Journal of Applied Science and Technology (JAST), Vol. 15, Nos. 1 & 2, 2010, pp. 115 - 119

IDENTIFICATION AND ANTIBIOTIC SENSITIVITY TEST OF BACTERIA FROM STOOLS OF PATIENTS WITH ACUTE DIARRHOEA

E.A. Ophori, C. Isibor †, J.A. Isibor †and M. Adu ‡
Department of Microbiology, University of Benin, PMB 1154, Ugbowo, Benin City, Nigeria
† Antirectroviral Therapy Centre, Central Hospital, Agbor, Delta State, Nigeria
‡ Medical Microbiology Department, Central Hospital Agbor, Delta State, Nigeria

ABSTRACT

One hundred and fifty stool samples from 65 female and 85 male patients with acute diarrhoea from the Central Hospital, Agbor (Nigeria) were examined to ascertain the likelihood of cholera outbreak in Agbor. The samples were preserved in Carey-Blair semi-solid medium, inoculated directly on blood agar, McConkey agar, deoxycholate citrate (DCA) agar and thiosulphate-citrate-bile-salt (TCBS) agar, and incubated aerobically for 24 h at 37 ° C. The isolates were characterised by sugar fermentation tests and cultural morphology. Seven bacterial genera were identified and *Escherichia coli* had an incidence of 58 %, *Vibrio cholerae* 36 %, *Salmonella* spp. 14.7 %, *Aeromonas hydrophila* 9.3 %, *Shigella dysenteriae* 7.3 % and *Klebsiella oxytoca* 7.3. % each and 3.3 % for *Shigella flexneri*. The *V. cholerae* isolates were designated VC01-VC07, and were serotyped using polyvalent, monospecific Ogawa and Inaba 01 and 0139 antisera. *V. cholerae* 01 biotype ETor serotype Ogawa was predominant and prevalent between April - May 2008, when most of the isolates were isolated. For the VC01, 39 isolates were from males (72.2 %) and 15 were from females (27.8 %). The bacteria isolates were tested against 9 antibiotics by the disc diffusion method. *E. coli*, *Salmonella* spp, *Shigella* spp and *Aeromonas hydrophila* were all resistant to gentamycin, tetracycline and penicillin (100 %) but were sensitive to chloramphenicol, ciprofloxacin and cefotaxime. All the *V. cholerae* strains (100 %) were highly sensitive to cephataxime, ciprofloxacin, chloramphenicol and erythromycin, but were resistant to streptomycin (71.4 %), tetracycline (71.4 %) and penicillin (57.1 %), while all isolates showed intermediate resistance or sensitivity to gentamycin. The study indicated that there was an ecological niche that supported *V. cholerae* 01 biotype in Agbor, which could become public health concern, unless detailed epidemiological investigation is conducted.

1. INTRODUCTION

Diarrhoea is a major cause of morbidity and mortality in developing countries. Several studies have been conducted to establish importance of different enteric bacteria in the aetiology of acute diarrhoea [1]. Bacteria causing diarrhoea include *Escherichia coli, Salmonella typhimurium, Campylobacter jejuni, Shigella* spp., *Klebsiella* spp. and *Enterobacter aerogenes* and *Vibrio cholerae* [1, 2]. Cholera continues to be a devastating disease, particularly, where endemic cholera has been reported to occur on larger-scale outbreaks [3].

Vibrio cholerae strains belonging to 01 and 0139 serogroups cause epidemics and pandemic cholera [4]. The 01 serogroup is subdivided into serotypes Ogawa. The Inaba sero group 0139 appeared in India in 1992, spread rapidly throughout Asia and was the potential eight pandemic strain of cholera [4]. In December 1996, 26 African countries reported cholera outbreaks; Nigeria, Senegal and Somalia reporting more than 1000 cases each, with high cases of fatality [5]. Transmission of *V. cholerae* to humans occurs through ingesting contaminated water or food [6].

The first recorded case of cholera in Nigeria occurred in a village near Lagos, on 26th December, 1970 leading to an important epidemic of 22,931 cases and 2,945 deaths in 1971 [5]. Between 1972 and 1990, Nigeria reported only few cases, however, between 1991 - 2009, many outbreaks of cholera were reported in Kano, Gbajimba, Benue, Edo State, and Delta State. In Agbor, ground water sources, such as wells are predominant and are of poor quality with regard to bacterial pathogens transmitted by the faecal-oral route. Such water sources are readily contaminated by faecal materials which indirectly or directly are passed on to food materials, and are potential means of spreading infections and pose threat to public health.

