroxime (30µg), amikacin (30µg), gentamycin (30µg), tetracycline (30µg), cotrimoxazole (30µg) and augmentin (30µg).

The Mueller-Hinton plates were flooded with isolated and identified *Pseudomonas aeruginosa* colony (which was mixed with sterilized drops of water) using a swap tick under aseptic condition. Antibiotic discs were gently placed on the well labeled sensitivity plate. The plates containing the discs were allowed to stand for at least 30 min before being incubated at 37 °C for 24hrs. Diameter of zones of inhibition seen round the antibiotics sensitivity discs were measured with a meter rule and categorized as resistant, intermediate and sensitive based on the Clinical and Laboratory Standards Institute (CLSI) standard for each bacteria isolate.

RESULTS

Microbial Count of Fish Sample from Okwan Estuary

Table 1 shows the microbial count of the Total Heterotrophic Bacterial Count (THBC), Total Coliform Count and *Pseudomonas aeruginosa* Count isolated from fish from Okwan Estuary. The result indicated that Intestine contained *Pseudomonas aeruginosa* of 5.4 x 10^5CFU/g, while gills and muscles had microbial count of 2.1x 10^5CFU/g and 1.1 x 10^5CFU/g respectively. Total coliforms count in the fish intestine was 7.5 x 10^5CFU/g, while gills and muscles had microbial count of 3.9 x 10^5CFU/g and 1.9 x 10^5CFU/g respectively. Total

Heterotrophic bacterial Counts were 1.02 x 10^6CFU/g, 8.6 x 10^5CFU/g and 3.0 x 10^5CFU/g in gills intestine and muscle respectively.

Table 1 Microbial Count of Fish Sample from Okwan Estuary

	THBC	**Enteric Bacterial Count**	***Pseudomonas aeruginosa* Count**
Gills	8.6 x 10^5	3.9 x 10^5	2.1 x 10^5
Intestine	1.02 x 10^6	7.5 x 10^5	5.4 x 10^5
Muscle	3.0 x 10^5	1.9 x 10^5	1.1 x 10^5

THBC = Total Heterotrophic Bacterial Count

Biochemical Characteristics of *Pseudomonas aeruginosa*isolated from Fish Okwan Estuary (using centrimide agar)

Table 2 shows the morphological and biochemical characteristics of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a gram-negative short rods bacterium. It also shows the positive reaction to oxidase test, catyalase test, and is motile, while showing negative reaction to indole production.

Table 2 Morphological and Biochemical Characteristics of *Pseudomonas aeruginosa*

Biochemical Test	***Pseudomonas aeruginosa***
Gram stain	-
Cell shape	Short rods
Motility	+
Colony color	Yellow-Green (Centrimide agar)
Oxidase test	+
Catalase	+
Indole production	-
Utilization of Glucose	-
Lactose	-
Mannitol	**AG**
Maltose	-

Keys

+ = Positive

- = Negative

AG = Acid and Gas

Morphological and Biochemical Characteristics of other Bacterial Isolated from Fish from Okwan Obolo Estuary

Table 3 shows other bacteria species isolated from fish in Okwan Obolo estuary. The following isolates were obtained; *Bacillus spp., Proteus spp., Shigella spp., Klebsiella spp., Enterobacter spp., Salmonella spp., and Escherichia coli*

Table 3 Morphological and Biochemical Characteristics of other Bacterial Isolated from Fish from Okwan Obolo Estuary

Gram Staining	Shape	Catalase	Oxidase	Indole	Motility	Lactose	Maltose	Mannitol	Glucose	Probable Organisms
+	Rods	+	+	-	+	AG	AG	-	AG	*Bacillus* spp.
-	Rods	+	-	+	+	-	-	-	AG	*Proteus* spp.
-	Rods	+	-	+	-	-	A	AG	AG	*Shigella* spp.
-	Rod	+	-	-	-	AG	AG	A	AG	*Klebsiella* spp.
-	Rods	+	-	-	+	A	-	-	-	*Enterobacter* spp.
-	Rod	+	-	-	+	-	AG	AG	-	*Salmonella* spp.
-	Rod	+	-	+	+	AG	-	AG	A	*Escherichia coli*

KEY

+ = Positive

- = Negative

AG = Acid and Gas production

A = Acid production.

Occurrence of *Pseudomonas aeruginosa* and other Bacterial isolates

The occurrence of microorganisms in the gill, muscles and intestine of the fish sample isolated from the Okwan estuary is shown in table 4. the result shows that the most widely distributed bacterial isolates were *P. aeruginosa* and *Salmonella* spp. With 100% (3) occurrence, while the least distributed was *Proteus* spp. And *Klebsiella* spp. With 33.3% (1) occurrence.

