Asian Journal of Epidemiology

ISSN 1992-1462

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Epidemiology 5 (1): 15-21, 2012
ISSN 1992-1462 / DOI: 10.3923/aje.2012.15.21

Prevalence, Biotypes and Antibiogram of *Vibrio* Associated Diarrhoea in Some Parts of Niger Delta Region of Nigeria

D.I. Udoh and A.Y. Itah
Department of Microbiology, University of Uyo, Uyo Akwa Ibom State, Nigeria

Corresponding Authour: D.I. Udoh, Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria Tel: 08023803639

ABSTRACT

The prevalence, biochemical characterization and antibiogram of *Vibrio* species associated with diarrhoea in some parts of Niger Delta region of Nigeria were studied using standard microbiological diagnostic procedures. The prevalence rate of 31.7% was obtained. Fifty one isolates of *Vibrio* species were isolated of which 39 (76.5%) were *Vibrio cholerae* while 12 (23.5%) were *Vibrio parahaemolyticus* and were identified as predominant species responsible for diarrhoeic cases in the region. Further characterization of *V. cholerae* identified the isolates as El Tor (48.7%), classical (38.5%) and non-01 *Vibrio cholera* (12.8%) biotypes. The distribution of the isolates according to age range of the subjects was studied and it differed statistically ($p<0.05$). Subjects 0-10 years of age had the highest percentage (46.3%) of positive cases while the lowest percentage of 9.68% was obtained from subjects of age range 21-30 years. Samples from female patients yielded higher percentage (62.7%) of positive cases than male subjects (37.3%) but there was no significant difference ($p<0.05$). Antibiogram of isolates revealed 100% sensitivity to Gentamycin with variable percentage resistance of 11.8 and 17.6% to Nitrofurantoin and Oflaxacin, respectively. However, 100% resistances of the isolates were observed with Amoxacillin and Cotrimoxazole antibiotics.

Key words: Prevalence, biotypes, antibiogram, *Vibrio*, diarrhoea

INTRODUCTION

The term diarrhoea in clinical practice is used to describe increased liquidity of stool usually associated with increased weight and frequency of more than three times per day (Soffer, 2001). Diarrhoeal diseases have been recognized throughout history as one of the commonest causes of human illness and they are the leading cause of childhood death and in some populous areas, they are responsible for more potential life loss than all other causes combined (WHO, 1995). According to Oquike (1997), diarrhoea is one of the leading causes of children mortality and morbidity in the tropics especially among children between ages of zero to ten years. Diarrhoeal diseases rank high as a major cause of illnesses and deaths among infants, young children and elderly especially in developing countries (Udoh and Uyah, 2010). Diarrhoea is a special problem for travelers, hospitalized patients, homosexual males, persons with underlying immuno-suppressed conditions such as HIV patients, children in day-care centres as well as those living in unhygienic environments and having exposure to contaminated water and foods (Itah and Ben, 2004).

One of the commonest bacterial agents of diarhoea is *Vibrio* species and belongs to the family Vibrionaceae (McLaughlin, 1995). These organisms are normally found in marine and estuarine environments throughout the world. *Vibrio cholerae* produces a potent enterotoxin called Choleragen that is responsible for the symptoms of cholera (Nester *et al*., 1995; Sack *et al*., 2004). The choleragen is heat labile and its protein molecule is composed of two parts namely A and B. The B fragment has toxic activity while fragment A has no toxicity activity, causing the activation of the enzyme adenylate cyclase which converts ATP to Cyclic Adenosine Monophosphate (CAMP). Accumulation of CAMP in the cell causes a markedly increased secretion of water and electrolytes. The massive form of it can amount to 2 L a day and because of its appearance, the watery diarrhoeal fluid has been described as rice water stool (Gilligan *et al*., 1992; Powell, 1999; Taylor, 1999). The abrupt onset of this disease is massive, painless followed by vomiting, severe dehydration and shock which may lead to death if prompt medical attention is not given.

This study examined the prevalence, biotypes and antibiogram of *Vibrio* species associated with diarrhoae in some parts of Niger Delta region Nigeria.

