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Research Article

Antibiotic sensitvity and plasmid profiles of bacteria isolated from water sources in Oproama community in the Niger Delta

Asiton-a A. Dick[1]*, Gideon O. Abu[2], Stella N. Ibe[2]

[1]Department of Science Laboratory Technology, School of Science, Rivers State College of Arts and Science, P.M.B. 5936, Rumuola, Port Harcourt, Nigeria.

[2]Department Of Microbiology, University Of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Nigeria.

*Correspondence: Asiton-a A.Dick; asitona@yahoo.com; +2348064131848, +2348055673874

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ABSTRACT: The antibiotic sensitivity pattern and plasmid profile of *Escherichia coli*, *Vibrio* and *Salmonella* species isolated from well and river water sources in Oproama Community were investigated. Antibiotic sensitivity profiles of the bacteria (*Escherichia coli*, *Vibrio* sp. and *Salmonella* sp.) isolated from the water showed high sensitivity to oflaxicin, nalidixic acid and nitrofurantoin and high resistance to amoxicillin, augumentin, cotrimazole and tetracycline. Multi antibiotic resistant index (MARI) as high as 0.375 (*Escherichia coli*: E9; *Vibrio* spp.: V3, V4, V10; *Salmonella* spp.: S1, S9), 0.5 (*Vibrio* spp.: V2; *Salmonella* spp.: S2, S4) and 0.75 (*Salmonella* spp.: S7) were recorded after curing the plasmids with sodium deodecyl sulphate (SDS). The plasmid profiles revealed that 60% of the isolates harboured detectable plasmids with sizes up to 23.130 kb.

KEYWORDS: Antibiotics, *Escherichia coli,* Plasmid, *Salmonella*, *Vibrio*.

INTRODUCTION

Bacterial resistance to antibiotics constitutes an emerging clinical problem due to the wide availability and frequent misuse of antibiotics (Davies and Amabile-Cuevas, 2003). The emergence of antibiotic resistant pathogenic bacteria in clinical environments has become a serious problem worldwide French (2005). However, drug resistant bacteria have also been detected from natural environments, where no direct exposure to antibiotics is known (Schwartz *et al*., 2003; Goni-Urizza *et al*., 2000). The presence of such non-clinical resistant bacteria poses a risk to humans and the environment as they may act as resistance reservoirs, contributing to the maintenance and spread of antibiotic resistance genes (Goni-Urizza *et al*., 2000).

Concerns have been raised on the spread of resistance from indigenous environmental bacteria to pathogenic organisms with the potential for compromising antimicrobial treatment of pathogenic organisms (Davies, 1996; Armstrong *et al*., 1995).

Antimicrobials themselves act as a selective pressure that allows the growth of resistant bacteria within a population and inhibits susceptible bacteria (Levy, 1994). Drug resistance property in bacteria is usually borne in R-plasmids, which can be disseminated, to diverse population and regions causing worldwide problems. R-plasmids from resistant strains of an organism may transfer to a sensitive counterpart, which can show the same drug resistance in the donor strain.

In this study, plasmid profiles and drug resistance pattern of multi drug resistant clinical isolates of *E. coli, Vibrio* and *Salmonella* species from well and river water sources were studied before and after curing to find out whether the resistant gene which makes *Escherichia coli, Vibrio* and *Salmonella* species resistant to multiple drugs (antimicrobials) is present in the plasmid DNA or in the chromosomal DNA.

MATERIALS AND METHODS

Water samples collection, processing and bacterial isolation

Water sample were collected from seven (7) wells and three (3) points along the Oproama River in the Niger Delta region of Nigeria. Samples were collected in sterile plastic sample bottles, labelled and kept in cool boxes. The samples were analysed within 24 hours of sample collection. Eosin methylene blue (EMB) Agar, Thiosulphate citrate bile salt (TCBS) Agar and Salmonella-Shigella (SS) Agar were used for the isolation of Escherichia coli, *Vibrio* and *Salmonella* species respectively. Membrane filtration technique was employed as 10 ml of the water samples were filtered through a sterile membrane filter (Millipore, 0.45 µm, 47 mm) (APHA, 1992). Representative discrete colonies were examined culturally, Gram-stained and subjected to biochemical characterisation and according to Cheesborough (1984).