Table 4 Occurrence of *Pseudomonas aeruginosa* and other bacterial isolates from fish sample

Isolates	Gills	Muscles	Intestine	Percentage of occurrence (%)
P. aeruginosa	+	+	+	3
Bacillus spp.	+	-	+	2

Proteus spp.	+	-	-	1
Shigella spp.	+	-	+	2
Klebsiella spp.	-	-	+	1
Enterobacter spp.	-	+	+	2
Salmonella spp.	+	+	+	3
Escherichia coli	+	-	+	2

KEYS

\+ = Presence

\- = Absence

Prevalence of *Pseudomonas aeruginosa* and other bacterial isolates in the fish samples studied

The prevalence of *Pseudomonas aeruginosa* and other bacterial isolates in the fish sample studied is shown in Table 5. The result shows that the most prevalent bacteria isolates were *Pseudomonas aeruginosa* and *Salmonella* spp., with 18.75% prevalence, while the least prevalent bacteria isolates were Proteus spp. and *Klebsiella* spp. with prevalence of 6.25%.

Table 5 Occurrence of *Pseudomonas aeruginosa* and other bacterial isolates from fish in Okwano Oboloestuary.

Isolates	**Prevalence**	**Percentage prevalence (%)**
Pseudomonas aeruginosa	3	18.75
Bacillus spp.	2	`12.50
Proteus spp.	1	6.25
Shigella spp.	2	12.5
Klebsiella spp.	1	6.25
Enterobacter spp.	2	12.5
Salmonella spp.	3	18.75
Escherichia coli	2	12.5
TOTAL	**16**	**100%**

Antibiotic susceptibility test for *Pseudomonas aeruginosa* and other bacteria isolated from fish sample

Table 6 shows the antibiotics susceptibility test result. The antibiotics used were Gentamycin(30µg), Ciprofloxacin(15µg), Tetracycline (30µg), Erythromycin (15µg), Streptomycin(10µg), and Neomycin (30µg). The isolates showed high resistant to Tetracycline, Neomycin, and Erythromycin, and most isolates were sensitive to Gentamycin, Ciprofloxacin, and Streptomycin.

Table 6 Antibiotic susceptibility test for *Pseudomonas aeruginosa* and other bacteria isolated from fish sample

Bacterial isolates	**Isolates codes**	**Gent (30µg)**	**Cipro (15µg)**	**Tetra (30µg)**	**Erythro (15µg)**	**Strep (10µg)**	**Neo** (30µg)
Pseudomonas aeruginosa	PA_G	15	22	7	20	18	NZ
	PA_M	19	24	NZ	8	22	NZ
	PA_I	NZ	21	NZ	11	17	NZ

Bacillus spp.	BA_G	27	21	NZ	10	NZ	12
	BA_I	NZ	23	NZ	12	NZ	7
Proteus spp.	PR_G	20	NZ	19	NZ	20	10
Shigella spp.	SH_G	18	25	NZ	23	NZ	11
	SH_I	20	NZ	11	NZ	NZ	NZ
Klebsiella spp.	KL_I	12	NZ	NZ	NZ	7	19
Enterobacter spp.	EN_M	21	21	22	13	23	NZ
	EN_I	18	NZ	NZ	NZ	24	10
Salmonella spp.	SA_G	NZ	23	NZ	NZ	NZ	11
	SA_M	NZ	25	16	9	12	17
	SA_I	NZ	21	NZ	NZ	NZ	NZ
Escherichia coli	ES_G	19	NZ	15	7	19	17
	ES_I	21	NZ	20	NZ	16	NZ

KEYS

NZ = No Zone of Inhibition

I = From intestine

M = From muscles

G = From gills

DISCUSSION

The microbial count of the total heterotrophic bacterial Count (THBC), Total Coliform Count and *Pseudomonas aeruginosa* count isolated from fish from Okwan Obolo Estuary is shown in Table 1. The result indicated that Intestine contained *Pseudomonas aeruginosa* count of $5.4x10^5$ CFU/g while gills and muscles had microbial count of $2.1x10^5$CFU/g and $1.1x10^5$ CFU/g respectively. Total Coliform count in intestine was $7.5x10^5$CFU/g while gills and muscles had microbial count of $3.9x10^5$CFU/g and $1.9x10^9$ CFU/g respectively. Total heterotrophic bacterial count was $1.02x10^6$ CFU/g, $8.6x10^5$ CFU/g and $3x10^5$ CFU/g in gills, intestine and Muscles respectively. The predominance of these bacteria including *P. aeruginosa* could be associated to poor management practices, increase dumping of waste and inappropriate man activities (such as defecating and regular bathing) in the Estuary (Nwanta *et al.*, 2008). The isolation of *Pseudomonas sp*. from collected fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. This is in accord with previously mentioned by Jeyasekaran *et al.* (2006) and Koutsoumanis, and Nychas (2000) who identified Pseudomonads as a good spoilage index.

