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WATER QUALITY OF SURFACE WATER BODIES AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *PSEUDOMONAS* SPECIES IN RIVERS STATE, NIGERIA

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ABSTRACT

The study was aimed at determining the antimicrobial susceptibility pattern of *Pseudomonas* species associated with surface water bodies in three selected rivers (Bonny, Mgboshimini and Ogbogoro) in Port Harcourt Metropolis, Rivers State. Thirty-six samples were collected from the rivers designated stations A, B, and C respectively amid two seasons (Dry and raining) and analyzed for physico-chemical and microbiological characteristics using standard analytical procedures. Result obtained from physico-chemical analysis varied within the two seasons, the pH of the samples from the three different Stations during dry season ranged from 7.10 -7.20 while that of rainy season was from 7.20 -9.10. Total suspended solids during dry season ranged from 32.70 – 53.50mg/L showing significant variation from WHO Standard which is 30mg/L, total dissolved solids for rainy season ranged from 640 -2800mg/L while Station A had the highest value of 2110mg/L for Total dissolved solids during dry season these were all significantly high in comparison with WHO standard. The mean total heterotrophic bacterial counts (CFU/ml) for the three stations during dry season were 5.04 ± 0.001^{a}, 4.97 ± 0.001^{b} and 4.96 ± 0.001^{b} respectively, Station A was significantly different from Station B and C. While the total heterotrophic bacterial counts during rainy season were 4.97 ± 0.001^{a}, 4.89 ± 0.001^{b} and 4.97 ± 0.001^{a} with Station B significantly different from Station A and C. The total Pseudomonad counts (CFU/ml) showed significant difference amongst the stations during dry season with 3.97 ± 0.10^{a}, 3.85 ± 0.10^{b} and 4.14 ± 0.10^{c} respectively also total pseudomonad count (CFU/ml) during rainy season showed significant difference ($P < 0.05$) within the stations, 2.43 ± 0.001^{a}, 3.17 ± 0.001^{b} and 4.02 ± 0.001^{c} respectively. Similarly total coliform count ranged from 6MPN/100ml to 11MPN/100ml and 11MPN/100ml to 21MPN/100ml for dry and raining season respectively. Ten genera of bacteria isolates were identified: *Alcaligenes* (3.3%), *Bacillus* (3.9%), *Escherichia coli* (15.6%), *Klebsiella* (14%), *Pseudomonas* (12.7%), *Salmonella* (8.8%), *Seratia* (2.3%), *Shigella* (17.9%), *Staphylococcus* (4.2%) and *Vibrio* (17.3%). The result of antimicrobial analysis revealed that of the ten antibiotic used the Pseudomonad isolates were susceptible to Amoxacillin (25%,), Gentamycin(22.9) and Amoxacillin (22.9%). The isolates were highly resistant to Augumentin (81.3%) and Streptomycin (64.6%). Isolate displayed site- difference in resistance and susceptibility pattern. The need to monitor water quality and improve awareness of the risk to infections associated with *Pseudomonas aeruginosa* is essential. Adherence to appropriate antibiotic use should be encouraged to minimize the emergence of multidrug resistant bacteria.

KEYWORDS: River water, *Pseudomonas* species, antimicrobial resistance.

INTRODUCTION

Water has and will always remain one of the most pivotal natural resources for the sustenance of life on earth and the importance of water; ground or surface cannot be overemphasized. Unlike ground water whose major function is serving as a source of drinking water, surface water is multipurpose; it functions in transportation, recreation, power generation, manufacturing industries, irrigation, food production and processing (Mackereth *et al.,* 2003). Surface water is regarded as water on land surface open to the atmosphere and subject to runoff. It may be running such as in streams and river or dormant such as in lake, reservoir and ponds. The versatility of surface water has made it the most impacted by the activities of man through which numerous substances may enter the water and cause ecological damage.

In Rivers state characterized by high rainfall and pockets of water bodies which are mostly rivers waste is often transported in open trucks and dumped in open dumpsites often located near residential areas, into creeks, ditches, open unused piece of land and rivers (Obire *et al.,* 2002). Unfortunately, river waters are being

polluted indiscriminately affecting their physico-chemical characteristics and microbiological quality (Koshy *et al.,* 1999; Obire *et al.,* 2002). Pollutants are foreign substances found in water in quantities high enough to cause harm or damage to living and non-living things. According to Nmom (2005), the environmental impact of pollutants depends on its concentration in the environment and its degree of toxicity. Surface water is reported to be constantly under threat from environmental contamination. Contamination of water is a global problem affecting both the industrialized and developing Nations (Chiras *et al.,* 1985).