Antibiotic Sensitivity Test

The isolates were then subjected to antibiotic sensitivity testing by the disc diffusion method on Sensitive Test Agar (LAB M) described by Bauer *et al*. (1979). Commercially available antimicrobial discs were used in the study and included: oflaxicin (5 µg), gentamicin (10 µg), nalixidic (30 µg), nitrofurantoin (200µg), cotrimazole (25 µg), amoxicillin (25µg), tetracycline (25 µg) and augumentin (30 µg). Plates were incubated at 35 ^{o}C for 18-24 hours. Zones of inhibition were interpreted as resistant, intermediate or sensitive using the interpretive chart of the zone size of the NCCLS (2005, 2006). Interpretation of results was done using the zone of inhibition.

Curing of Plasmid

The curing of plasmid was done according to modified methods of Vyvyan *et al*. (1972) and Kai *et al*. (2002) using sodium dodecyl sulphate (SDS). One hundred millilitres of nutrient broth was inoculated with 0.5 ml of the test isolate from overnight culture broth incubated for 24 hours at 37 ^{o}C. The freshly inoculated nutrient broth culture was incubated for 3-4 hours to allow for minimal growth of the organism. Then sterile 10% (w/v) sodium dodecyl sulphate (SDS) solution was added, sufficient to bring the concentration to 1% (w/v). This was then incubated for 48 hours at 37 ^{o}C to ensure there was growth of the test organism. Freshly prepared nutrient broth was again prepared and then inoculated with the test culture and incubated for 24 hours at 37 ^{o}C. Antibiotic sensitivity test was then done for the cured organism using the disk diffusion method described by Bauer *et al.* (1979)

Multi Antibiotic Resistant Index (MARI)

This was carried out as described by Matyar *et al*. (2007) with slight modification. MARI = resistant antibiotics ÷ total antibiotics tested. MARI value > 0.2 indicate existence of isolate(s) from high-risk contaminated source with frequency use of antibiotic(s) while values ≤ 0.2 show bacteria from source with less antibiotics usage (Krumperman, 1985).

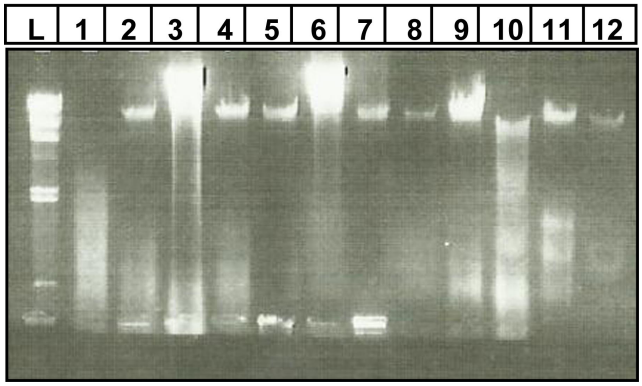

Figure 1: Agarose gel electrophoresis showing plasmid profile of isolates. L, is a DNA ladder of size; 1. *Vibrio* sp. (No plasmid); 2. *Escherichia coli* (<0.125 kb , 23.130 kb,); 3. *Salmonella* sp. (<0.125 kb, 23.130 kb); 4. *Escherichia coli* (0.125 kb, 23.130 kb); 5. *Salmonella* sp. (<0.125 kb, 23.130 kb); 6. *Salmonella* sp. (<0.125 kb, 23.130 kb); 7. *Escherichia coli* (23.130 kb); 8. *Escherichia coli* (23.130 kb); 9. *Salmonella* sp. (0.125 kb, 23.130 kb); 10. *Escherichia coli* (0.125, >0.125 kb 2.322kb, 9.416 kb); 11. *Salmonella* sp. (0.125 kb,>0.125 kb, <2.027 kb, 23.130 kb); 12. *Vibrio* sp. (>0.125 kb, 9.146 kb).

