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Non-plasmid mediated multi-drug resistance in *Vibrio* and *Aeromanas spp*. isolated from seafoods in Lagos.

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Abstract : Fifty seafood samples comprising of shrimps, crabs and cuttlefish collected from fishing companies in Lagos between September 2007 and May 2008, were cultured on TCBS agar after enrichment in APW. Using API 20E system and complementary biochemical test, 83 *VIbrio* species and 3 *Aeromonas hydrophila* were identified from the samples. The *Vibrio* species encountered comprised of *V. cholerae* (3) *V. parahaemolyticus* (5) *V. mimicus* (16) V. *alginolyticus* (37) and *V. harveyi* (12), *V. vulnificus* (10) and *Aeromonas hydrophila* (3). Antimicrobial susceptibility carried out using standard procedures revealed that all the 86(100%) isolates were resistant to,Augmentin, Chloramphenicol and Amoxicillin (Amx-Au - Chl). Ten(11.6%) of the isolates had multiple resistance to all the 10 antibiotics tested: Gentamycin, Nitrofuratoin, Tetracycline, Chloramphenicol, Amoxicillin, Ofloxacin, Cotrimoxazole, Augmentin, Ciprofloxacin, Ceftriazone (Amx-Aug-Chl-Nit-Cip-Tet-Ofl-Gen-Cot-Cro).Plasmid characterization revealed that only four (4.7%) of the Eighty-six isolates haboured plasmid DNA. These were two strains of *V. alginolyticus* with plasmids of molecular weight of 25.0 Kbp and 9.416 Kbp each and two strains of *V. mimicus* with plasmids of molecular weight 4.361 Kbp each.None of the Aeromonas specie haboured plasmids. This shows that antibiotic resistance in Vibrio and Aeromonas species in this environment is not plasmid mediated.

keywords: *Vibrio*, *Aeromonas spp.*, resistance, non-plasmid, Lagos, Nigeria

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Introduction

Seafood, especially the shellfish can readily harbour pathogenic microorganisms because of the texture of their flesh and also their microbe laden habitat they inhabit. In the case of being contaminated with pathogenic bacteria, shellfish pose a serious threat to public health.

Vivekanandhan *et al*., (2005) reported that of the 536 samples of fishes and 278 samples of prawns from the major fish market of Coimbatore, South India, analyzed for the prevalence of *Aeromonas hydrophila* over a two year period, the prevalence level of *A. hydophila* varied from 17.62% in prawns to 33.58% in fishes. Considering the pychrotrophic nature and the role of *A. hydrophila* as a pathogen of emerging importance, the considerably high levels of this organism in popular food item such as fish and prawn raises serious concern.

Eja *et al*., (2008) reported the prevalence *Vibrio parahaemoliticus, V. cholerae*, and *V. alginolyticus* in shellfish harvested from the Great Kwa River estuary in Calabar, Nigeria and their seasonal variability. The estuary was constantly faecally polluted, coupled with high rate of infection of shellfish by these *Vibrio* species thus posing a health risk. Recent study carried out by Adeleye *et al*., (2008) revealed the prevalence *Vibrio* species in seafood consumed in Lagos which were found to be multi-drug resistant.

This present study was carried out to determine whether observed drug resistance was plasmid mediated, the knowledge of this will determine whether environmental isolates of *Vibrio* species and *Aeromonas hydrophila* may act as reservoir for potential spreading of virulent

genes to other bacterial specie in this environment.

Materials and methods

Fifty samples comprising shrimps, crabs and cuttle fish were collected from different fishing companies in Lagos and ten samples from local fishermen at Agboyi-Ado and Oworonshoki areas of Lagos between September 2007 and May 2008. These samples were collected in sterile stainless steel plates and transported to the lab in cooler boxes. Microbiological analysis was carried out immediately after collection by culturing samples on TCBS agar after enrichment in APW. Using conventional biochemical characterization methods and API 20E systems as previously described (Adeleye *et al*., 2008).

Antimicrobial susceptibility of isolates was tested against the following antibiotics; Gentamycin, Nitrofuratoin, Tetracycline, Chloramphenicol Amoxicillin, Ofloxacin, Cotrimoxazole, Augmentin, Ciprofloxacin, Ceftriazone using disk diffusion method on Mueller Hinton agar. Inhibition zones were interpreted using NCCLS recommendations (NCCLS, 2000).

