International Scholars Journals

Advanced Journal of Environmental Science and Technology ISSN 7675-1686 Vol. 2 (6), pp. 001-008, June, 2011. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The current pollution status of the new Calabar river in the Niger Delta region of Southern Nigeria: A survey of antibiogram profiles of its bacterial isolates

Gideon O. Abu* and Chidiebere Egenonu.

Microbiology Department, University of Port Harcourt, P. M. B 5323, Port Harcourt, Nigeria.

Accepted 12 April, 2011

The incidence of environmental pollutants on bacterial strains isolated from the New Calabar River in the Niger Delta region of Southern Nigeria were investigated in order to ascertain the possible effects on antibiotic resistance and patterns. The physico-chemical parameters of the water samples assayed included temperature, pH, biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, salinity, chloride, nitrate, phosphate, total dissolved solids, sulphate, oil and grease, cadmium, copper, nickel, lead, mercury and iron. Antibiogram of bacterial isolates from the water samples were sought using the disk diffusion method. Results showed multiple antibiotic resistance patterns among the bacterial isolates. The levels of resistance exhibited by the isolates to specific antibiotics were: Ampicillin 66.7%, Rifampicin 66.7%, Tetracycline 53.3%, Cephalothin 46.7%, Erythromycin 46.7%, Novobiocin 40%, Chloramphenicol 33.3%, Nalidixic acid 33.3%, Streptomycin 33.3%, Cotrimoxazole 26.7%, Norfloxacin 13.3%, Ciprofloxacin 6.7%, Ofloxacin 6.7%, Amikacin 0%, Gentamycin 0% and Pefloxacin 0%. Higher incidence of antibiotic resistance was observed at sites with pronounced industrial and human activities, suggesting possible effect of pollutants on the ecosystem. There was weak correlation (r = 0.28) between incidence of antibiotic resistance and faecal coliforms. This suggests that the antibiotic resistance patterns of these bacterial isolates may be due to factors that are not linked to faecal pollution. The presence of chemical pollutants may have contributed to the increased antibiotic resistance observed at sites with pronounced industrial and human activities.

Key words: Antibiogram, New Calabar river, pollution, coliforms, physico-chemical.

INTRODUCTION

In Nigeria, the input of environmental pollutants in aquatic systems is a common phenomenon. The New Calabar River is among the important water resources in the Niger Delta region of Southern Nigeria; it is in the vicinity of the rapidly expanding oil city of Port Harcourt in Rivers State, Southern Nigeria. Most communities within this area are directly dependent on the river for their agricul-tural, recreational, and sometimes, domestic water sup-plies. The river is subjected to effluent discharge from Industries sited along its banks. Also, surface run-off resulting from soil erosion, lumbering activities, forestry operations, dredging activities, and domestic sewage inputs may lead to wide scale contamination of the river. A previous study (Ogan and Nwiika, 1993), revealed multiple antibiotic resistance in bacterial populations of some lower Niger Delta rivers including the New Calabar River. This was attributed to indiscriminate waste disposal in the fresh and brackish water bodies. Ten years later, Odokuma and Ijeomah (2003) reported on heavy metal resistance among bacterial populations of the New Calabar River. Odokuma and Okpokwasili (1997) had also reported on the organic pollution of the New Calabar River, and showed that seasonal changes as well as industrial effluent discharges influenced the organic load of the river. The concern over water quality relates not just to the water itself, but also to the danger of diffusion of toxic substances into other ecosystems (Pretorius, 2000; Bezuidenhout et al., 2002). The aquatic environment for living organisms can be affected and bioaccumulation of harmful substances in the water-dependent food chain can occur (Alam et al., 2006). Surface water is vulnerable to pollution from untreated industrial effluents and munici-

*Corresponding author. E-mail: gideonabu1@yahoo.com.