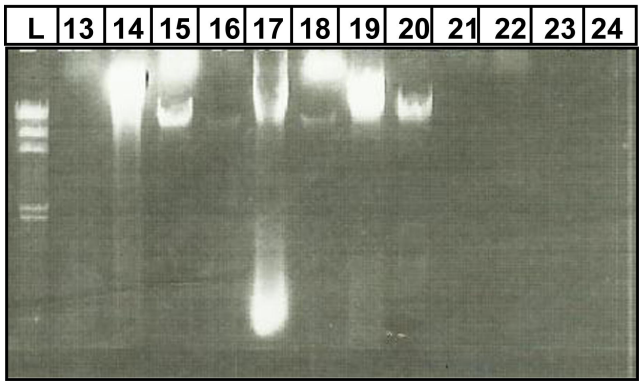

Figure 2: Agarose gel electrophoresis showing plasmid profile of isolates. L, is a DNA ladder of size; 13. *Escherichia coli* (No plasmid); 14. *Salmonella* sp. (23.130 kb); 15. *Salmonella* sp. (23.130 kb); 16. *Vibrio* sp. (23.130 kb); 17. *Salmonella* sp. (0.125 kb, 23.130 kb); 18. *Escherichia coli* (23.130 kb); 19. *Vibrio* sp. (23.130 kb); 20. *Vibrio* sp. (23.130 kb). 21. *Escherichia coli* (no plasmid); 22. *Salmonella* sp. (no plasmid); 23. *Salmonella* sp. (no plasmid); 24. *Vibrio* sp. (no plasmid)

Figure 3. Agarose gel electrophoresis showing plasmid profile of isolates. L, is a DNA ladder of size; 25. *Escherichia coli* (no plasmid); 26. *Vibrio* sp. (no plasmid); 27. *Vibrio cholerae* (no plasmid); 28. *Escherichia coli* (no plasmid); 29. *Vibrio* sp. (no plasmid); 30. *Vibrio* sp. (no plasmid).

Plasmid Isolation and Profiling

Plasmid extraction was carried out by the method described by Birnboim and Doly (1979). Plasmid profiling was carried out on 1% agarose gel using 50µl of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) buffer. A *Hind*III digest of λ DNA was used as molecular weight marker and the gel was electrophoresed in a horizontal tank at a constant voltage of 60 V for about 1 h 30 min. Plasmid DNA bands were identified by fluorescence of bound ethidium bromide using a short wave ultraviolet light transilluminator. Photographs were taken using a digital camera. The result is shown in Figures 1−3.

RESULTS AND DISCUSSION

The antimicrobial susceptibility profiles of isolates before and after curing their plasmids are presented in Tables 1−3. The susceptibility profiles obtained revealed varying degrees of resistance and sensitivity to the antibiotics used in the screening

Table 1 shows the antimicrobial susceptibility profiles of ten (10) *Escherichia coli* isolates (E1-E10) representing isolates from ten different stations. The result indicated that out of the ten *Escherichia coli* isolates, all the isolates were resistant at least to one antibiotic particularly Amoxicillin (AMX). Before curing, the resistant antibiotics is as follows: E1 (AMX: 12.5%), E2 (AMX: 12.5%), E3 (COT, AMX, AUG: 37.5%), E4 (COT, AMX, TET, AUG: 50%), E5 (COT, TET, AMX, AUG: 50%), E6 (AMX, AUG: 25%), E7 (GEN, COT, AMX, TET, AUG: 62.5%), E8 (GEN, COT, AMX, TET, AUG: 62.5%), E9 (COT, AMX, TET: 37.5%) and E2 (COT, AMX, TET, AUG: 50%).

After curing the plasmids, the resistance of the isolates observed is as follows: E1 (none), E2 (none), E3 (none), E4 (COT, TET: 25%), E5 (AMX: 12.5%), E6 (none), E7 (COT, AMX: 12.5%), E8 (GEN, COT, AMX, AUG: 50%), E9 (COT, TET: 25%) and E10 (COT: 12.5%). Generally, the result shows that there was a significant reduction in resistance while sensitivity increased after curing the plasmids.

Table 2 shows the sensitivity profiles of *Vibrio* species. Before curing the plasmids, resistance observed is as follows: V1 (AMX, AUG: 25%), V2 (COT, AMX. TET, AUG: 50%), V3 (COT, AMX, TET, AUG: 50%), V4 (GEN, COT, AMX, TET, AUG: 62.5%), V5 (AMX, TET, AUG: 37.5%), V6 (GEN, AMX, AUG: 37.5%), V7 (AMX, AUG: 25%), V8 (COT, AMX, AUG: 37.5%), V9 (COT, AMX, TET: 37.5%) and V10 (GEN, COT, AMX, TET, AUG: 62.5%).