Microbial pollution in aquatic environments is one of the crucial issues with regard to the sanitary state of water bodies used as drinking water supply, recreational activities and harvesting seafood due to a potential contamination by pathogenic bacteria, protozoa or viruses (René *et al.,* 2006). Bacterial organism inhabit a variety of environments, some of these environments serve as their natural habitat , the load and presence of certain organism due to human activity determines contamination (Adokiye *et al.,* 2006).

Pseudomonas is a gram-negative rod shaped bacteria containing 191 validly described species (Todar, 2012). The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of places such as water, soil, inert materials and vegetative environments (Kiska *et al.,* 2003). The organism is able to grow and multiply in a variety of water sources including river water, seawater and wastewater (Kimata *et al.,* 2004). Their ease of culture in-vitro and the availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research (Goldberg, 2000). It is an opportunistic pathogen; there is hardly any tissue that it cannot infect when the tissue defenses are compromised. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections (Todar, 2012). *Pseudomonas* possesses several virulence factors which aid in its pathogenicity and resistance to antimicrobial agents (Cane *et al.,* 1999; Lee, *et al.,* 2003).

The objective of this study therefore was to determine the antimicrobial susceptibility pattern of *Pseudomonas* species associated with surface water bodies in Rivers State, Nigeria as to highlight the emergence of antibiotic resistant strains of bacteria and the dangers associated with the indiscriminate use of antibiotics and treat to public health.

MATERIALS AND METHODS

STUDY AREA

The three rivers selected for the purpose of this study were Bonny, Mbgoshimini and Ogbogoro Rivers. The economic significance and the frequency of human activities around the rivers were considered in selection. The Mgboshimini river located in Mgboshimini community, is predominantly fishing site and it also serves as a transport route with its course cutting across several communities, it also serve as a main channel for water runoff in the communities. The Ogbogoro river is strategic for its economic value, major dredging activities take place there, it also has links to the Choba River which is a major fishing site connected to the Imo river, the Bonny river is the main transport route for travellers going to Bonny, also it serves as a mini port for the deposition of several agricultural produce from different communities, the river also serves as a recipient of domestic (sewage) wastes and agricultural waste run offs along the bank of the river.

SAMPLE COLLECTION

A total of thirty six water samples were collected from the three rivers, from points with the highest frequency of human activity on the river. Collection of samples was done twice monthly within a period of six months amid two seasons (Dry and Rainy) using 250ml of sample bottle. The samples were appropriately labeled and were immediately transported to the laboratory in an ice packed cooler. All samples were processed according to microbiological standards within four hours after sample collection.

PHYSICO-CHEMICAL ANALYSIS

A number of physicochemical parameters were determined on each water sample, using standard methods. They included, colour ,conductivity, turbidity, total hardness, total alkalinity ,chloride, total solids, nitrate, sulphate, calcium, magnesium dissolved oxygen (DO), pH, total dissolved solids (TDS), total suspended solids (TSS) and others were nitrate, Magnesium, Chloride, oil, grease, total iron, lead, copper, biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The pH was measured *in-situ* using Hach pH meter (Model EC1O); total dissolved solids was measured *in-situ* using Hach conductivity meter (Model CO150). The dissolved oxygen was also measured *in-situ* using Hach DO meter Model DO175 (Eze *et al.,* 2015). Sulphate was determined using Barium chloride (Turbidimetric) method. Nitrate was determined using Cadmium reduction method. Alkalinity and phosphate were measured using potentiometric titration. The conductivity of the water samples were measured by using pre-calibrated conductivity meter model 611. The measurement was taken at room temperature, samples were put into beaker in adequate volume to dip the electrode and then the scale was set before the conductivity of each sample was noted. Using titration method with EDTA solution total hardness was determined. Using dichromate refluction method Chemical oxygen demand was determined. (COD) and biochemical oxygen demand (BOD) were carried out by

using alkali azide method. All manipulations were done under controlled conditions to avoid contamination.