Plasmid DNA extraction was done using Alkaline lyses method of Birnboim and Dolly, (1979). The fragments were visualized by UV transillumination. Sizes of plasmids were estimated by comparing with previously characterized plasmids.

Results

From these biochemical tests results the isolates were subsequently identified as *Vibrio cholerae* (3), *V. parahaemolyticus* (5), *V. mimicus* (16), *V. harveyi* (12), *V. alginolyticus* (37), *V. vulnificus* (10) and *Aeromonas hydrophila* (3). The later appeared as slightly raised circular yellow colonies on TCBS plates. It is important to note that the *Aeromonas hydrophila* isolated were not deliberately sought for; they grew on the plates along with the *Vibrio* specie isolated.

Tables 1 and 2 show the antimicrobial susceptibility patterns and the antibiogram of the isolates. Multiple drug resistance was prevalent among the *Vibrio* and *Aeromonas* species. Ten (11.6%) of the total number of isolates obtained in this study were resistant to all 10 drugs tested and this represents the highest multiple drug resistance observed and least common (Amx-Aug-Chl-Nit-Cip-Tet-Ofl-Gen-Cot-Cro). At least 10(11.6%) of the isolates were resistant to 8 drugs. All the 86(100%) isolate were resistant to three drugs, which represented the most common pattern (Amx-Aug-Chl).

Only four of the isolates carried plasmid DNA, figure 1 shows plasmid profile of the two plasmid carrying strains discovered, these are *V. alginolyticus* that carried plasmids of molecular weight 25.0 Kbp and 9.416 Kbp each. Two other isolates of *V. mimicus* harbored very small plasmids of molecular weight 4.361 Kbp each.

Discussion

This study revealed a high prevalence of antibiotic resistances in the *Vibrio* and *Aeromonas* isolates. The resistance patterns detected ranged between three and ten drugs. Ten (11.6%) of the total number of isolates obtained in this study were resistant to all 10 drugs tested showing the following profile; Amx-Aug-Chl-Nit-Cip-Tet-Ofl-Gen-Cot-Cro and this represents the highest multiple drug resistance observed. Other multi-drug resistance profiles include Amx-Aug-Chl-Nit-Cip-Tet-Ofl-Gen-Cro shown by at least 14(16.2%) of the isolates and Amx-Aug-Chl-Nit-Tet-Gen-Cot-Cro seen in at least 17(19.7%) of the isolates. The observations are in agreement with previous studies by Adeleye *et al* (2008) where resistance to 10, 9, 8 drugs occurred in the majority of the *Vibrio* isolates from same origin. Multi-drug resistant environmental *Vibrio* and *Aeromonas* species have been frequently reported from many part of the world (Ansary et al., 2006, Chowdhurry *et al*., 1986, Ibara and Avarado, 2006, Periska *et al*., 2003, Wang *et al*., 2006).

In order to determine whether the observed multi drug resistance pattern observed in the isolates was plasmid mediated, the isolates were all screened for the presence of large conjugative plasmids. The presence of plasmids was detected in only four of the isolates (4.7%). The incidence of plasmids conferring resistance to a particular antibiotic(s) and/or to other harmful agents among acquatic bacterial population has been studied. However, according to Radu et al.,(2000)the possible role of plasmid mediating resistance to antibiotics among Vibrio spp., has

not been established. Since the antimicrobial susceptibility patterns in the strains of *V. alginolyticus* and *V. mimicus* that carried plasmids did not differ from strains which did not habour plasmids it can be inferred that antimicrobial resistance observed in our *Vibrio* isolates may be chromosomally mediated. Similar conclusions had been drawn by Jun *et al*., (2003) while observing low plasmid carriage in fifty one multi-drug resistant *Vibrio* species isolated in Hong Kong. None of the three strains of the *Aeromonas hydrophila* haboured plasmid. Similarly, Ansary *et al;* (2006) had reported that of 34 *Aeromonas hydrophila* isolated from various fish species caught in Malaysia, only 5(14.7%) carried plasmid DNA and that there was no correlation between the presence of plasmids and antibiotic resistance. Similarly, Chang and Bolton, (1987) reported that of 75 clinical isolates of *Aeromonas hydrophila* only one harbored plasmid DNA which mediated resistance to 10 antibiotics.

Over all, this current study suggest that multi-drug resistance observed in environmental isolates of potentially pathogenic *Vibrio* and *Aeromonas* species contaminating seafood in Lagos is non- plasmid mediated and therefore may not act as reservoir for spread of virulent genes.