The result shows that after curing the plasmids, the resistance pattern observed is as follows: V1 (none), V2 (COT, TET: 12.5%), V3 (COT, AMX, TET, AUG: 50%), V4 (COT: 12.5%), V5 (AMX, TET: 25%), V6 (AMX: 12.5%), V7 (AMX: 12.5%), V8 (none), V9 (none) and V10 (TET: 12.5%). Generally, the result shows that there was a significant reduction in resistance while sensitivity increased after curing the plasmids, although V3 was still resistant to the number of antibiotics.

Table 3 shows the susceptibility profiles of *Salmonella* species. The result shows that before curing the resistance pattern is as follows: S1 (COT, AMX, TET, AUG: 50%), S2 (COT, AMX, TET, AUG: 50%), S3 (GEN, AMX: 25%), S4 (COT, AAMX, TET, AUG: 50%), S5 (AUG: 12.5%), S6 (AMX, AUG: 25%), S7 (GEN, NAL, COT, AMX, TET, AUG: 75%), S8 (COT: 12.5%), S9 (GEN, COT, AMX, TET, AUG: 62.5%) and S10 (COT, AMX, TET, AUG: 50%).

After curing the plasmid, resistance level observed is as follows: S1 (COT, AMX, AUG: 37.5%), S2 (COT, AMX, TET, AUG: 50%), S3 (none), S4 (COT, AMX, AUG: 37.5%), S5 (none), S6 (none), S7 (GEN, NAL, COT, AMX: 50%), S8 (none), S9 (AMX, AUG: 25%) and S10 (COT). Although some isolates (S2, S7) are still resistant to the same number of antibiotics after curing, the result showed the degree of resistance was reduced.

Infections caused by resistant pathogens result in significant morbidity and mortality and contribute to escalating healthcare cost worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem with many pathogens throughout the world (Keith and John, 2005). All the isolates used in the present study namely; *Escherichia coli*, *Vibrio* sp and *Salmonella* sp have been shown to cause different known water-related infections or diseases (Akinyemi *et al*., 2006). The high susceptibility of *Escherichia coli* isolates to oflaxicin, nalixidic acid, nitrofurantoin, tetracycline and gentamicin is a welcome relief, since it is an indication of effectiveness of the antibiotic against the *Escherichia coli* isolates. However, amoxicillin still exhibited high resistance among the isolates after the curing showing that their resistance was chromosomally-borne.

Table 1: Antibiotic Susceptibility Patterns of the *Escherichia coli* isolates

Code No.	Status	Antibiotic Zone of Inhibition (mm)											
		OFL	GEN	NAL	NIT	COT	AMX	TET	AUG	R	I	S	MARI
E1	Natural	25S	14I	20S	18S	20S	12R	15I	17I	1(12.5%)	3(37.5%)	4(50%)	0.125
	Cured	27S	16S	21S	19S	22S	14I	16I	20S	0(0%)	2(25%)	6(75%)	0
E2	Natural	27S	14I	29S	16I	13I	12R	14R	15I	2(25%)	4(50%)	2(25%)	0.25
	Cured	30S	16S	32S	20S	14I	15I	18I	17I	0(0%)	4(50%)	4(50%)	0
E3	Natural	23S	13I	25S	19S	0R	0R	17I	0R	3(37.5%)	2(25%)	337.5%)	0.375
	Cured	25S	13I	28S	20S	13I	13R	19S	16I	1(12.5%)	3(37.5%)	4(50%)	0.125
E4	Natural	19S	15S	25S	18S	0R	0R	0R	0R	4(50%)	0(0%)	4(50%)	0.5
	Cured	20S	16S	22S	16I	12I	16I	12R	16I	1(12.5%)	4(50%)	3(37.5%)	0.125
E5	Natural	26S	18S	30S	19S	0R	11R	0R	0R	4(50%)	0(0%)	4(50%)	0.5
	Cured	27S	17S	31S	20S	14I	11R	15I	14I	1(12.5%)	3(37.5%)	4(50%)	0.125
E6	Natural	36S	14S	35S	32S	21S	10R	17I	12R	2(25%)	1(12.5%)	5(62.5%)	0.25
	Cured	38S	18S	37S	31S	23S	13R	19S	16I	1(12.5%)	1(12.5%)	6(75%)	0.125
E7	Natural	18S	12R	15I	16S	0R	0R	0R	0R	5(62.5%)	1(12.5%)	2(25%)	0.625
	Cured	18S	14I	17I	20S	11I	12R	16I	13R	2(25%)	4(50%)	2(25%)	0.25
E8	Natural	20S	0R	13R	13R	0R	0R	0R	0R	7(87.5%)	0(0%)	1(12.5%)	0.875
	Cured	18S	15S	20S	19S	11I	13R	16I	17I	1(12.5%)	3(37.5%)	4(50%)	0.125
E9	Natural	23S	14I	16I	20S	0R	0R	0R	14I	3(37.5%)	3(37.5%)	2(25%)	0.375
	Cured	22S	16R	17I	20S	11I	13R	12R	16I	3(37.5%)	3(37.5%)	2(25%)	0.375
E10	Natural	17S	13I	21S	18S	0R	0R	0R	0R	4(50%)	1(12.5%)	3(37.5%)	0.5