MICROBIOLOGICAL ANALYSIS

MEDIA PREPARATION

Six media were used for the study; Nutrient Agar (NA), Cetrimide Agar, MacConkey Agar (MAC), Salmonella Shigella Agar (SSA), Thiosulfate citrate bile salts-sucrose Agar (TCBS) and Eosin Methylene blue Agar (EMB). The different agar was prepared according to the manufacturer's instructions.

ISOLATION OF MICROORGANISMS

Tenfold serial dilution was carried out on each water sample (dilution factor was between 10^{-1} to 10^{-3}). An aliquot (0.1ml) was collected from test tube labeled 10^{-2} using a pipette and dropped at the center of the dried medium (Agar plate), with the aid of a spreader (bent glass rod) the aliquot was spread evenly on the entire surface of the medium. The inoculated plates were turned lid down during incubation at 37^{o}C for 18 to 24 hours after which distinct colonies that developed on the plate were counted and recorded as colony forming unit per milliliter (CFU/ml). Pure cultures were obtained from the distinct colonies for further analysis. Coliform counts were ascertained using most probable number technique.

IDENTIFICATION OF ISOLATES

Morphological identification, Grams reaction and Biochemical characterization carried out includes: citrate utilization, indole, catalase, sugar fermentation, coagulase, oxidase and motility test. The results were interpreted using the Bergeys Manual of Systematic Bacteriology (2003).

ANTIMICROBIAL SENSITIVITY TESTING OF *PSEUDOMONAS* ISOLATES

Antimicrobial sensitivity test was performed using modified Kirby-Bauer method to measure the ability of an antibiotic to inhibit bacterial growth in vitro by disc diffusion (Bauer *et al,*. 1996). Pseudomonas was aseptically sub-cultured, 3ml of distilled water was measured and dispensed into the test tube and corked with cotton wool after which it was autoclaved for at (121^{o}C for 15minutes, it was allowed to cool after cooling a loop full of organism was dispensed into the solution and mixed by agitating. Nutrient Agar plates were prepared, sterile swab stick was dipped into the test tube and used to smear on the prepared agar plate, the smear was made all over the plate after smearing a forceps was sterilized using alcohol and spirit lamp, using sterile forceps, a sensitivity paper disc, containing selected chemotherapeutic agents were then soaked on the media. After 24hours, the plates were observed for the zones of inhibition. The distinct antibiotics used for screening includes; Oflaxacin (10µg), Streptomycin (30µg), Septrin (30µg), Sparfloxacin (10µg), Chloramphenicol (30µg), Ciprofloxacin (10µg), Amoxacillin (30µg) Augmentin (30µg), Gentamycin (10µg) and Pefloxacin (10µg). Zones of inhibition of growth around discs indicated susceptibility to the various antimicrobial agents while no visible zone showed that the isolate is resistant. The result of the test was interpreted according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2017).

RESULT

The result of physico-chemical analysis is as represented in Table 1 and compared to the WHO standard for potable and recreational water. The parameters showed varying results. The pH of the water sample ranged from 7.10 – 9.10. With sample A having the highest pH of 9.10 during raining season which is high compared to WHO standard. Total suspended solid, total dissolve solid and total solids in sample A and C were significantly high when compared to WHO standard, while that of sample B fell within the acceptable limit. Elements such as nitrite, sulphate, calcium, magnesium and chloride were all within the acceptable limit when compared, while the highest level of turbidity 11.00NTU was recorded in sample A during raining season. Results for Biochemical Oxygen Demand and Chemical Oxygen Demand were higher than the standard, Sample C showed a COD of 136mg/ml while the WHO value is pegged at 40Mg/ml. Overall from the study , using physic-chemical parameters to ascertain the quality of the river water, the rivers did not comply to the WHO standard for portable and recreational water.

The result of the total heterotrophic bacterial count during the dry season and raining season are as shown in Figure 1a and Figure 1b respectively. Total heterotrophic bacterial count ranged from 4.79 – 5.15 Log_{10}CFU/ml with samples A showing the highest load during raining season.

Whereas total Pseudomonad count during the dry season and raining season are as shown in Figure 2a and Figure 2b respectively ranged from 0.00 – 4.32 Log_{10}Cfu/ml with sample C showing the highest load during dry season. Samples A and B showed zero growth of *Pseudomonas* in-between sampling period.