municipal wastewater, run-off from chemical fertilizers and pesticides, as well as oil and lube spillage in the coastal area from the operation of sea and river ports (Odokuma and Okpokwasili, 1997; Krantz and Kifferstein, 1998; Morrison et al., 2001; Alam et al., 2006). Similarly, increase of faecal pollution in source water is also a problem in developing as well as developed countries (Sinton et al., 1993; Bezuidenhout et al., 2002). This problem is further aggravated where there is lack of sanitation systems, thus posing an increased risk for the outbreak of waterborne diseases (Pretorius, 2000). According to Bahe et al. (2005) many compounds can impose effects at low concentrations within chemical mixtures occurring in the environment. This poses unique monitoring and measurement challenges as well as ways of understanding risks. Exposure to environmental pollutants and changes in nutrient composition could lead to selective pressures favoring certain organisms or genotypes (Lin et al., 2004), resulting in the existence of organisms "resistant" to stressors posing a threat to their very own survival (Phillips et al., 2004). Among the novel selective pressures that face environmental bacterial populations from the industrial revolution include heavy metals, xenobiotic compounds, antibiotics, agrochemicals, as well as organic solvents; these can have a remarkable role on the environmental selection of antibiotic resistance genes (Alonso et al., 2001). Rivers contaminated with urban effluent and agricultural run-offs have been observed to harbor greater antibiotic resistant bacterial populations than areas upstream of the contamination source (Leff et al., 1993; Wiggins et al., 1999; Goni- Urriza et al., 2000; McArthur and Tuckfield, 2000). Such systems show positive correlation between the presence of pollutants and spatial distribution of antibiotic resistance among the microbial populations (Goni-Urriza et al., 2000; McArthur and Tuckfield, 2000). Ogan and Nwiika (1993) suggested that the contribution of faecal bacteria, in the New Calabar River, to multiple antibiotic resistance gene pools might be quite significant. Nevertheless, there is very little information on environmental isolates antibiogram profiles within this region, as limited surveys have been conducted. In the present study, the impact of environmental pollutants on the distribution of antibiotic resistance in bacterial populations within georeferenced sites on the New Calabar River was ascertained without differentiating transferable and non-transferable resistance traits. We georeferenced the sampling sites even though the river is tidal.

MATERIALS AND METHODS

Study area

Ten sites within the New Calabar River were selected for the study (Figure 1). Site 1 is Emohua village, and close to it is an extension base of Wilbros Nigeria Limited (WNL), an oil services industry. The activities here include the building and repair of oil pipelines as well as mini-oil rigs. Dredge pipes laid by WNL mainly surround site 2, although the area formerly harboured a fibre industry whose activity included the manufacture of fibre and allied materials. Within site 3 is WNL and located near it is the Choba market with its terminals close to the river. Site 4 sustains a jetty adjoining the University of Port Harcourt Teaching Hospital (UPTH) staff quarters. The region surrounding site 5, which is Aluu village, is used mainly for dredging as well as other domestic and recreational activities. Site 6 is a narrow creek linking the discharge point of the farming activities within the African Regional Aquaculture Centre (ARAC) into the river. Site 7 is the immediate upstream of the discharge point of ARAC. The ARAC operates on integrated poultry and fish farm. Site 10 is the upstream of the discharge point of ARAC. Sites 8 and 9 are located on a creek adjoining the New Calabar River, between sites 7 and 10. These sites (8 and 9) are considered to be removed from industrial and domestic activities.

Sample collection

Water samples were aseptically taken from the river using 1 sterile screw-capped bottles at different sites. The bottles were opened at about 15 cm depth, allowed to fill, closed under water, and quickly transferred into an ice container. Temperature and pH of the water samples were measured *in situ* at different sites using a mercury bulb thermometer and pH indicator strips non-bleeding (color pHast® pH 5 – 10) respectively. All sites were georeferenced using a handheld global positioning system (GPS) receiver unit (Megallan GPS 315) to generate geographic coordinates (longitudes and latitudes) on the New Calabar River. The water samples were then transported to the laboratory and analyzed within 8 h of collection.

Isolation of bacteria

A ten-fold serial dilution of the water samples from different sites were used for the enumeration of bacterial species using the spread plate technique; and membrane filtration technique was employed for total and faecal coliform counts. Tryptone Soy Agar (TSA), Eosin Methylene Blue (EMB) agar, Thiosulphate Citrate Bile Sucrose (TCBS) agar and *Salmonella-Shigella* Agar (SSA) were used to determine total aerobic heterotrophic bacterial (THB) population, total and faecal coliforms, *Vibrio* species and *S. shigella* species respectively. All plates were incubated at 35^{o}C for 24 h with the exception of EMB plates meant for the isolation of faecal coliforms, which were incubated at 44.5^{o}C for 24 h. Analyses of water samples were performed in triplicates.

Characterization and identification of bacterial isolates

Single colonies of bacteria were randomly selected from different media plates based on their morphology. These bacterial cultures were subsequently isolated in pure forms. Representative bacterial isolates were characterized and identified as described by Holt et al. (1994). This was based on Gram-reaction, Acid-fast staining reaction, spore staining, motility test and biochemical reaction tests.

Physicochemical analysis

The physico-chemical parameters such as dissolved oxygen (DO), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chloride, salinity, sulphate, phosphate, nitrate, oil and grease, cadmium, copper, nickel, lead, mercury and iron analyses of the water samples were determined as described by the American Public Health Association (APHA ,1992).