OFL, Oflaxicin (R: ≤12; I: 13-15; S: ≥16) GEN-Gentamicin (R: ≤12; I: 13-14; S: ≥15), NAL-Nalidixic acid (R: ≤13; I: 14-18; S: ≥19), NIT-Nitrofurantoin (R: ≤14; I: 15-16; S: ≥17), COT-Cotrimazole (R: ≤10; I: 11-15; S: ≥16), AMX-Amoxicillin (R: ≤13; I: 14-17; S: ≥18), TET-Tetracycline (R: ≤14; I: 15-18; S: ≥19), AUG-Augumentin (R: ≤13; I: 14-17; S: ≥18). S: Sensitive; R: Resistant; V1-V10: *Vibrio* spp from stations 1-10; MARI: Multi Antibiotics Resistance Index

The *Vibrio* isolates exhibited complete susceptibility to oflaxacin, nitrofurantoin and a good percentage to nalixidic acid. The isolates also showed high resistance to amoxicillin, augumentin, cotrimoxazole and tetracycline. This study shows that susceptibility increased, although some were still resistant to amoxillicin, cotrimoxazole and tetracycline. However, despite the isolates still resistant to some antibiotics after the curing, the results show that zone sizes actually increased indicating that the curing actually had effect on the isolates. The antibiotic profiles for *Salmonella* sp. reveal that before curing, all the isolates were susceptible to oflaxicin and nitrofurantoin and nalixidic acid, although, the isolates were also susceptible to the other antibiotic based on their zone size. The highly effective antibiotics to the isolates include amoxicillin, cotrimazole, tetracycline and augumentin. The results show that susceptibility increased greatly after curing, which is the process of elimination of plasmid from host cells. The study shows that the *Escherichia coli*, *Vibrio* and *Salmonella* isolates were highly sensitive to oflaxicin, nalixidic acid, nitrofurantoin and were highly resistant to amoxicillin, augumentin and cotrimazole. This profile study is important because it helps the clinician to specific antibiotic which may be expected to give the most satisfactory results in treatment. High resistance of bacterial isolates in this study to amoxicillin, augumentin and cotrimoxazole corroborate the findings of Obi *et al*. (2004) who showed that at least 20% of bacterial isolates from water supply in rural Venda communities of South Africa demonstrated antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol. Again, in this study, differences were observed in the incidence of antibiotic resistance within the different stations. These differences may be attributed to the impact of human activities on the bacterial isolates within these stations (Abu and Egenonu, 2008). There are reports demonstrating the role played by industrial and human activities on the antibiotic resistance distribution of bacterial isolates in the environment (Lin *et al*., 2004). Abu and Egenonu (2008) from their study on the current pollution status of the New Calabar River in the Niger Delta suggested that the antibiotic resistance patterns of bacterial isolates may be due to factors that are not linked to faecal pollution based on weak correlation between antibiotic resistance and faecal coliforms.