Table 1: Physico-chemical constituents of the river water samples.

Parameter \ Season	River Water Front Samples						WHO Standard
	Bonny (A)		Mgboshimini (B)		Ogbogoro (C)		
	Dry	Raining	Dry	Raining	Dry	Raining	
Odour	Unobjectionable		Objectionable		Unobjectionable		Unobjectionable
Taste	Objectionable (salty)		Objectionable		objectionable		Unobjectionable
Colour	6.00	33.00	21.00	21.00	12.00	16.00	15 Hazen units
pH	7.10	9.10	7.20	7.20	7.20	8.20	6.5 - 8.5
Conductivity	2770.00	3310.00	60.00	60.00	1140.00	2450.00	1000uS/cm
Turbidity	2.00	7.00	9.00	11.00	1.00	5.00	5NTU
Total Hardness	20.60	65.60	32.90	110.90	22.70	101.70	100mg/L
Total Alkalinity	22.15	53.85	27.80	58.40	19.90	21.00	200mg/L
Chloride	301.00	420.00	9.50	9.50	288.10	340.70	250mg/L
Total Suspended Solids	48	65.00	53.50	75.50	32.70	77.30	30mg/L
Total Dissolved solids	2110.00	2800.00	220.00	640.00	1160.00	2100.00	500mg/L
Total Solids	2159.00	2670.00	273.50	700.50	1192.70	2512.30	500mg/L
Nitrate	0.55	6.50	1. 95	4. 20	0.88	11.60	10mg/L
Sulphate	11.40	47.50	22. 10	20. 10	16.60	65.60	250mg/L
Calcium	9.95	35.10	14. 30	85. 80	11.006	9.06	70mg/L
Magnesium	2.70	9.20	4. 90	7. 60	2.20	11.3	30mg/L
Oil and Grease	<0.001	3.70	0. 45	0. 88	0.01	2.01	0. 1mg/L
BOD_5	23	43	37	40	14	18.20	14mg/L
COD	136	157	112	210	63	83.10	40mg/L
Total Iron	0.24	0.44	0. 39	0. 49	0.18	0.15	0. 3mg/L
Lead	0.27	0.31	0. 02	0. 24	0.30	1.39	0.01mg/L
Copper	0.64	0.98	0. 73	1. 85	0.55	2.65	1.0mg/L

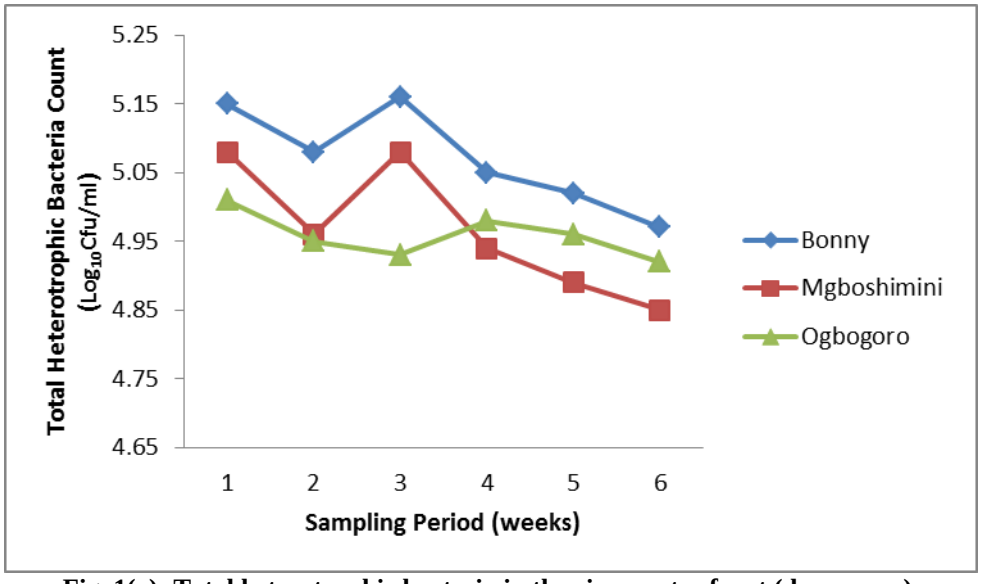

Fig. 1(a): Total heterotrophic bacteria in the river water front (dry season).