MATERIALS AND METHODS

Collection of samples: Stool samples were collected from diarrhoeal patients in hospitals, clinics and medical diagnostic centers visited in some parts of Niger Delta region of Nigeria. Niger Delta is oil producing areas of the country. The two states sampled were Akwa Ibom and Cross River States of region. A loopful of stool samples were collected into a leak-proof wide mouth sterile container with cap containing alkaline peptone water as transport medium (Cheesbrough, 2002). Samples were transported within six hours to Microbiology laboratory, University of Uyo for analysis.

Inoculation of samples: A loopful of each diarrhoeal stool was transferred from alkaline peptone water onto Thiosulphate-citrate-Bilesalt-sucrose (TCBS) agar plate using surface plating technique. The plates were incubated at 37°C for 24 h after which they were observed for growth colonies. Pure cultures were stocked in Nutrient agar.

Isolation and maintenance of pure cultures: Isolation of *Vibrio* species was carried out based on the technique described by Cheesbrough (2002). This included colony morphology and pigmentation. Representative of all yellow and green colonies on TCBS agar plates were subcultured onto Nutrient agar using the streak plating technique. Purified discrete colonies were refrigerated in Nutrient agar slants at 4°C for further studies.

Identification and biochemical characterization of isolates: The isolates were identified and characterized based on colony formation, staining reaction and biochemical tests such as Oxidase test, catalase test, indole test, urease test, methyl red-voges proskauer test, string test, growth in the presence of some percentages of NaCl, sugar fermentation test, immobilization in distilled water, motility in peptone water. The identification and characterization of the isolates were carried out as described by Holt *et al*. (1994) and Cheesbrough (2002).

Biotyping the *Vibrio cholerae* isolates obtained in the study: Further biochemical characterization and serological analysis of *Vibrio cholerae* species into biotypes were carried out using heamagglutinations on chicken and sheep blood cells, agglutination of *V. cholerae* -01 polyvalent antisera, voges proskauer test reaction and sensitivity test with polymyxin-B (Cheesbrough, 1984; Chart, 2007).

Antibiogram on the isolates: The susceptibility of the isolates to antimicrobial agents was tested using agar-disc diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS, 2005 now Clinical Laboratory Standard Institute (CLSI). Six to eight hours old broth cultures of the test organisms were carefully spread uniformly over iso-sensitest agar (Oxoid Ltd, England, Code No. CM 471). Using a sterile forceps, each antibiotic disc was picked from its container and placed at the center of the plate. The discs were carefully pressed to ensure direct contact with the organisms on the plates. All plates were incubated at 37°C for 24 h after which observations were made.

Statistical analysis: Simple percentages were employed to expressed prevalence as well as percentage of occurrences of variables. Difference in the distribution of the isolates according to age range and sex were analyzed using chi-square and Analysis of Variance (ANOVA) to determine the level of significance. This made use of MINITAB statistical software (MINITAB, USA).

RESULTS AND DISCUSSION

The prevalence rate of *Vibrio* associated diarrhoea is reported as 31.7% from the areas sampled in the region (Table 1). Fifty one positive cases with *Vibrio* species was obtained out of 161 diarrhoeal cases screened.

The identification and biochemical characterizations of the isolates are presented in Table 2. The *Vibrio* species exhibited positive reaction to some tests such as catalase, oxidase, indole, methyl red and motility test. Various percentages and zero percentage were obtained in other tests depending on the strains of *Vibrio* organisms. The colonial appearance of the isolates on the TCBS agar at 37°C for 24 h were yellow with large, smooth and raised colonies of about 2-3 mm in diameter as well as green with large, smooth and raised morphology of about 3-5 mm in diameter. The species of *Vibrio* identified were *Vibrio cholerae* and *Vibrio parahaemolyticus*. Further biochemical characterization of *Vibrio cholerae* into biotypes carried out in the work showed different strains of *Vibrio cholerea* namely 19 (48.7%) *V. cholerae* El Tor, 15 (38.5%) *V. cholerea* classical biotypes and 5 (12.8%) non-01 *cholerae* strains (Table 3). The isolation of these *V. cholera* biotypes from diarrhoeic stool samples in some parts of the country is of public health

Table 1: Percentage prevalence of *Vibrio* associated diarrhoea in Akwa Ibom and Cross River States of the Niger delta region of Nigeria