Antibiotic susceptibility test

Antibiogram of the bacterial isolates from water samples in this

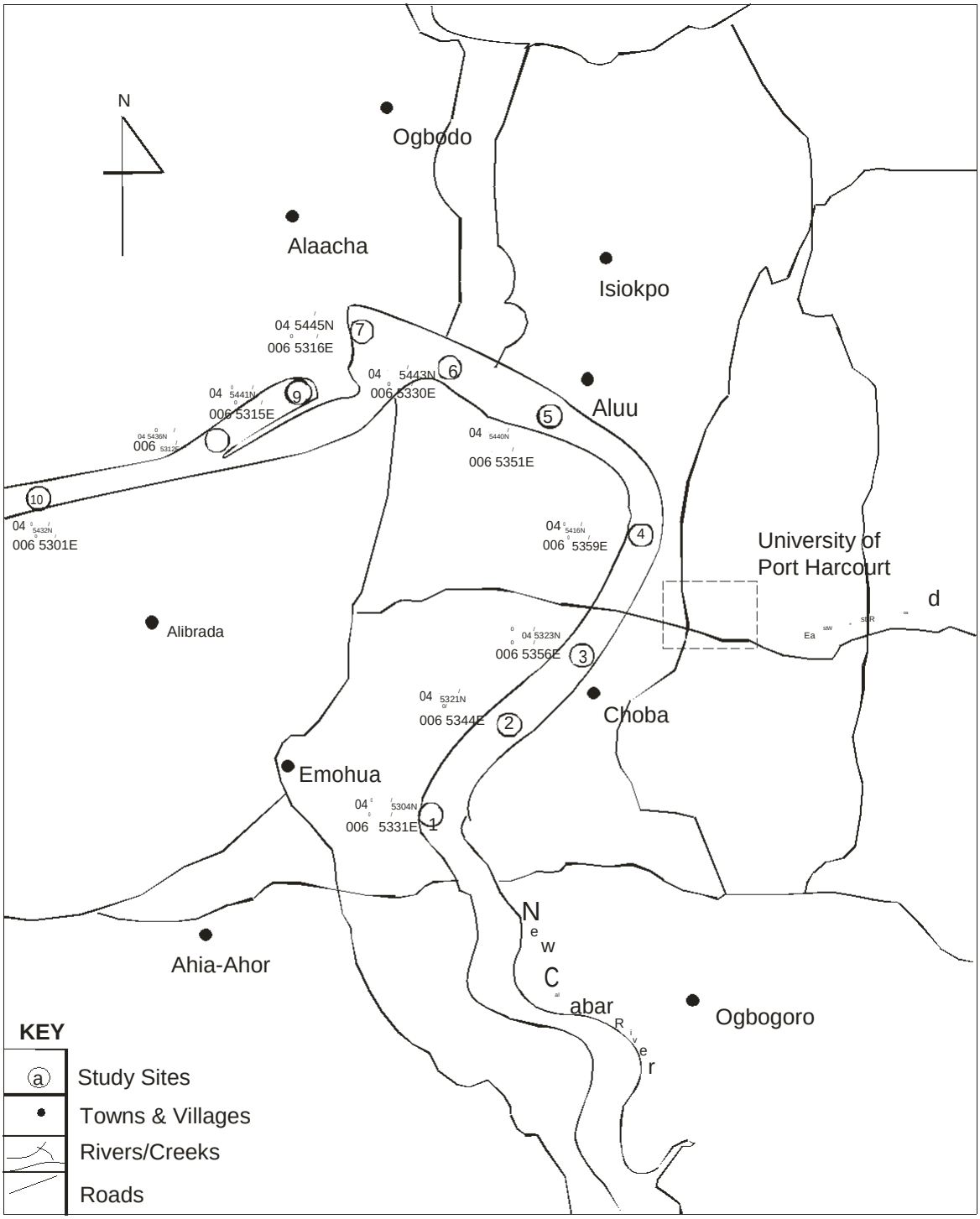

Figure 1. A map showing the ten (1-10) georeferenced sites on the New Calabar River.

study was ascertained on Mueller-Hinton agar using the Kirby-Bauer disc diffusion method as described by Harley and Prescott (1990) . A total of 16 antibiotics corresponding to drugs most commonly used in the treatment of human and animal infections caused by Gram-negative and Gram- positive bacteria were employed in this study. The antibiotics and their concentrations included Ampicillin (ß-lactamases) 30 µg, Cotrimoxazole (Folate inhibitors) 30 µg, Nalidixic acid (Quinolones) 30 µg, Cephalothin (Cephalosporins) 30 µg, Streptomycin (Aminoglycosides) 30 µg, Novobiocin (Aminocoumarins) 30 µg, Pefloxacin (Fluoroquinolones) 10 µg, Gentamycin (Aminoglycosides) 10 µg, Tetracycline (Tetracyclines) 30 µg, Ofloxacin (Fluoroquinolones) 10 µg, Amikacin (Aminoglycosides) 30 µg, Ciprofloxacin (Fluoroquinolones) 10 µg, Rifampicin (Ansamycins) 20 µg, Erythromycin (Macrolides) 30 µg, Norfloxacin (Fluoroquino-