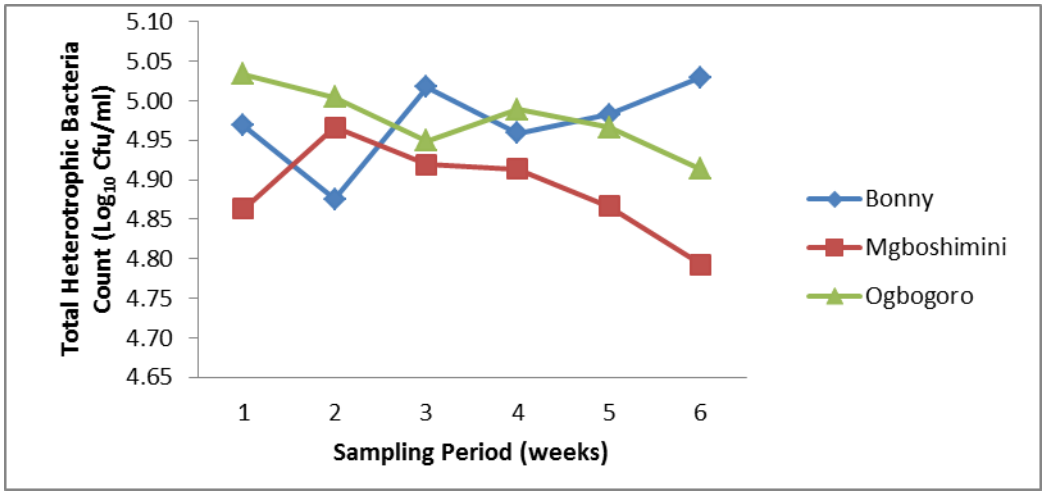

Fig. 1(b): Total heterotrophic bacteria for the river water front (rainy season).

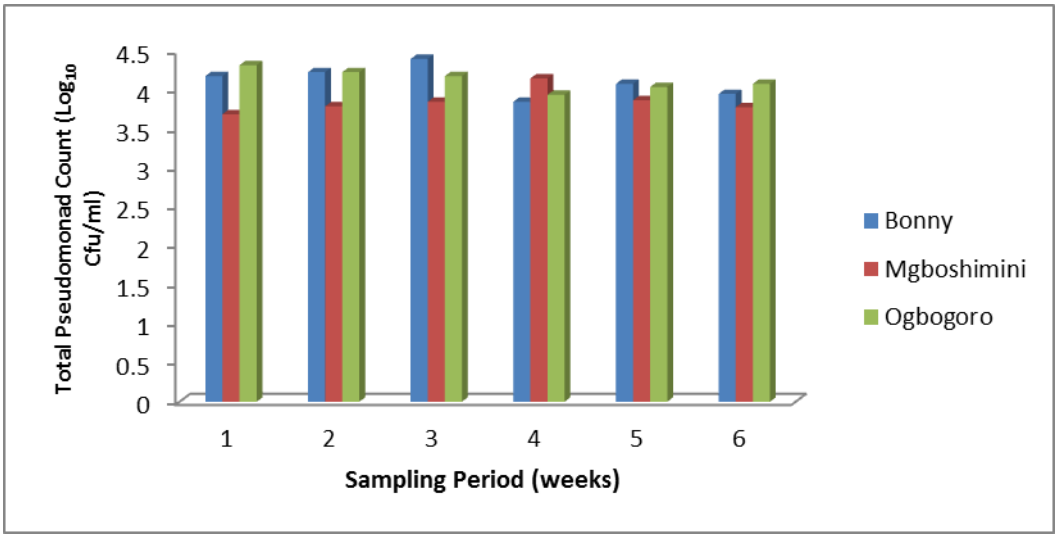

Fig. 2(a): Total Pseudomonad count for the river water front (dry season).