Foods and water contaminated with antibiotic resistant bacteria is a major threat to public health, as the antibiotics resistance determinants can be transferred to other bacteria of human clinical significance. The emergence of bacteria resistant to antibiotics is common in areas where antibiotics are commonly used. The progressive increase in antimicrobial resistance in enteric pathogens in developing cou-

Table 2. Prevalence of bacteria isolates from stools of patients with acute diarrhoea

Bacteria isolates	No. of isolates	% Prevalence of isolate
Escherichia coli	87	58.0
Vibrio cholerae	54	36.0
Salmonella spp.	22	14.7
Aeromonas hydrophila	14	9.3
Shigella dysentariae	11	7.3
Klebsiella oxytoca	11	7.3
Shigella flexneri	5	3.3
Total	204	

E. coli had the highest percentage of prevalence (58 %), followed by *Salmonella* spp. (14.7 %), *Shigella dysenteriae* and *Klebsiella* spp (7.32 % each). The least prevalent bacterium was *Shigella flexneri* (3.3 %). Table 3(a) shows the antibiotic susceptibility pattern of *V. cholerae* isolates showing the diameter of zones of inhibition produced. Different strains isolated were designated VC01 - VC07 after serologic testing. All 7 isolates of *V. cholerae* were resistant to streptomycin (71.4 %), tetracycline (71.4 %), penicillin (57.1 %) and augumentin, but were 100 % sensitive to cephataxime, ciprofloxacin, chloramphenicol and erythromycin. All 7 isolates show intermediate resistance and sensitivity to gentamycin.

The antibiotic resistant pattern of other bacteria is shown in Table 3(b). *E. coli*, *Salmonella* spp., *Shigella* spp. and *Aeromonas hydrophila* were highly resistant to gentamycin, tetracycline and penicillin, but were sensitive to chloramphenicol, ciprofloxacin and cefotaxime. *Vibrio cholerae*, *E. coli*, *Salmonella* spp., *Shigella* spp and *Aeromonas hydrophila* were resistant to tetracycline and penicillin.

Table 3(a). Diameter of zone of inhibition (in mm) of identified *V. cholerae (VC)* strains

Antibiotics Discs	Mean diameter of zones of inhibition of VC1-VC7						
	VC1	VC2	VC3	VC4	VC5	VC6	VC7
Control (blank)	0	0	0	0	0	0	0
Chloramphenicol (30 µg),	31	30	30	30	31	28	28
Streptomycin (10 µg)	00	16	00	00	20	00	00
Erythromycin (15 µg),	27	29	26	26	29	27	29
Tetracycline (30 µg),	00	00	00	00	10	00	14
Penicillin (10 µg),	18	00	00	10	10	14	00
Gentamycin (30 µg),	24	24	21	20	21	19	24
Ciprofloxacin (5 µg)	30	31	30	29	31	30	30
Augumentin (30 µg)	14	12	12	14	12	10	12
Cefotaxime (30 µg)	31	30	32	32	28	32	30

Table 3(b). Antibiotic resistant pattern of other bacteria isolates from stool samples

Organisms / Antibiotics	*Salmonella* spp	*Aeromonas hydrophila*	*E.coli*	*Klebsiella oxytoca*	*Shigella flexneri*	*Shigella dysenteriae*
Control (blank)	0	0	0	0	0	0
Chloramphenicol (30 µg),	4(18.2)	2(14.3)	13(14.9)	1(9.1)	0	0
Streptomycin (10 µg)	20(90.9)	2(14.3)	56(64.4)	6(54.6)	3(60)	9(81.8)
Erythromycin (15 µg),	12(54.6)	4(28.6)	84(96.6)	4(36.4)	1(20)	0(100)
Tetracycline (30 µg),	18(81.8)	11(78.6)	78(89.7)	11(100)	4(80)	11(100)
Penicillin (10 µg),	18(81.8)	12(85.7)	74(85.1)	2(18.2)	3(60)	7(63.6)
Gentamycin (30 µg),	22(100)	11(78.6)	63(72.4)	4(36.4)	3(60)	7(63.6)
Ciprofloxacin (5 µg)	2(9.1)	1(7.1)	10(11.5)	0(100)	0	1(9.1)
Augumentin (30 µg)	3(13.6)	1(7.1)	32(36.8)	2(18.2)	0	0
cefotaxime (30 µg)	2(9.1)	29(14.3)	6(6.9)	1(9.1)	0	1(9.1)