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Table I: Antimicrobial susceptibility patterns o f *Vibrio* species and *Aeromonas hydrophila* isolated from fishing companies and local fishermen at Agboyi-Odo and Oworonshoki areas of Lagos state.

Antibiotics tested	Number of resistant strains (%)							Number of sensitive strains (%)					
ųg	VA	VP	VM	VH	VC	VV	AH	VA	VP	VM	VH	VC	VV
Amoxycillin (25)	37(100)	5(100)	16(100)	12(100)	3(100)	10(100)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Augmentin (30)	37(100)	5(100)	16(100)	12(100)	3(100)	10(100)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Chloramphenicol (10)	37(100)	5(100)	16(100)	12(100)	3(100)	10(100)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Nitrofurantoin (200)	19(51.4)	5(100)	16(100)	12(100)	3(100)	10(100)	3(100)	18(48.6)	0(0)	0(0)	0(0)	0(0)	0(0)
Ciprofloxacin (10)	21(56.7)	2(40)	0(0)	7(58.3)	1(33.3)	2(20)	0(0)	16(43.2)	3(60)	16(100)	5(41.7)	2(66.7)	8(80)
Tetracyclin (50)	29(78.3)	5(100)	13(81.3)	12(100)	3(100)	0(0)	0(0)	8(21.6)	0(0)	3(18.7)	0(0)	0(0)	10(10
Oflaxacin (5)	29(78.3)	2(40)	0(0)	12(100)	0(0)	4(40)	0(0)	8(21.6)	3(60)	16(100)	0(0)	3(100)	6(60)
Gentamycin (10)	37(100)	5(100)	12(75)	12(100)	3(100)	10(100)	3(100)	0(0)	0(0)	2(25)	0(0)	0(0)	0(0)
Cotrimoxazole (25)	24(64.3)	5(100)	2(12.5)	12(100)	1(33.3)	4(40)	0(0)	13(35)	0(0)	14(87.5)	0(0)	2(66.7)	6(60
Ceftriazone (30)	2(14.3)	1(20)	2(12.5)	11(91.7)	1(33.3)	0(0)	0(0)	35(94.5)	4(80)	14(87.5)	1(8.3)	2(66.7)	0(0)

VA =	*Vibrio alginolyticus*				VV =	*Vibrio vulnificus*									
VP =	*Vibrio parahaemolyticus*				AH =	*Aeromonas hydrophila*									
VM =	*Vibrio mimicus*														
VH =	*Vibrio harveyi*														
VC =	*Vibrio cholera*														

Table 2: Antimicrobial resistance profiles (Antibiograms) of *Vibrio* species and *Aeromonas hydrophila* isolated from seafoods in Lagos

Antimicrobial resistance profiles										Number of strains showing profiles						
										VA	VP	VM	VH	VC	VV	AH
Amx	Aug	Cl	Nit	Cip	Tet	Ofl	Gen	Cot	Cro	2	1	0	7	0	0	0
Amx	Aug	Cl	Nit	Cip	Tet	Ofl	Gen	Cot		19	2	0	7	0	2	0
Amx	Aug	Cl	Nit	Tet	Ofl	Gen	Cot	Cro		2	1	0	11	0	0	0
Amx	Aug	Cl	Nit	Tet	Ofl	Gen	Cot			19	2	0	12	0	3	0
Amx	Aug	Cl	Nit	Cip	Tet	Gen	Cot			12	2	0	7	1	2	1
Amx	Aug	Cl	Nit	Cip	Tet	Gen	Cro			2	1	0	7	0	0	0
Amx	Aug	Cl	Nit	Tet	Gen	Cot	Cro			2	1	2	11	1	0	0
Amx	Aug	Cl	Nit	Cip	Tet	Ofl	Gen			19	2	0	7	0	2	0
Amx	Aug	Cl	Nit	Tet	Gen	Cro				2	1	2	11	1	0	0
Amx	Aug	Cl	Nit	Tet	Gen	Cot				19	5	2	12	1	3	0
Amx	Aug	Cl	Nit	Tet	Gen					29	5	8	12	3	3	0
Amx	Aug	Cl	Nit							19	5	16	12	3	10	3
Amx	Aug	Cl								37	5	16	12	3	10	3