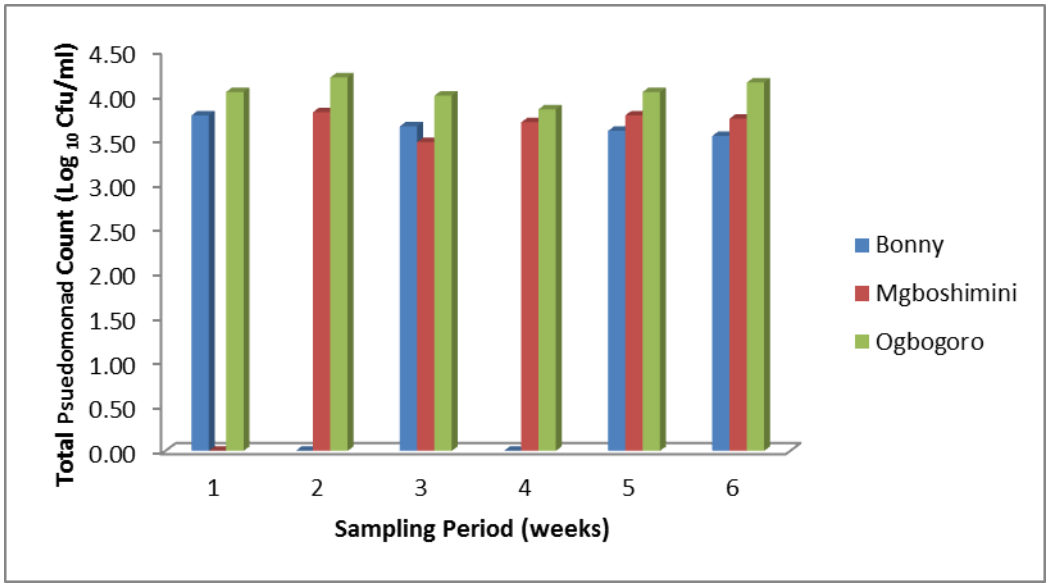

Fig. 2(b): Total Pseudomonad count in the river water front (raining season).

The distribution of bacteria isolated from the river water front samples is shown in Table 2. Based on colony and cellular morphology, and biochemical characteristics, the bacterial isolates identified belonged to 10 genera

namely *Alcaligenes, Bacillus, Escherichia, Klebsiella, Peudomonas, Salmonella, Serratia, Shigella, Staphylococcus* and *Vibrio* (Bergeys Manual of Systematic Bacteriology, 2003).

Table 2: Distribution of bacteria isolated from the river water front samples.

Bacterial genera	River water front		
	Bonny	Mgboshimini	Ogbogoro
Alcaligenes	+	-	+
Bacillus	+	+	+
Escherichia	+	+	+
Klebsiella	+	+	+
Pseudomonas	+	+	+
Salmonella	+	+	+
Serratia	+	+	+
Shigella	+	+	+
Staphylococcus	+	+	+
Vibrio	+	+	+

The frequency of occurrence of bacterial isolate displayed in Fig 3, shows *Shigella* with the highest occurrence of 17.9% and the least occurring was *Alcaligenes* species with a frequency of 3.3 % while *Pseudomonas* species occurred at the frequency of 12.7%.