Table 2: Antibiotic Susceptibility Patterns of the *Vibrio* sp. Isolates

Code No.	Status	Antibiotic Zone of Inhibition (mm)											
		OFL	GEN	NAL	NIT	COT	AMX	TET	AUG	R	I	S	MARI
V1	Natural	32S	14I	30S	20S	15I	0R	13R	0R	3(37.5%)	2(25%)	3(37.5%)	0.375
	Cured	35S	14I	31S	20S	18S	18S	14R	14I	1(12.5%)	2(25%)	5(62.5%)	0.125
V2	Natural	19S	13R	17I	16I	0R	0R	0R	0R	5(62.5%)	2(25%)	1(12.5%)	0.625
	Cured	17S	15R	16I	19S	10R	18S	12R	13R	4(50%)	1(12.5%)	3(37.5%)	0.50
V3	Natural	20S	13I	18I	16I	0R	0R	0R	0R	4(50%)	3(37.5%)	1(12.5%)	0.50
	Cured	24S	16S	20S	18S	12I	12R	10R	12R	3(37.5%)	1(12.5%)	4(50%)	0.375
V4	Natural	19S	0R	23S	14I	0R	0R	0R	0R	5(62.5%)	1(12.5%)	2(25%)	0.625
	Cured	21S	13I	25S	15I	10R	12R	13R	18S	3(37.5%)	2(25%)	3(37.5%)	0.375
V5	Natural	21S	14I	24S	27S	14I	0R	0R	0R	3(37.5%)	2(25%)	3(37.5%)	0.375
	Cured	20S	16S	28S	30S	14I	10R	11R	16I	2(25%)	2(25%)	4(50%)	0.25
V6	Natural	15I	0R	19S	16S	17S	0R	13R	0R	4(50%)	1(12.5%)	3(37.5%)	0.50
	Cured	16S	16S	20S	18S	19S	11R	15I	16I	1(12.5%)	2(25%)	5(62.5%)	0.125
V7	Natural	21S	13I	19S	18S	13I	0R	15I	0R	2(25%)	3(37.5%)	3(37.5%)	0.25
	Cured	22S	14I	17I	19S	16S	10R	18I	17I	1(12.5%)	4(50%)	3(37.5%)	0.125
V8	Natural	23S	16S	14I	16I	0R	0R	13R	0R	4(50%)	2(25%)	2(25%)	0.50
	Cured	25S	18S	19S	15I	16S	18S	15I	14I	0(0%)	3(37.5%)	5(62.5%)	0
V9	Natural	20S	15S	23S	19S	10R	8R	10R	14I	3(37.5%)	1(12.5%)	4(50%)	0.375
	Cured	22S	17S	25S	21S	13I	14I	16I	17I	0(0%)	4(50%)	4(50%)	0
V10	Natural	17S	11R	23S	15I	0R	0R	0R	0R	5(62.5%)	1(12.5%)	2(25%)	0.625

OFL, Oflaxicin (R: ≤12; I: 13-15; S: ≥16) GEN-Gentamicin (R: ≤12; I: 13-14; S: ≥15), NAL-Nalidixic acid (R: ≤13; I: 14-18; S: ≥19), NIT-Nitrofurantoin (R: ≤14; I: 15-16; S: ≥17), COT-Cotrimazole (R: ≤10; I: 11-15; S: ≥16), AMX-Amoxicillin (R: ≤13; I: 14-17; S: ≥18), TET-Tetracycline (R: ≤14; I: 15-18; S: ≥19), AUG-Augumentin (R: ≤13; I: 14-17; S: ≥18). S: Sensitive; R: Resistant; V1-V10: *Vibrio* spp from stations 1-10; MARI: Multi Antibiotics Resistance Index

Matyar *et al*. (2007) and Krumperman (1985) stated that multi antibiotic resistant index values greater than 0.2 indicate existence of isolate(s) from high-risk contaminated source with frequency use of antibiotics while value ≤ 0.2 show bacteria from source with less antibiotic usage. This value (MARI > 0.2) shows indiscriminate use of antibiotics among rural dwellers in Oproama Community. Also, Subashkumar *et al*. (2006) stated that isolates that exhibit multi antibiotics resistance index (MARI) value greater than 0.2 depicting high level of antibiotics resistance due to either indiscriminate use of antibiotic of horizontal gene transfer. It could also be combination of the two factors.

The plasmid profiles of ten (10) *Escherichia coli*, *Vibrio* sp. and *Salmonella* isolates each from water samples are presented in Plates 1-3. Plate 1 reveals detectable plasmid profiles of eleven (11) out of twelve (12) isolates with band size ranging from 0.125 kb to 23.130 kb. The *Escherichia coli* isolates are 2(E6) < 0.125 kb, 23.130 kb; 4(E2), 0.125 kb, 23.130 kb; 7(E7) <0.125 kb, 23.130 kb; 8(E5) 23.130 kb; 10(E9) 0.125 kb, >0.125 kb, 2.322 kb, 9.416 kb.