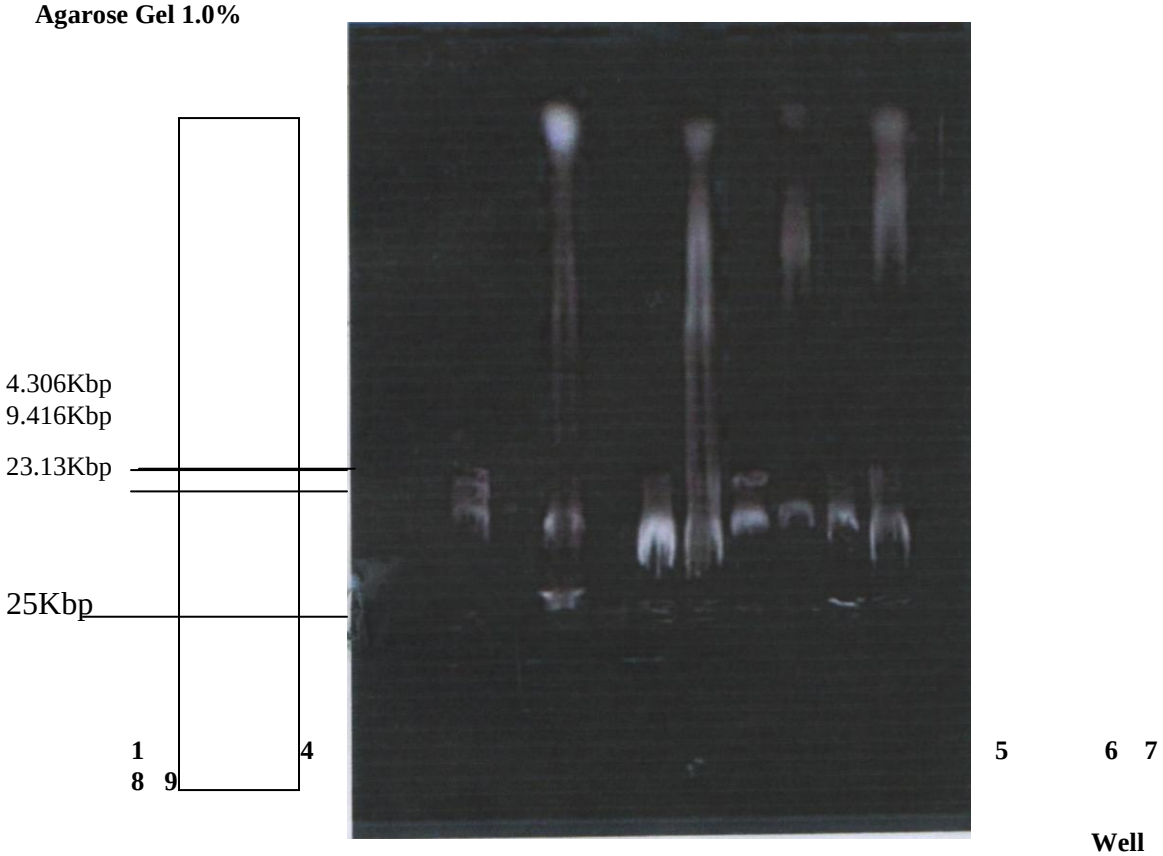
Amx = Amoxycillin
Aug = Augmentin
Cl = Chloramphenicol
Ofl = Ofloxacin
Nit = Nitrofurantoin
Cip = Ciprofloxacin
Tet = Tetracycline

VA = *Vibrio alginolyticus*
VP = *Vibrio parahaemolyticus*
VM = *Vibrio mimicus*
VH = *Vibrio harveyi*
VC = *Vibrio cholera*
VV = *Vibrio vulnificus*
AH = *Aeromonas hydrophila*

Gen = Gentamycin
Cot = Cotrimoxazole
Cro = Ceftriazone

Agarose Gel electrophoresis showing plasmid profile of *Vibrio* species.

Agarose Gel 1.0%



1	**DNA Marker**	**Well 3 & 7**
2	***V. alginolyticus***	**Plasmids with molecular weight**
3	***V. alginolyticus***	**25.0Kbp and 9.416Kbp respectively**
4	***V. alginolyticus***	
5	***V. alginolyticus***	
6	***V. alginolyticus***	
7	***V. alginolyticus***	
8	***V. alginolyticus***	
9	***V. alginolyticus***	
10	***V. alginolyticus***	