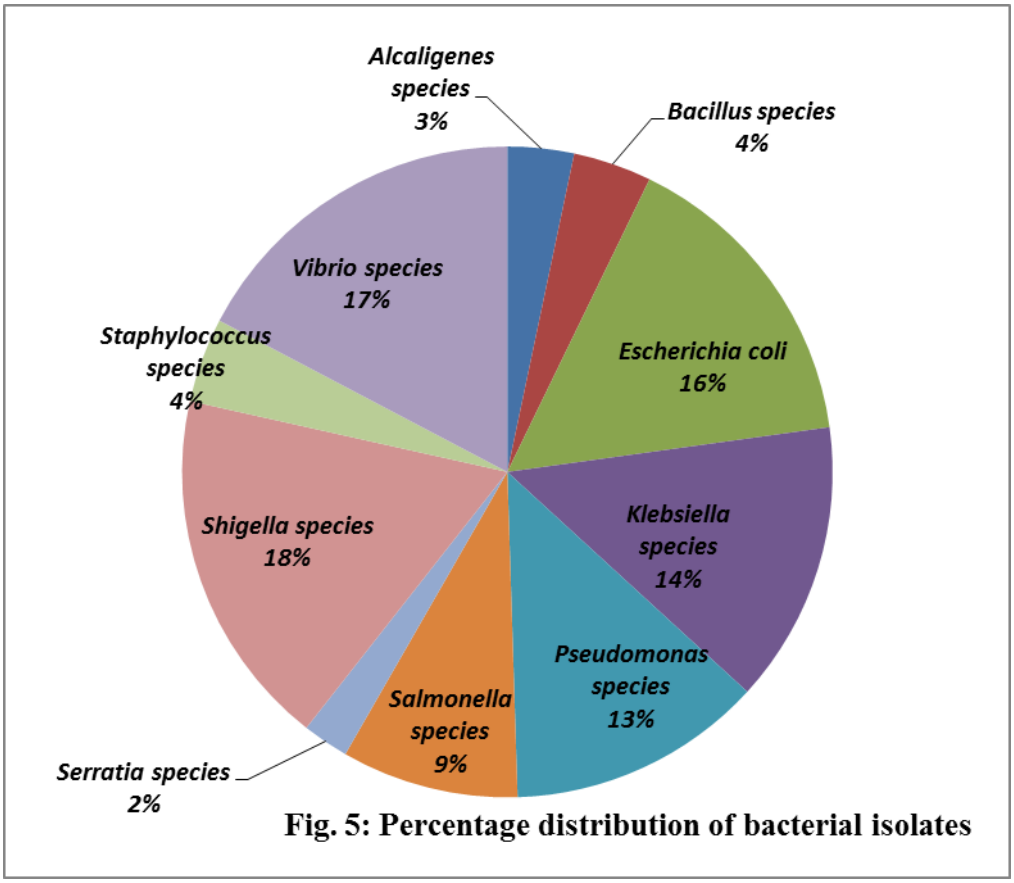

Fig. 5: Percentage distribution of bacterial isolates

Pseusomonas Species isolated from the three different rivers showed variable degree of susceptibility and resistance to antibiotics, isolate from station A showed susceptibility to gentamycin and septrin at 28.6%, while increased resistance to oflaxacin and augmentin at 76.2% and 90.5% respectively, Table 3. *Pseodomonas* isolate from station B also displayed low susceptibility to amoxicillin and ciprofloxacin. Augmentin and Chloramphenicol showed resistance at 72.7% and 63.6% respectively. Table 3 The highest level of resistance was recorded in station C with augmentin showing resistance up to 90.5%,Table 3 while pefloxacin and septrin showed the least susceptibility overall at 18.8% Table 3.

Table 3: Antimicrobial sensitivity and frequency (%) of *pseudomonas species* isolated from three water fronts.

		Antimicrobial Agent – number (%)									
Species (No. of isolates =21)	Resistance Profile	S	OFX	PEF	CN	AU	AM	CPX	SP	CH	SXT
Pseudomonas species (21,Station A)	S	2 (9.5)	-	2 (9.5)	6 (28.6)	-	5 (23.8)	4 (19.0)	3 (14.3)	3 (14.3)	6 (28.6)
	I	6 (28.6)	5 (23.8)	12 (57.1)	10 (47.6)	2(9.5)	9 (42.9)	15 (71.4)	10 (47.6)	8 (38.1)	7 (33.3)
	R	13 (61.9)	16 (76.2)	7 (33.3)	5(23.8)	19 (90.5)	7 (33.3)	2 (9.5)	8 (38.1)	10 (47.6)	8 (38.1)
Pseudomonas species (11,Station B)	S	1 (9.1)	-	1(9.1)	2 (18.2)	-	3 (27.3)	3 (27.3)	1 (9.1)	1 (9.1)	2 (18.2)
	I	4 (36.4)	6 (54.5)	6 (54.5)	6 (54.5)	3 (27.3)	6 (54.5)	7 (63.6)	6 (54.5)	3 (27.3)	4 (36.4)
	R	6 (54.5)	5 (45.5)	4 (36.4)	3 (27.3)	8 (72.7)	2 (18.2)	1 (9.1)	4 (36.4)	7 (63.6)	5 (45.5)
Pseudomonas species (16,Station C)	S	-	-	-	3 (18.8)	-	4 (25.0)	-	2 (12.5)	2 (12.5)	3 (18.8)
	I	4 (25.0)	12 (75.0)	10 (62.5)	12 (75.0)	4 (25.0)	8 (50.0)	12 (75.0)	8 (50.0)	6 (37.5)	6 (37.5)
	R	12 (75.0)	4 (25.0)	6 (37.5)	1 (6.3)	12 (75.0)	4 (25.0)	4 (25.0)	6 (37.5)	8 (50.0)	7 (43.8)
Total	S	3(6.3)	0(0.00)	3(6.3)	11(22.9)	0 (0.00)	12 (25.0)	7 (14.6)	6 (12.5)	6 (12.5)	11 (22.9)
	I	14(29.2)	23(47.9)	28(58.3)	28(58.3)	9 (18.8)	23 (47.9)	34 (70.8)	24 (50.0)	17 (35.4)	17 (35.4)
	R	31(64.6)	25(52.1)	17(35.4)	9(18.8)	39 (81.3)	13 (27.1)	17 (14.6)	18 (37.5)	25 (52.1)	20 (41.2)