The *Vibrio* isolates includes 1(V9) no plasmid, 12 (V10) >0.125 kb, 9.416kb. The Salmonella isolates includes 3(S7) <0.125 kb, 23.130 kb; 5(S10) <0.125 kb, 23.130 kb; 6(S4) <0.125kb, 23.130 kb; 9(S9) 0.125 kb, 23.130 kb; 11(S1) 0.125 kb, >0.125 kb, <2.027 kb, 23.130 kb. Plate 2 reveals that of the eleven (11) isolates, plasmids were detected in only seven (7). The *Escherichia coli* include 13(E8) no plasmid; 18(E10) 23.130 kb; 21(E2) no plasmid. *Vibrio* isolates include 16(V3)23.130 kb; 19(V5) 23.130 kb; 20(V2) 23.130 kb while the Salmonella isolates include 14(S5) 23.130 kb; 15(S6) 23.130 kb; 17(S7) 0.125 kb, 23.130 kb; 22(S8) no plasmid; 23(S2) no plasmid. Plate 3 reveals that none of the seven (7) isolates carried plasmid. The *Escherichia coli* isolates include 25 (E4) and 28 (E3). *Vibrio* isolates include 24(V4), 26(V7), 27(V1), 29(V4) and 30(V6).

Plasmid DNA isolation was performed on ten *Escherichia coli*, ten *Vibrio* and ten *Salmonella* isolates from environmental sources (drinking well and river water) in Oproama Community exhibiting multiple resistance markers.

Table 3: Antibiotic Susceptibility Patterns of the *Salmonella* sp. Isolates

Code No.	Status	Antibiotic Zone of Inhibition (mm)											
		OFL	GEN	NAL	NIT	COT	AMX	TET	AUG	R	I	S	MARI
S1	Natural	20S	15S	21S	19S	0R	0R	0R	0R	4(50%)	0(0%)	4(50%)	0.5
	Cured	18S	16S	20S	20S	12I	9R	13R	11R	3(37.5%)	1(12.5%)	4(50%)	0.375
S2	Natural	15I	13I	14I	17S	0R	0R	0R	0R	4(50%)	3(37.5%)	1(12.5%)	0.5
	Cured	16S	14I	16I	17S	11R	11R	11R	12R	4(50%)	2(25%)	2(25%)	0.5
S3	Natural	18S	11R	16I	15I	16S	12R	13R	13R	4(50%)	2(25%)	2(25%)	0.5
	Cured	20S	15S	18I	16I	17S	15I	16I	18S	0(0%)	4(50%)	4(50%)	0
S4	Natural	20S	13I	16I	14R	0R	0R	0R	0R	5(62.5%)	2(25%)	1(12.5%)	0.625
	Cured	21S	14I	18I	15I	10R	9R	14R	12R	4(50%)	3(37.5%)	1(12.5%)	0.5
S5	Natural	28S	14I	24S	21S	14I	15I	14R	12R	2(25%)	3(37.5%)	3(37.5%)	0.25
	Cured	26S	15S	24S	21S	17S	15I	19S	13R	1(12.5%)	1(12.5%)	6(75%)	0.125
S6	Natural	19S	13I	22S	16I	13I	0R	14R	0R	3(37.5%)	3(37.5%)	2(25%)	0.375
	Cured	20S	14I	19S	16I	14I	13R	18I	14I	1(12.5%)	5(62.5%)	2(25%)	0.125
S7	Natural	18S	0R	0R	20S	0R	0R	0R	0R	6(75%)	0(0%)	2(25%)	0.75
	Cured	20S	11R	12R	24S	10R	11R	14R	13R	6(75%)	0(0%)	2(25%)	0.75
S8	Natural	19S	15S	17I	19S	12I	13R	14R	15I	2(25%)	3(37.5%)	3(37.5%)	0.25
	Cured	20S	18S	19S	20S	14I	16I	13R	19S	1(12.5%)	2(25%)	5(62.5%)	0.125
S9	Natural	20S	12R	16I	15I	0R	0R	0R	0R	5(62.5%)	2(25%)	1(12.5%)	0.625
	Cured	21S	13I	18I	17S	14I	12R	13R	12R	3(37.5%)	3(37.5%)	2(25%)	0.375
S10	Natural	23S	18S	25S	18S	0R	0R	0R	0R	4(50%)	0(0%)	4(50%)	0.5