Table 1. Mean values of physico-chemical indicators of the pollution status of the New Calabar River.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	WHO standard
Temperature (^{0}C)	27	26	27	26	26	26	26	25	25	24	NA
pH	5.5	6.0	6.0	5.0	5.5	5.5	5.0	5.0	5.0	5.0	6.5 – 8.5
BOD (mg/)	2.42	2.40	2.72	2.34	2.60	2.50	2.30	2.00	1.62	1.50	0 – 6
COD (mg/)	7	11	9	6	9	7	5	4	3	3	NA
DO (mg/)	6.24	6.00	5.08	6.45	5.20	5.72	6.92	7.00	7.40	7.10	0–20
Salinity (ppt)	0.019	0.015	0.017	0.011	0.0096	0.0093	0.0089	0.0049	0.0022	0.0021	0.5
Chloride (mg/)	10.42	8.24	9.31	6.00	5.32	5.12	4.90	2.72	1.21	1.17	250
Nitrate (mg/)	0.35	0.33	0.32	0.24	0.54	0.41	0.42	0.27	0.10	0.20	50
Phosphate (mg/)	0.24	0.14	0.25	0.10	0.29	0.32	0.13	0.10	0.05	0.07	2
TDS (mg/)	1.34	1.25	1.64	1.14	1.52	1.47	1.12	1.08	1.02	1.04	1000
Sulphate (mg/)	29.4	25.1	30.4	23.2	30.5	31.2	17.4	16.6	12.2	13.5	250
Oil and Grease (mg/)	0.020	0.042	0.024	ND	0.009	0.005	0.005	0.001	ND	ND	NA
Cadmium (mg/)	0.020	0.072	0.065	0.010	0.097	0.055	0.045	0.036	ND	ND	0.003
Copper (mg/)	0.072	0.104	0.090	0.060	0.090	0.076	0.050	0.040	0.010	ND	2
Nickel (mg/)	ND	0.006	0.0072	ND	0.009	ND	ND	ND	ND	ND	0.07
Lead (mg/)	0.124	0.230	0.180	0.120	0.180	0.160	0.135	0.120	0.09	0.1	0.01
Mercury (mg/)	ND	ND	ND	ND	ND	0.01	ND	ND	ND	ND	0.006
Iron (mg/)	0.23	0.31	0.37	0.22	0.40	0.21	0.18	0.16	0.12	0.15	1 – 3

ND = Not detected; NA = Not available.

lones) 10 µg and Chloramphenicol (Phenicols) 30 µg.

Statistical analysis

Analysis of Variance (ANOVA) was used to ascertain significant variations of parameters within different sites of the New Calabar River. Pearson's correlation coefficient (r) was used to represent the relationship between bacterial/physico-chemical parameters of the New Calabar River and incidence of antibiotic resistance within different sites. Probability was set at P = 0.05.

RESULTS

Variation of bacterial groups in water samples from different sites of the New Calabar River is as shown in Figure 2. The total aerobic heterotrophic bacterial (THB) counts of the water samples ranged from $1.0 \times 10^4 – 9.2 \times 10^8$ cfu/m . Total coliform and faecal coliform counts of the water samples ranged from $2.0 \times 10^2 – 6.0 \times 10^5$ cfu/100m and $2.0 \times 10^1 – 1.0 \times 10^5$ cfu/100m respectively. Results on physico-chemical parameters (Table 1) revealed that higher values in BOD, COD and TDS were observed in sites 3, 5, 6, 2 and 1. Contrarily, lower values in DO were observed in these sites as compared to other sites. The pH range of 5.0 – 6.0 which is moderately acidic was observed in this study. The values of nitrate, sulfate and phosphate were higher in sites 5 and 6. The salinity and chloride levels were 0.0021 – 0.019 ppt and 1.21 – 10.42 mg/m respectively indicative of brackish water. Concentrations of individual heavy metals in the water samples were in the order: Fe > Pb > Cu > Cd > Ni > Hg. With the exception of cadmium, lead and pH (which is acidic at a mean value of 5.35), the mean values of the physico-chemical parameters of the New Calabar River fell within acceptable standard guidelines for potable water (WHO, 2006). Analysis of variance (ANOVA) at P = 0.05 showed significant differences in physico-chemical parameters for water samples from the different sites.