Names of local government area	No. of samples screened	No. of samples associated with *Vibrio* species (%)	No. of non-*Vibrio* samples
Uyo	10	2	8
Ikot Ekpene	15	3	12
Essien Udim	18	6	12
Oron	12	5	7
Mbo	12	4	8
Ikot Abasi	10	3	7
Etinan	15	3	12
Etim Ekpo	22	9	13
Calabar South	5	2	3
Akpabuyo	11	4	7
Odukpani	7	2	5
Akamkpa	9	3	6
Ikom	15	5	10
Total	161	51 (31.7)	110 (68.3%)

Table 2: Characteristics of 51 *Vibrio* isolates from diarrhoeal stool samples

Test carried out	No. of isolates positive (%)	No. of isolates negative (%)
Gram staining	0 (0)	51 (100)
Oxidase	51 (100)	0 (0)
Catalase	51 (100)	0 (0)
Indole	51 (100)	0 (0)
Urease	0 (0)	51 (100)
Methyl red	51 (100)	0 (0)
Motility in peptone H2O	51 (100)	0 (0)
Immobilization in distilled H2O	0 (0)	51 (100)
String test	39 (76.5)	12 (23.5)
Growth in nutrient broth		
+ 0% NaCl	51 (100)	0 (0)
+ 2% NaCl	51 (100)	0 (0)
+ 4% NaCl	51 (100)	0 (0)
+ 6% NaCl	12 (23.5)	39 (76.5)
+ 8% NaCl	12 (23.5)	39 (76.5)
+ 10% NaCl	0 (0)	51 (100)
Sugar fermentation test		
Glucose	51 (100)	0 (0)
Sucrose	39 (76.5)	12 (23.5)
Mannitol	51 (100)	0 (0)
D-arabinose	45 (88.2)	6 (11.8)
L-arabinose	4 (7.8)	47 (92.2)
D-mannose	51 (100)	0 (0)
D-galactose	51 (100)	0 (0)
Lactose	0 (0)	51 (100)
Voges proskauer	19 (37.3)	32 (62.7)

Various percentages observed in each test used for characterization showed different species or strains of *Vibrio* isolates

Table 3: Biochemical characterization of *Vibrio cholera* into biotypes

	Reaction of		
Test	*V. cholera* El Tor biotype	*V. cholera* classical biotype	Non-01 *cholerae* strain
Agglutination of *V. cholera*-01 Polyvalent antisera	+	+	-
Haemolysis on sheep red blood cells	+	-	-
Haemagglutination of chicken red blood cells	+	-	-
Haemaglutination of sheep red blood cells	+	-	-
Haemagglutination on human group O red blood cells	+	-	-
Voges proskauer test	+	-	-
Sensitivity to polymyxin B	-	+	+

+: Presense, -: absence

concerns as this strain is responsible for invasive diarrhoae. El Tor biotype was the prominent strain of Vibrio. El Tor biotype was identified as the highest isolated strain of *Vibrio cholera* in this study. However, classical biotypes and non-01 biotype of *V. cholerae* were also obtained in this work and it is discovered that these biotypes were implicated and responsible for some cases of secretory diarrhoea in the sampled areas in the country.

The low frequency of occurrence of *Vibrio* species from diarrhoeal stools suggests that diarrhoea could be associated with other infectious agents such as rotavirus, *Clostridium difficile*, *Shigella*,

Table 4: Distribution of *Vibrio* isolates according to age range in the study areas

Age range in years	No. of samples screened	No. of samples with *Vibrio* sp. (%)
0-10	80	37 (46.3)
11-12	22	5 (22.7)
21-30	31	3 (9.68)
31-40	16	4 (25.0)
41 and above	12	2 (16.7)
Total	161	51

Table 5: Distribution of *Vibrio* according to age range in each state of the niger delta region screened

Age range in years	No. of samples screened in Akwa Ibom State	No. of samples screened in Cross River State	No. of samples with *Vibrio* sp. In Akwa Ibom State	No. of samples with *Vibrio* sp. In Cross River State
0-10	50	30	24 (48.8)	13 (43.3)
11-12	18	4	4 (22.2)	1 (25.0)
21-30	25	6	2 (8.0)	1 (16.7)
31-40	12	4	4 (33.3)	0 (0.00)
41 and above	9	3	1 (11.1)	1 (33.3)
Total	114	25	35	16

Salmonella, *Aeromonas*, *Proteus*, *Campylobacter* and *Yersinia* organisms (Fekety, 1997). The recovery of *Vibrio* species from these stool samples when no cases of epidemic was reported showed that fact that toxins from *Vibrio* species are confined by a lysogenic phage that can be transferred between other bacteria.