4. DISCUSSION

The study on enteric bacteria causing diarrhoea from stools of patient with acute diarrhoea showed that *E. coli* (58 %) and *V.cholerae* (36 %) accounted for most of the bacteria isolated and identified, and have been reported to cause diarrhoea in humans [1, 9]. *Aeromonas hydrophila* was another enteric bacterium that could cause diarrhoea in humans, but the incidence was low (9.3 %) when compared with *E.coli* and *V.cholerae* and has not been reported as a serious pathogen that posed serious threat, although has been isolated (3.0 %) and reported to cause septicaemia both in human and catfish [10]. *Salmonella* and *Shigella flexneri* had incidence of 14.7 and 3.3 % respectively, while incidence of *V. cholerae* at 36 % might not indicate an alarming situation in the

study area, but would be of concern for public health. The incidence of *V. cholera* have been reported in different environments in Nigeria [5, 9] and in stools [11, 12].

Diarrhoea is one of the most common illnesses among children in Nigeria, causing a high number of morbidity and mortality. Due to lack of hygiene knowledge in rural areas, unsafe drinking water and widespread of faecal contamination of the environment are associated with high risk of children illnesses.

Incidence of *V. cholerae* was found to be 36 %, and males had higher rate of isolation (72.2 %), while females had lower rate (27.8 %). Similar results had been reported in India [13, 14], but in contrast to the incidence of 41.7 % and 42.2 % reported in Kathmanju (India) [12], 20.4 % reported in Uyo [14] and 4.39 % in Jos [15], where cholera has been endemic. The research findings indicated an ecological niche which supports *V. cholerae* survival in Agbor region, and the sources might be sewage, water, and food products.

One significant finding is confirmation of antibiotic resistance of *V. cholerae* to streptomycin, tetracycline, penicillin and augumentin, commonly used for treatment of cholera onset [2, 16]. All isolates were sensitive to cephataxime, ciproflaxin, chloramphenicol and erythromycin. The resistance of *E. coli* to penicillin and streptomycin has also been reported and found to be plasmid mediated [17].

Comparatively, the results of resistance to tetracycline reported in a study conducted in Nigeria [18] and the research findings show that most enteric bacteria, such as *E.coli, Salmonella* spp., *Aeromonas hydrophila* are resistant to tetracycline [1, 19], a relatively cheap and affordable antibiotic used by low income patients. The ease of affordability may lead to abuse, since antimicrobial abuse is a common feature in most parts of Nigeria (including Agbor), as advertisements encourage self-medication. Abuse and excess use of antibiotics encourage development of antibiotic resistance [20].

There have not been any previous reports of screening for *V. cholerae* in Agbor, and while prevalence may not be a serious clinical problem, the presence of the organism is of public health importance, and will require more detailed epidemiological studies.

5. CONCLUSIONS

The incidence of cholera causing pathogen *V. cholerae* in stools of patients with acute diarrhoea in Agbor, and antibiotics resistance pattern were investigated. There have not been previous screening for *V. cholerae* in patients with acute diarrhoea disease in Agbor. The presence of the organism is of public health importance and extensive epidemiological studies are required.

The domestic sources of water supply and food vendors should be screened for *V. cholerae*, and personal hygiene must be improved by hand washing and water handling practices. Also, judicious and correct use of antibiotics should be promoted to combat the high rate of antibiotic resistance among pathogenic bacteria.