The results revealed marked differences among bacterial isolates in their susceptibility and resistance patterns to a particular antibiotic. All isolates were susceptible to amikacin, gentamycin and pefloxacin. These isolates were also sensitive to ofloxacin with the exception of some *Bacillus* species. Only *Alcaligens* species show-ed resistance to ciprofloxacin. Similarly, only some spe-cies of *Bacillus* and *Pseudomonas* were resistant to norfloxacin. The bacterial isolates were generally resistant to ampicillin and rifampicin. In general, over 70% of all bacterial isolates were susceptible to ofloxacin, pefloxacin, norfloxacin, ciprofloxacin, cotrimoxazole, gentamycin and amikacin. Over 50% were sensitive to cephalothin, novobiocin, chloramphenicol, nalidixic acid, streptomycin and erythromycin. At least 20% of bacterial isolates demonstrated susceptibility to ampicillin, tetracycline and rifampicin. The level of resistance shown by bacterial isolates to specific antibiotic is as follows: Ampicillin 66.7%, Rifampicin 66.7%, Tetracycline 53.3%, Cephalothin 46.7%, Erythromycin 46.7%, Novobiocin 40%, Chloramphenicol 33.3%, Nalidixic acid 33.3%, Streptomycin 33.3%, Cotrimoxazole 26.7%, Norfloxacin 13.3%, Ciprofloxacin 6.7%, Ofloxacin 6.7%, Amikacin 0%, Gentamycin 0% and Pefloxacin 0%. The result shows that susceptibility of bacterial isolates to antibiotics was generally less for the older and widely used antibiotics. Figure 3 shows the incidence of antibiotic resistance among bacterial isolates within the

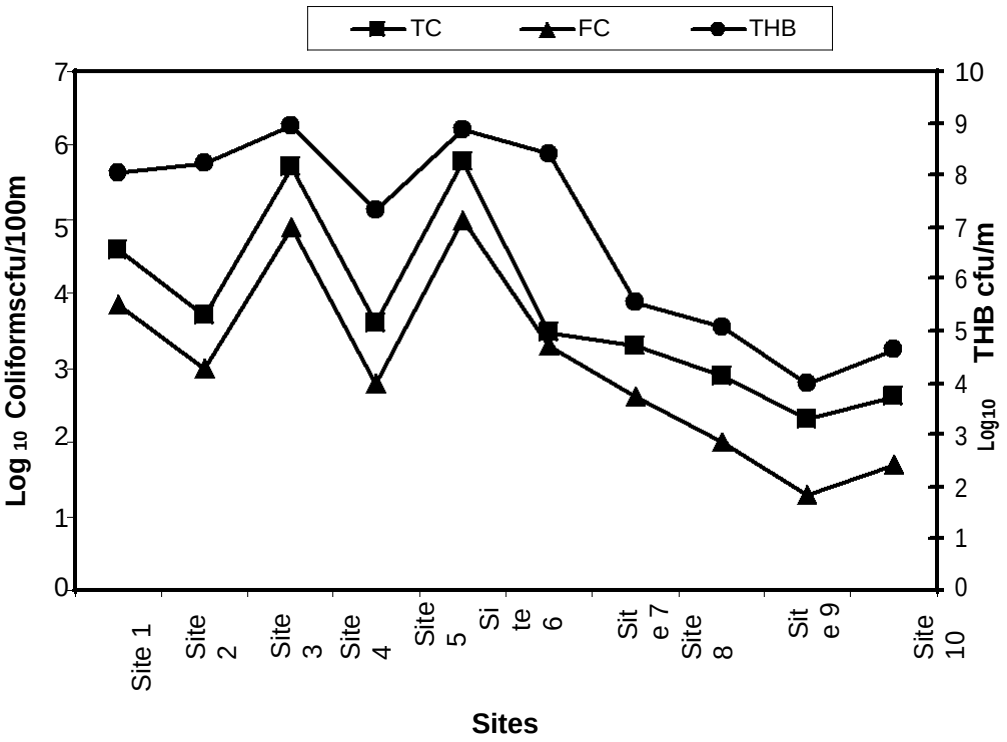

Figure 2. Variatons of total aerobic heterotrophic bacterial (THB) counts ( ), total coliforms (TC) counts ( ) and faecal coliforms (FC) counts ( ) in water samples of the New Calabar River.

different sites. High incidence of antibiotic resistance patterns was found in the order: site 3 > site 2 > site 5 > site 1 > site 6 > site 4 > site 8 > site 9 > site 7 > site 10. From the result, slight variations were observed in the antibiotic resistance patterns of individual bacterial isolates within the different sites. The resistance patterns of different bacterial isolates from the New Calabar River to tested antibiotics are shown in Figure 4. It indicated that *Pseudomonas* exhibited the highest resistance to the antibiotics. *Salmonella*, *Shigella*, *Alcaligens*, *Micrococcus* and *Klebsiella* had the least level of resistance. Statistical analysis (ANOVA) showed significant variations in the incidence of antibiotic resistance within the different sites and the resistance patterns of bacterial isolates to antibiotics respectively.