OFL, Oflaxicin (R: ≤12; I: 13-15; S: ≥16) GEN-Gentamicin (R: ≤12; I: 13-14; S: ≥15), NAL-Nalidixic acid (R: ≤13; I: 14-18; S: ≥19), NIT-Nitrofurantoin (R: ≤14; I: 15-16; S: ≥17), COT-Cotrimazole (R: ≤10; I: 11-15; S: ≥16), AMX-Amoxicillin (R: ≤13; I: 14-17; S: ≥18), TET-Tetracycline (R: ≤14; I: 15-18; S: ≥19), AUG-Augumentin (R: ≤13; I: 14-17; S: ≥18).). S: Sensitive; R: Resistant; V1-V10: *Vibrio* spp from stations 1-10; MARI: Multi Antibiotics Resistance Index

The result shows that plasmid was detected in six *Escherichia coli* isolates (60%), four *Vibrio* isolates (40%) and eight *Salmonella* isolates (80%) of the 30 environmental isolates examined in this study. All the isolates had plasmids within the range of 0.125kb to 23.130kb. Large molecular weight plasmids (90kb) have commonly been associated with toxigenic strains (Boop *et al*., 2003). Some of the isolates shared the same plasmid size (0.125 kb and 23.130 kb) and were isolated from the same source.

Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance (Meyer, 1988). In this study, 18 (60%) plasmid profiles were detected which indicates that plasmid profiling can also be used as an epidemiological tool for typing *Escherichia coli*, *Vibrio* and *Salmonella* sp. as described by Meyer (1988). Son *et al*. (1998) stated that generally epidemiologically unrelated isolates contain different plasmid profiles whereas related isolates could also display variation in plasmid profiles. The more plasmids exist in an organism, the more specific is the plasmid profile as a marker for a single isolate. This may be true for isolate10 (E9) which has four plasmid bands. The largest size of plasmid detected in all the plasmid positive isolates was 23.130kb. Bacterial antibiotics resistance patterns are sometimes associated with the presence of large plasmids and ability of plasmids for conjugation process (Alitheen *et al*., 2009). However, for other isolates that had no plasmid (40% of isolates), they also showed the multiple antibiotics resistance patterns with high number of antibiotics which indicates that resistance to most of these antibiotics is of chromosomal origin or on mobile genetic elements that may help in the dissemination of the resistant genes to other bacteria of human clinical significance (Son *et al*., 1998). According to Carattoli (2003) and Yah *et al.* (2007), the antibiotic resistance in those isolates that seem not to possess plasmids was associated with chromosome and/or transposons instead of being plasmid-mediated. This therefore implies that there is no consistent relationship between antibiotic resistance pattern and the number of plasmid bands present except for isolate 6 (S4), where one plasmid coded for tetracycline, resistance to various antimicrobial agents was not associated with presence of

plasmids. This could be attributed to the sources of the water samples from which the study organisms were isolated.

The results could not correlate the antibiotic resistance among the isolates with a specific plasmid detected because no genetic transfer study was performed. According to Ottaviani *et al*. (2001). Although in most cases antimicrobial resistance in *Vibrio* spp. is intrinsic to the species rather than acquired through plasmid transfer or through antibiotic exposure.

Conclusion

Most isolates in this study were resistant to amoxillicin, augumentin and cotrimazole but higher sensitivity was shown towards oflaxicin, nitrofurantoin and nalixidic acid. It is expected that oflaxicin, nitrofurantoin and nalixidic acid can be the drug of choice for effective management of disease caused by such bacteria. Multiple antibiotic resistant bacteria also occurred in microorganisms in the various water samples. In addition, the study also revealed that curing of the plasmid increased susceptibility (sensitivity) to the test antibiotics although, resistance was still observed.

The study also revealed that 60% of test isolates possess plasmids of various sizes, even though resistance to various antimicrobial agents was not associated with presence of plasmids as no particular molecular size could be associated with any particular antimicrobial resistance. Resistance was also observed in isolates with various molecular size plasmids as well as in those (40%) that had no plasmids.

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