The high *Pseudomonas aeruginosa* and other bacterial count in intestine compared to the gills and muscles agreed with the work of Igbinosa *et al.* (2017). The higher microbial count in the intestine of fish is suggested by various researcher, including Olafsen (2001) and Austin (2002) to be due to the movement of contaminated water and food with higher microbial population into the fish intestine. Fish also directly take in waste deposited by human activities, these wastes possess various microorganisms, and these microorganisms moves directly into the intestine of the fish (Olafsen, 2001). The presence of coliform bacteria in the fish gills and intestine directly indicates the high level of pollution of the water body by feacal contamination as stated by Halkman (2014). The morphological and biochemical characteristics of *P. aeruginosa* is shown in Table 2. The result revealed that *P. aeruginosa* is a Gram-negative short rods bacterium. It also shows positive action to oxidase test, catalase test and is motile, while showing negative reaction to indole production. The characteristics obtained is further confirmed by Yagoub, S. (2009) and Igbinosa *et al.*, (2017). Table 3 shows the morphological and biochemical characteristics of other bacteria species isolated from fish in Okwan Obolo

Estuary. The following isolates were obtained, *Bacillus* spp., *Proteus* spp., *Shigella* spp., *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp. and *Escherichia coli.* This result agrees with the works of Yagoub (2009); Heinitz *et al.* (2000) and Hwang *et al*. (2004) as similar microorganisms were isolated from fresh fish samples from several rivers.

The occurrence of microorganisms in the gill, muscles and Intestine of the fish sample isolated from Okwan Obolo Estuary is shown in Table 4. The result shows that the most widely distributed bacterial isolates were *P. aeruginosa* and *Salmonella spp*. with 100% (3) occurrence, while the least distributed was *Proteus* spp. and *Klebsiella* spp. with 33.3 (1) occurrence; Table 5 further shows the prevalence of *Pseudomonas aeruginosa* and other bacterial isolates in the fish sample. The result shows that the most prevalent bacteria isolates were *Pseudomonas aeruginosa* and Salmonella spp with 18.75% prevalence, while the least prevalent bacteria isolates were *Proteus* spp., and *Klebsiella* spp. with 6.25% prevalence. The high prevalence and occurrence of *P. aeruginosa* and *Salmonella* spp. aligned with the research work of Yagoub, (2009).

Table 6 shows the antibiotics susceptibility test result. The antibiotics used were Gentamycin, Ciproflaxacin, Tetracycline, Erythromycin, Streptomycin and Neomycin. The isolates showed high resistant to Tetracycline, Neomycin and Erythromycin, and most isolates were sensitive to Gentamycin, Ciprofloxacin and Streptomycin. The result clearly shows that isolated bacteria species were generally resistant to different antibiotics used, indicating prevalence of multiple resistant species from the fish sample analyzed. Similar antibiotic resistance profile was also detected by Odjadjare *et al.* (2012). *Pseudomonas aeruginosa* and other bacteria the fish from Okwan Obolo Estuary had multiple antibiotics resistance characteristics and reduced susceptibility. This reduced susceptibility is usually connected to a strenuous action of multiple antibiotic efflux pumps coupled with chromosomally mediated antibiotic resistance determinants such as mexXY, mexAB-oprM) and the reduced permeability of the bacterial cellular envelopes by antimicrobial agents (*Igbinosa et al.,* 2012). Asides the intrinsic factors, Igbinosa and Obuekwe (2014) stated that *P. aeruginosa* can easily develop acquired resistance resulting from the change the genetic makeup in chromosomally encoded determinants or by the horizontal determinants transfer of antibiotic resistance genes (Igbinosa and Obuekwe, 2014). The high prevalence of multiple drug resistant (MDR) *Pseudomonas aeruginosa* and other bacteria isolated from Okwan estuary can be linked to the indiscriminate dumping of waste around the surrounding and inside the estuary and poor management practices. In the process of the bacteria to survive, they mutate and acquire different genes which could be associated to their high resistance to antibiotics (Yagoub, 2009),

CONCLUSION

The research on antibiotics susceptibility profile of Pseudomonas aeruginosa and other bacteria from fish in Okwan Obolo Estuary revealed the isolation of multiple antibiotics resistant *Pseudomonas aeruginosa* and other bacteria (*Bacillus* spp., *Proteus* spp., *Shigella* spp., *Klebsiella* spp., *Enterobacter spp., Salmonella* spp. and *Escherichia coli*) from fish in Okwan Obolo Estuary. This is an epidemiological concern, as the isolates are capable of causing infection in human.

RECOMMENDATIONS

From the research, the following recommendations are made;

i. Federal and State Agencies such as Nigeria Integrated Water Resources Commission, National Water Resources Institute (NWRI) and River Basin Development Authorities should ensure that the dumping of waste in water bodies and other poor practices as regards water should be highly prohibited.

ii. Estuary and Streams should be analyzed regularly and properly treated, if possible, to curb the number of pathogenic microorganisms prevalent in the water bodies.

iii. Fish should be properly and efficiently cooked before consumption, as to prevent passage of infection from fish to humans (final consumers).

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