The distribution of *Vibrio* isolates according to age range in the study area .has revealed 46.3% as the highest percentage distribution with patients of 0-10 years of age while the lower percentage (9.68%) was recorded in 21-30 years (Table 4). These differed statistically ($p<0.05$). Probably the feeding habit and hygienic status of parents and caregivers might have been responsible for the high prevalence rate obtained among children (Itah, 1999).

The distribution of *Vibrio* according to age range in each state studied showed that in Akwa Ibom State, a total of 114 diarrhoeal stool samples screened, 35 (30.7%) samples were positive with *Vibrio* species and in Cross River State, a total of 47 diarrhoeal stool samples were screened from subjects of various age ranges in which 16 (34.7%) were associated with *Vibrio* organisms (Table 5). The highest percentage distribution of 48.8% with 0-10 years of age and lowest percentage distribution of 8.0% with 21-30 years of age in Akwa Ibom State while in Cross River State, the highest percentage of 43.3% with 0-10 years of age was observed and lowest percentage of zero (0%) with 31-40 years of age was observed during the study.

The total percentage distribution of *Vibrio* isolates according to sex revealed that the 40% of the positive cases were obtained from male patients while 60% of it came from females counterparts in Akwa Ibom State. In Cross River, a total percentage 31.2% of positive samples came from male subjects while 68.8% were from female counterparts. The cumulative or the overall percentage distribution of the positive cases for the two states showed 37.3% from male cases and 63.7% from female subjects (Fig. 1).

Although, higher percentages of *Vibrio* species obtained in female populations in each state and the two states combined, the distribution of the isolates according to sex revealed no significant difference ($p<0.05$) between states. These showed that sex has no influence in both epidemic and

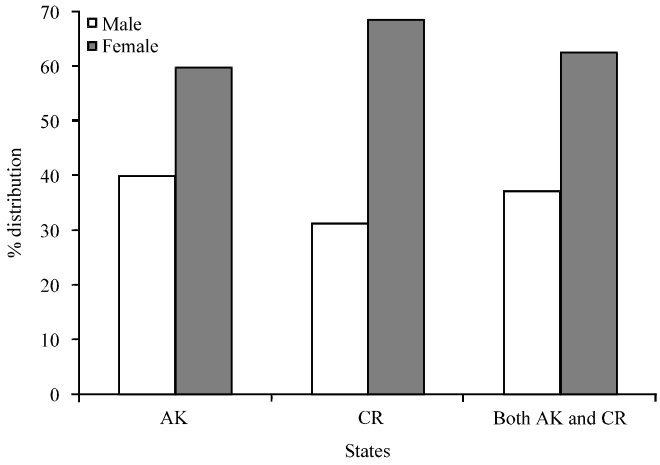

Fig. 1: Percentage distribution of positive cases according to sex of patients

Table 6: Antibiogram of the *Vibrio* isolates

Drugs	Abbreviation	Drug potency (µg)	Zone of inhibition (mm)	Results	No. of isolates sensitive (%)	Percentage resistance
Nitrofurantoin	NAT	300	15-28	S	42 (82.4)	17.6
Tetracycline	TET	30	10-15	M	30 (58.8)	41.2
Ofloxacin	OFL	30	15-20	S	45 (88.2)	11.8
Augmentin	AUG	30	13-15	M	51 (100)	0
Gentamycin	GEN	10	20-31	S	51 (100)	0
Amoxcicillin	AMX	25	<10	R	51 (100)	100
Cotrimoxazole	COT	25	<10	R	51 (100)	100
Nalidixic acid	NAL	30	>12	M	51 (100)	0

Keys: S: Strongly sensitive, M: Moderately sensitive, R: Resistance

persistence of diarrhoea caused by *Vibrio* organisms. This is in agreement with earlier reported by Joseph (1980) who stated that diseases caused by *Vibrio* organisms show no specific attachment to sex.