Keys (Antimicrobial agent): S: Streptomycin, OFX: Tarivid, PEF: Pefloxacin, CN: Gentamycin, AU: Augmentin, AM: Amoxacillin, CPX: Ciprofloxacin, SP: Sparfloxacin, CH: Chloramphenicol and Septrin. Resistance Profile: S: Susceptibility (≥17mm), I: Intermediate (14 – 16mm), R: Resistant (≤13mm).

DISCUSSION

Water quality is determined by the physico-chemical characteristics of the constituents which are affected by actions in and around the water body. Variation in this constituents leads to alteration in the microbial community of the water (Chilton, 1996).This study revealed several variations in the physico- chemical parameters measured in comparison to WHO standard for potable water. The three rivers studied were not in compliance, despite certain parameters being within acceptable standard, overall quality is affected by other parameters having varying values from the WHO standard. Factors like dumping of effluents in water ways, seepage, runoffs and indiscriminate use of water bodies contributes greatly to its contamination. This study showed high and low levels of physico–chemical constituents. High turbidity according to Shittu *et al.*, (2008) which is often associated with higher levels of disease causing microorganism such as bacteria and other parasites was recorded in two of the sampling cites. Heterotrophic count measures a range of bacteria that are naturally present in the environment, the analysis of THB count in the river water samples revealed the presence of heterotrophic bacterial in all three water sources. Results obtained from this study showed that THB count during dry season ranged from 4.85 – 5.15 Log_{10}CFU/ml whilst the value obtained during rainy season ranged from 4.97 – 5.03 Log10CFU/ml. Total Pseudomonad count also ranged from 3.69 -4.32 Log10CFU/ml and 0.00 – 4.20 Log10CFU/ml for dry and rainy season respectively. The vulnerability of surface water to multiple pathogenic bacteria species is revealed by the isolation of 10 genera of bacteria including *Escherichia coli* and Salmonella which are known pathogenic bacteria species. Bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics (Sharma *et al.*, 2012; Verma *et al.*, 2011; Chitanand *et al.*, 2010). The distribution of susceptible and resistant pseudomonas species to antibiotics from the study showed site specific differences. The three sites showed varying degree of susceptibility to different antibiotics, this is a validation of the multi-drug resistant pattern of *Pseudomonas* due to its genetic makeup (Girlich *et al.*, 2011; Czekalski *et al.*, 2012; Tissera *et al.*, 2013; Blaak et *al.*, 2015). Results showed high resistance of *Pseudomonas* to Augmentin; although isolates in all three river water samples displayed intermediate susceptibility to most antibiotics, susceptibility was low. Pseudomonad isolates were susceptible to Amoxacillin (25%,), Gentamycin(22.9) and Amoxacillin (22.9%). Also, the isolates were highly resistant to Augumentin (81.3%) and Streptomycin (64.6%). Isolates displayed site-difference in resistance and susceptibility pattern. The need to monitor water quality and improve awareness of the risk to infections associated with Pseudomonas aeruginosa is essential. Adherence to appropriate antibiotic use should be encouraged to minimize the emergence of multidrug resistant bacteria. The result further confirms the occurrence of waterborne antibiotic resistant *Pseudomonas* species in surface water bodies in Port Harcourt metropolis.

CONCLUSION

The findings of this study has revealed that the quality of the three rivers sampled are not in compliance with WHO standard and, these rivers are also polluted with antibiotic resistant bacteria pathogen, *Pseudomonas* which is an emerging pathogen of clinical relevance due to its antibiotic resistant pattern. Efforts should therefore be made in limiting the effect of these bacteria in the environment so as to limit the spread of antibiotic resistance. Operational hygienic practices should also be implemented along water bodies to help improve the quality of the water thereby reducing the risk of hazardous effect on humans.

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