DISCUSSION

The current level of faecal coliforms suggests that the New Calabar River is unfit for domestic purposes includeing human consumption. Ogan and Nwiika (1993) published results on the ecology of aquatic bacteria of some lower Niger Delta Rivers in Nigeria, including the New Calabar River which indicated that the rivers had high levels of faecal coliforms. The levels of faecal coliforms in the river water could be associated with defaecation into the river by inhabitants of densely populated settlements in and around the region. Washing of faecal material deposited within adjoining land into the river by rain has its own contribution to the level of contamination of the river. The high values of nitrates, sulphates and phosphate within sites 5 and 6 could be attributed to agricultural and human activities in the area. Although most physical and chemical parameters obtained in the present study were of acceptable World Health Organization (WHO) standards, sites 3, 5, 2, 6 and 1 were of poor quality compared to other sites. Cumulatively, human (Industrial, agricultural and domestic) activities around these sites were pronounced during the study period. These factors could have contributed independently or in combination, to the very high levels of BOD, COD and TDS as well as lower levels of DO observed within these sites. Odokuma and Ijeomah (2003), stated that the pollution potential of industrial effluent discharges in the New Calabar River was negligible and as such did not contribute to the seasonal variation in the heavy metals content of the river water and sediment during the course of their study. The pollution trend may have changed between 2003 (Odokuma and Ijeomah, 2003) and the period of our study. Thus, the antibiotic susceptibility testing of bacteria isolated from water samples of the New Calabar River in our study showed that a large proportion was resistant to antibiotics. High resistance of bacterial iso-lates in this study to ampicillin, tetracycline, rifampicin, cephalothin and erythromycin corroborates the findings of Obi et al., (2004) who showed that at least 20% of bacte-

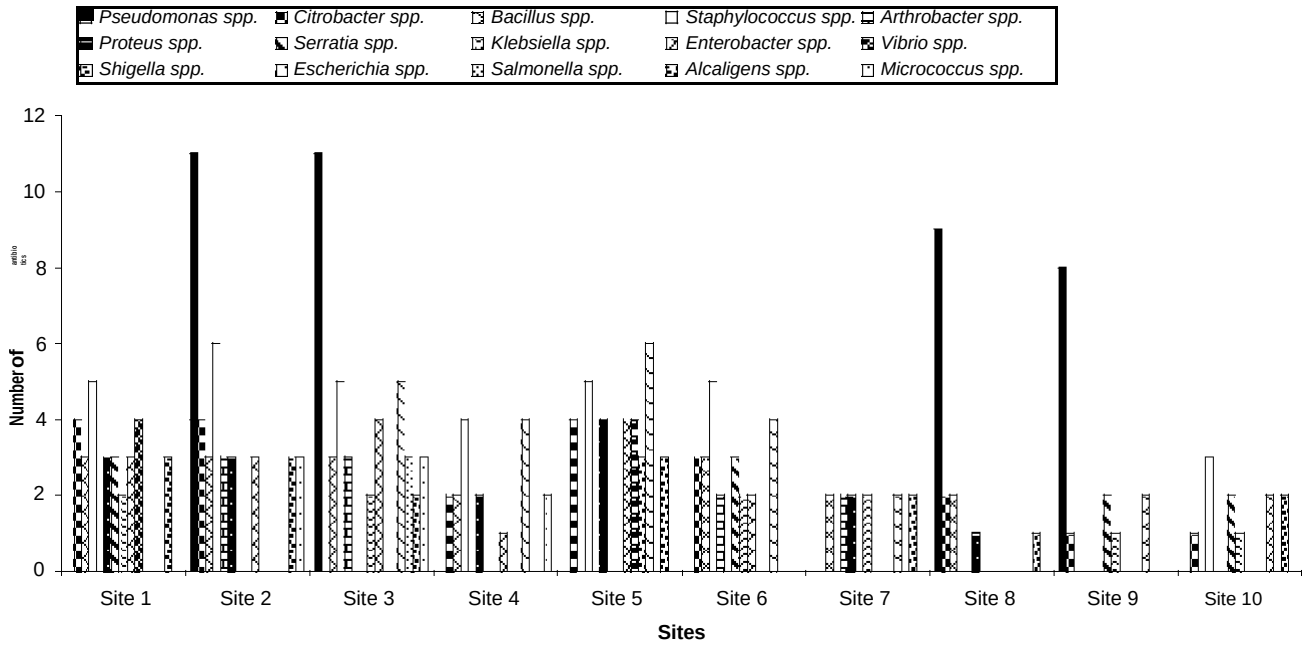

Figure 3. Incidence of antibiotic resistance in water samples of the New Calabar River.