All the isolates obtained were highly sensitive (100%) to Gentamycin antibiotics. Forty-two (82.4%) were sensitive to Nitrofurantoin and 45 (88.5%) were sensitive to Ofloxacin. However percentage resistance of 17.6 and 11.8%, respectively was observed in the study but 100% resistances of the isolates were observed with Amoxacillin and Cotrimoxazole antibiotics (Table 6). Their 100% resistance to some antibiotics commonly used in daily practice such as Amoxcicillin and Cotrimoxazole is a cause for concern. Their resistance may have resulted from spontaneous mutation, acquisition of plasmids or may be other factors during subculturing. Gilligan *et al.* (1992) reported that *Vibrio* species especially *V. parahaemolyticus* is typically resistance to Ampicillin and Carbenicillin. Thus, for proper management of diarrhoeal diseases, antibiotics susceptibility test of the organisms involved are required and indiscriminate taking of antibiotics without susceptibility test is discouraged.

CONCLUSION

There is also need for development, implementation and evaluation of effective measures such as prompt administration of rehydration treatment, adequate health facilities and capable

personnel, high standard of personal and environmental hygiene as well as intervention programmes as these will help in lowering cases of diarrhoea morbidity and mortality rates and even eradicate them.

ACKNOWLEDGMENT

The authors are duly grateful to the Department of Microbiology, University of Uyo, Uyo Akwa Ibom State, Nigeria for providing administrative support.

REFERENCES

Chart, H., 2007. Vibrio, Mobiluncus, Garderella and Spirillum Cholerae. Vaginosis, Rat Bite Fever. In: Medical Microbiology, Greenwood, D., R. Black, J. Peutherer and M. Barer (Eds.). Churchhill Livingstone, Elsevier, New York, pp: 309-314.

Cheesbrough, M., 1984. Medical Laboratory Manual for Tropical Countries: Microbiology. Vol. 2 Tropical Health Technology, Combridgeshire, England, Pages: 479.

Cheesbrough, M., 2002. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, Cambridge, UK., ISBN: 0-521-66546-9, pp: 157-234.

Fekety, R., 1997. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. Am. J. Gastroenterol., 92: 739-750.

Gilligan, P.H., J.M. Janda, M.A. Karmalli and J.M. Miller, 1992. Cumitechn 12a. Laboratory Diagnosis of Bacterial Diarrhoea. American Society for Microbiology Press, Washington DC.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., The Williams and Wilkins Company, Baltimore, Maryland, USA, Pages: 787.

Itah, A.Y. and A.E. Ben, 2004. Incidence of enteric bacteria and *Staphylococcus aureus* in day care centers in Akwa Ibom State, Nigeria. Southeast Asian J. Trop. Med. Public Health, 35: 202-209.

Itah, A.Y., 1999. Ileal loop reaction *E.coli* serotypes isolated from infantile diarrhoea stools in Calabar, Nigeria. J. Sci. Eng. Tech., 6: 1577-1588.

Joseph, T., 1980. Bacteriology of diarhoea in infants and children. Indian J. Microbiol., 20: 189-193.

McLaughlin, J.C., 1995. *Vibrio*. In: Manual of Clinical Microbiology. Murray, P.R., E.J. Garon, M.A. Pfeller, F.C. Tenover and R.H. Yolked (Eds.). American Society for Microbiology, Washington, DC., pp: 465-476.

NCCLS, 2005. Performance standards for antimicrobial susceptibility testing tenth informational supplement. M100-S15. Wayne (PA): The Institute.

Nester, E.W., C.E. Roberts and M.T. Nester, 1995. Microbiology: A Human Perspective. WMC Brown Publishers, USA., pp: 518-520.

Oquike, J.U., 1997. Gaastroenteritis: A case for the routine search for clostridium diffile in infant stools. Nig. J. Microbiol., 2: 1-4.

Powell, J.L., 1999. *Vibrio* species. Clin. Laboratory Med., 19: 537-552.

Sack, D.A., R.B. Sack, G.B. Nair and A.K. Siddique, 2004. Cholera. Lancet, 17: 223-233.

Soffer, E.E., 2001. Diarrhoeal. In: Essentials of Medicine andreoli, T.E. (Ed.). W.B. Saunders, Philadelphia, pp: 316-320.

Taylor, R.K., 1999. *Vibrio* bacterium. Nature, 399: 312-313.

Udoh, D.I. and G.E. Uyah, 2010. Prevalence of diarrhoeic agents among hospitalized children in some hospitals in parts of Southeast Nigeria. World J. Applied Sci. Technol., 2: 150-156.

WHO, 1995. Diarrhoea and acute respiratory disease control. Interim Report 1994, Pages: 122.