6. REFERENCES

1. Jindal N., Arora S. And Arora R., Enterotoxigenic enteric bacteria causing secretory diarrhoea, *Indian J. Pathol. Microbiol.*, Vol. 38, No. 2, 1995, pp. 177-180
2. Kumar P.A., Patterson J. and Karpagam P., Multiple antibiotic resistance profiles of *Vibrio Cholerae* non-01 and non-0139. *Japan., J. Infect. Dis.*, Vol. 62, 2009, pp. 230-232
3. Swerdlow D.L., Malienga G., Begkoyian G., Nyangwu G.,Toole M., Waldman R.J., Puhr D.N. and Taux R.V., Epidemic cholera among refuges in Malawi, Africa: treatment and transmission, *E. idemiol. Infect.*, Vol. .118, 1997, pp. 207-214
4. Nato F., Boutonnier A., Rajerison M., Grosjean P., Dartevelle S., Guenole A., Bhuyan N.A., Sack D.A., Nair G.B., Fournier J.M. and Chanteau S., One-step Immunochromatographic dipstick tests for rapid detection of V. Cholerae 01 and 0139 in stool samples, *Clin. Diagn. Lab. Immunol.*, Vol. 10, No. 3, 2003, pp. 476-478
5. WHO, Global Task Force on cholera control, Cholera country Profile: Nigeria, *WHO Bull.*, Vol. 1, 2008, pp. 1- 2.
6. Taylor R.K., Use of PhoA Fene fusions to identtify a polus colousation factor coordinately regulated with cholera toxin, in *Proc. Nat. Acad. Sci.*, Vol. 84, 1987, pp. 2833-2837.
7. Mackie P. and Carthney M.C., *Practical Microbiology* (XIVth edt), Churchill Livingstone, 1996.
8. CLSI, *Performance Standards for Antimicrobial Susceptibility Testing*, 15th informalizal supplement CLSI/NCCLS M100-SIS., Wayne, PA, 2005.

9. Hutin Y, Luby S, and Paquet C.A., Large cholera outbreak in Kano City, Nigeria: the importance of hand washing with soap and the danger of street vendered water, *J. Water and Health*, Vol. 1, No. 1, 2003, pp. 45-52.
10. Nair G.B., *Vibrio cholera*, National Institute of Cholera and Enteric Diseases, Calcutta, India, 2004.
11. Dashtbani A., Bakhshi B., Oskoni M., Pourshafie M.R., *Vibrio cholerae* among stool samples of patients with diarrhea and PCR detection of two housekeeping genes hly rec A, *ESCMD*, 1992, pp. 1-4.
12. Maharjan R., Lekhak B., Shrestha C.D. and Shrestha J, Detection of enteric bacterial pathogens (*Vibrio cholerae* and *E. coli* 6197) in childhood diarrhoeal cases, *Scientific World*, Vol. 5, No. 5, 2007, pp. 23-26.
13. Huilan S., Zhen L.G. and Mathan M.M., Etiology of acute diarrhea among children in developing countries, A multi centre study in five countries, *Bull. WHO*, Vol. 60, No. 5, 1991, pp. 549-555.
14. Ndon J.A., Udo S.M. and Wehrenberg W.B., Vibrio-associated gastroenteritis in the Lower Cross-River Basin of Nigeria, *J. Clin. Micro biol., Vol.* 30, No. 10, 1992, pp. 2730-2732.
15. Opajobi S.A., Kandakai-Olukemi Y.T., Mawak J.D., Olukemi M.A. and Bello C.S.S., *Vibrio cholerae 01* infection in Jos, Nigeria, *Afr. J. Clin. Exper. Microbiol.*, Vol. 5, No. 3, 2004, pp. 260-264.
16. Khan W.A., Hossain J.S. and Uddin N.S., Isolation, identification and determination of antibiotic susceptibility of *Vibrio parahaemolyticus* from shrimp at Khinlna region of Bangladesh, *Research J. Microbiol.*, Vol. 2, No. 3, 2007, pp. 216-227.
17. Parvez M.K.U., Hakim M.A., Chowdhury D.K. and Raham M.S., Study on antibiotic resistance by pathogenic bacteria isolated from clinical specimen, *Pakistan J. Biol. Sci.*, Vol. 7, No. 11, 2004, pp. 2005-2008.
18. Okeke I.N., Lamikanra A. and Edelman R., Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries, *Emerg. Infect. Dis.*, Vol. 5, 1999, pp. 18-27
19. Kilonzo-Nthenge A., Nahashon S.N., Chen F. and Adefope N., Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and Guinea fowl, *Poultry Science*, Vol. 87, 2008, pp. 1841-1848
20. Molla B., Mesfin A. and Alemayehu D., Multiple antimicrobial resistant *Salmonella* serotype isolated from chicken carcasis and giblets in Debre Zeit and Addis Ababa, Ethiopia, *Ethiopian J. Health Der.*, Vol. 17, 2003, pp. 131-149.

ACKNOWLEDGEMENT

The authors wish to thank the patients who gave consent to participate in the study, and are also highly indebted to the Management and Staff of Central Hospital, Agbor, valuable cooperation.