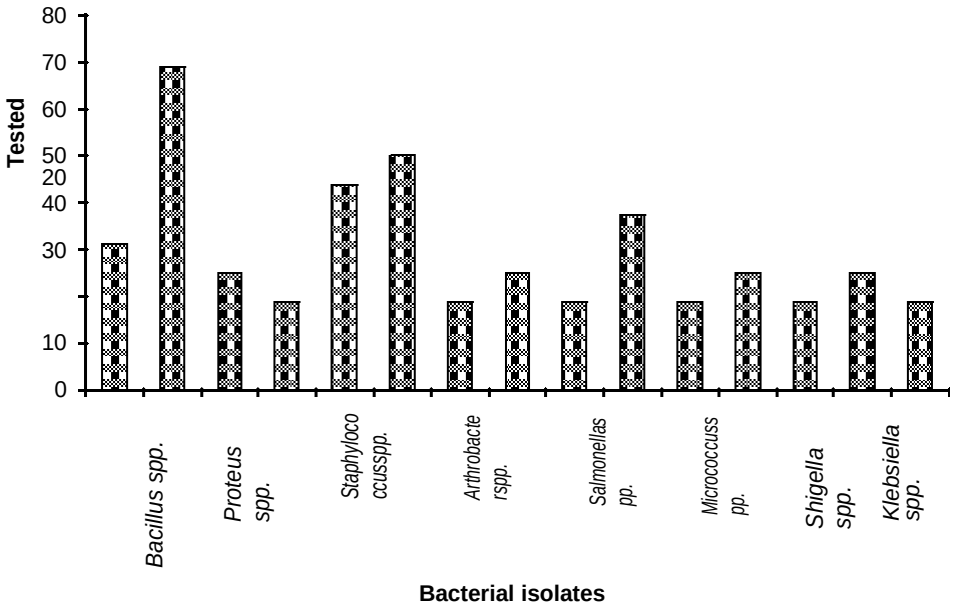

Figure 4. Antibiotic resistance patterns of different bacterial isolates in water samples of the New Calabar River.

terial isolates from water supply in rural Venda communities of South Africa demonstrated antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol. However, in the current study, differences were observed in the incidence of antibiotic resistance within the different sites. Bacterial isolates from sites 3, 2, 5, 6 and 1 showed higher level of resistance against several antibiotics compared to isolates from other sites. These differences in incidence of antibiotic resistance may be attributed to the impact of Industrial and human activities on the bacterial isolates within these sites. This is in harmony with the strong correlation observed between incidence of antibiotic resistance and physico- chemical parameters of the sites within the New Calabar River. There are reports demonstrating the role played by industrial and human activities on the antibiotic resistance distribution of bacterial isolates in the environment (Davidson, 1999; Lin et al., 2004). McArthur and

Tuckfield (2000) demonstrated that chemically contaminated streams might contribute to increased antibiotic resistance. Goni-Urizza et al. (2000) found a correlation between antibiotic resistant bacteria in rivers and the input of urban effluents. In the present study, sites 8 and 9, which have little or no industrial and human activities within them, were observed to have high incidence of antibiotic resistance. The presence of *Pseudomonas* species, which are naturally multi-resistant organisms (Quinn, 1998; Goni-Urizza et al., 2000) within these sites, may explain that situation. This should imply that species composition might have influenced the frequency and distribution of antibiotic resistance in the environment. Ogan and Nwiika (1993) tended to attribute the prevalence of antibiotic resistance in river water to faecal bacteria such as the coliforms. But, the weak correlation (r = 0.28) between faecal coliforms and incidence of antibiotic resistance observed in our study, suggests that the impact of faecal coliforms on antibiotic resistance among isolates within the New Calabar River is insignificant. However, a wide variation in antibiotic resis-tance patterns was found among the different bacteria genera isolated in our study. These variations may be attributed to predisposition of isolates to the prevailing selective pressure in the river or to pre-existing such as genetic composition and molecular mechanisms including cell permeability in the organisms (Guardabassi and Dalsgaard, 2002; Kummerer, 2004). This could possibly explain why resistance to ofloxacin and ciprofloxacin in our study was seen only among *Bacillus* species and *Alcaligens* species respectively. It is therefore imperative to note that susceptibility of bacteria to antibiotics could be altered by the impact of environmental and human activities on such isolates. This possibly results in the development and selection of antibiotic resistant strains. This is a health risk as infections of such resistant strains are more difficult to treat. Heavy metals such as mercury can be mobilized into the food chain when they are methylated by bacteria under anaerobic conditions.

Conclusion

Evidence provided in this study indicated that high levels of antibiotic resistance were observed within sites with pronounced industrial and human activities. It showed that the presence of environmental pollutants in the New Calabar River might have contributed to the observed phenomenon. Observation of antibiotic resistance around areas with little or no indicators of pollution suggests that the expression of multiple antibiotic resistance property by bacteria is a function of several inter-related factors. These could mainly be due to intrinsic disposition of isolates and environmental impacts. However, there is need for further studies in order to ascertain the underlying factors responsible for this trend within the New Calabar River. Thus, careful measures should be adopted with regards to the input of environmental pollutants, because their potential impact on the ecosystem may be much greater than its direct effect on human life.

ACKNOWLEDGEMENTS

Dr. C. A. Tse of Department of Geology, University of Port Harcourt is acknowledged for use of the GPS.

REFERENCES

Alam MN, Elahi F, Didar-UL-Alam Md (2006) Risk and Water Quality Assessment Overview of River Sitalakhya in Bangladesh. Academic Open Internet J. 19 ISSN 1311-4360. [online]. http://www.acadjournal.com

Alonso A, Sanchez P, Martinez JL (2001) Environmental selection of antibiotic resistance genes. Environ. Microbiol. 3 (1): 1-9.

American Public Health Association (APHA, 1992) Standard Methods for the Examination of Water and Wastewater. 18th ed. American Public Health Association, Washington, D. C.

Bahe AR, Classen JJ, Williams B, Stavely J (2005). Emerging Environmental Contaminants and Antibiotic Resistance: Science and Policy Concerns. Animal Waste Management Symposium, pp. 246-259.

Bezuidenhout CC, Mthembu C, Puckree T, Lin J (2002). Microbiological evaluation of the Mhlathuze River, KwaZulu-Natal (RSA). Water SA. 28(3): 281-286.

Davidson J (1999) Genetic exchange between bacteria in the environment. Plasmid. 42: 73- 91.

Goni-Urriza M, Capdepuy M, Arpin C, Raymond N, Caumette P, Quentin C (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp. Appl. Environ. Microbiol. 66(1): 125- 132.

Guardabassi L, Dalsgaard A (2002). Occurrence and fate of antibiotic resistant bacteria in sewage. Environmental Project No. 722, Danish Environmental Protection Agency, Danish Ministry of the Environment.

Harley JP, Prescott LM (1990). Laboratory Exercises in Microbiology. Wm. C. Brown Publishers, USA.

Holt JG, Krieg NR, Sneath PHA, Stanley JT Williams ST (1994). Bergey's manual of determinative bacteriology. 9th ed. Baltimore, Md: Williams and Wilkins Pub. Co., Maryland. **pp???**

Krantz D and Kifferstein B (1998). Water Pollution and Society. [online]. http://www.umich.edu/~gs265/society/waterpollution.html.

Kummerer K (2004). Resistance in the environment. *J. Antimicrob. Chemother*. 54(2): 311-320.

Leff LG, Dana JR, McArthur JV, Shimkets LG (1993). Detection of Tn5 – like sequences in kanamycin-resistant stream bacteria and environmental DNA. Appl. Environ. Microbiol. 59: 417- 421.

Lin J, Biyela PT, Puckree T (2004). Antibiotic resistance profiles of environmental isolates from Mhlathuze River, KwaZulu-Natal (RSA). Water *SA*. 30(1): 23-28.

McArthur JV, Tuckfield RC (2000). Spatial Patterns in Antibiotic Resistance among Stream Bacteria: Effects of Industrial Pollution. Appl. Environ. Microbiol. 66(9): 3722-3726.

Morrison G, Fatoki OS, Persson L, Ekberg A (2001). Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River - pH, electrical conductivity, oxygen-demanding substances (COD) and nutrients. Water *SA*. 27(4): 475-480.

Obi CL, Bessong PO, Momba MNB, Potgieter N, Samie A, Igumbor EO (2004). Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa. Water SA. 30(4): 515-519.

Odokuma LO, Okpokwasili GC (1997). Seasonal Influences of the Organic Pollution Monitoring of the New Calabar River, Nigeria. Environ. Monitor. Assess. 45: 43-56.

Odokuma LO, Ijeomah SO (2003). Seasonal changes in the heavy metal resistant bacteria population of the New Calabar River, Nigeria. Global J. Pure and Appl. Sci. 9(4): 425- 433.

Ogan MT, Nwiika DE (1993). Studies on the ecology of aquatic bacteria of the lower Niger Delta: Multiple antibiotic resistance among the standard plate count organisms. J. Appl. Bacteriol. 74: 595-602.

Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightgale C, Preston R, Waddell J (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53: 28-52.

Pretorious L (2000). An investigation into the effect of various levels of sanitation on surface water quality in a typical developing community. SA Waterbulletin. 26(23):10-12.

Quinn JP (1998) Clinical problems posed by multi-resistant non-fermenting Gram-negative pathogens. *Clin. Infect. Dis*. 27 S117-S124.

Sinton LW, Donnison AM, Hastie CM (1993). Faecal streptococci as faecal pollution indicators: a review. II. Sanitary significance, survival, and use. N. Z. J. Mar. Freshwater Res. 27: 117-137.

WHO (2006). Guidelines for drinking-water quality (electronic resource). Incorporating first addendum – 3rd ed. Volume 1. Recommendations. World Health Organization, Geneva.

Wiggins BA, Andrews RW, Conway RA, Corr CL, Dobratz EJ, Dougherty DP, Eppard JR, Knupp SR, Limjoco MC, Mettenberg JM (1999). Use of antibiotic resistance analysis to identify non-point sources of faecal pollution. Appl. Environ. Microbiol. 65(8): 3483-3486.