

Prevalence of *Vibrio Cholerae* in Northern Nigeria

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Abstract

One hundred and fifty six (156) stool samples were analyzed using selective cultural methods for the isolation of *Vibrio* species. They were from epidemic areas namely; Jos (Plateau State), Argungu (Kebbi State), Kwali and Gwagwa (Abuja) and Suleja (Niger State) all in Northern Nigeria. 123 (78.8%) were males and 33 (21.1%) females.

Eight of these samples were positive for *Vibrio cholera* with the distribution as follows; Jos – 2, Argungu – 5 and Kwali – 1.

Serotyping showed that all were of the Ogawa serotype.

Antibiotic sensitivity pattern showed that all eight (100%) samples were sensitive to Ceftazidime, Cefuroxime Axetil, Gentamycin, Sparfloxacin and Tetracycline, 5 (62.5%) sensitive to Erythromycin and 4 (50%) sensitive to Chloramphenicol. The antibiotics that all the isolates were resistant to were Ampicillin, Cloxacillin, Streptomycin and Penicillin.

The study showed that *Vibrio cholerae* is one of the causes of gastroenteritis epidemics in Northern Nigeria.

KEY WORDS: PREVALENCE, *VIBRIO CHOLERA*, NORTHERN NIGERIA

Introduction

Vibrio cholerae is usually the cause of several gastrointestinal epidemics that breakout in the Northern Nigeria. This can be attributed to several factors which include; standard of living, poor sewage management, poor state of personal hygiene, unavailability portable water and occasional floods.

The particular strain, serogroup, and antibiogram of the *Vibrio cholerae* that cause epidemics in these areas are rarely available. The aims and objectives of this research are:

- (i) To isolate the *Vibrio species* responsible for cholera outbreaks in the Northern Nigeria.
- (ii) To determine the most prevalent of the serotypes
- (iii) To determine the antibiogram of the isolates.

Methodology

The study area was Northern region of Nigeria, West Africa. The region consists of nineteen(19) states together with The Federal Capital Territory Abuja. Occasional outbreaks have been reported in this area year after year. The vegetation in the region is Guinea savannah with moderate rainfall recorded (WHO 2009).

Purposive sampling method was used arriving at a sample size of 156 stool samples which were collected from in and out patients of both sexes and different age groups in five different locations with reported cases of gastro enteritis in 2010/2011 namely; Argungu (Kebbi State), Jos (Plateau State), Suleja (Niger State), Kwali and Gwagwa (FCT).

Stool samples in epidemic areas of gastroenteritis were collected in sterile, clean, wide mouthed, screw cap bottles, patients on chemotherapy treatment and yet to be treated ones were considered.

Samples were processed according to the guidelines on laboratory method for the diagnosis of *Vibrio cholera* by the Centre for Disease Control (CDC) which includes; Macroscopy, Microscopy, Culture, Biochemical testing, Serology and Antimicrobial susceptibility testing.

For culture, samples were inoculated into 10mls of Alkaline Peptone Water (APW) and also directly onto Thiosulphate Citrate Bile salt Sucrose Agar (TCBS) and incubated for 24

hours at 37°C. After 6 hours of incubation, subcultures were made from the surface growth on the APW onto TCBS and incubated overnight at 37°C.

After overnight incubation, suspected colonies appear as large as 2-4 mm in diameter, slightly flattened, yellow as a result of sucrose fermentation with opaque centre and translucent periphery. Gram staining was done on them and read under ×100 objective, Gram negative straight or comma shaped colonies were further identified.

Suspicious colonies from TCBS Agar were picked and inoculated into Alkaline Peptone Water, Nutrient Agar (N.A.) and Mac Conkey Agar. Identities of the isolates were determined by biochemical and serological tests, the serological tests performed on the samples were:

Oxidase Test, Biochemical Tests like, Nitroso Indole Test, Citrate Utilization Test, Sugar Fermentation Test, Urease Test and Serotyping

Antibiotic susceptibility testing was done using Agar disc diffusion method. The following discs were used; Ampicillin 12µg, Chloramphenicol 10µg, Tetracycline 10µg, Streptomycin 10µg, Cotrimoxazole 25µg, Erythromycin 5µg, Ofloxacin 5µg, Ciprofloxacin 30µg, Ceftraxine 30µg and Gentamicin 10µg.

Zone of inhibition ≥25mm was confirmed sensitive, ≥18mm - moderately sensitive, 13-17mm - intermediate and ≤12mm - resistant.

Results

Stool samples were collected from one hundred and fifty six (156) people from epidemic areas (Jos, Argungu, Kwali, Gwagwa and Suleja). The number of samples collected from each location were as follows; Jos -36(23%), Argungu – 27(17.3%), Kwali – 43(27.6%), Gwagwa – 35(22.4%) and Suleja -15(9.6%) samples each. This is made up of 123 (78.8%) male and 33 (21.1%) female patients (Table 1).

Eight (5.13%) of these samples were positive for *Vibrio cholerae* and the distribution was as follows; Jos – 2(25%), Argungu – 5(62.5%) and Kwali – 1(12.5%) [Table 2].

The distribution of the positive samples with regards to in and out patients were 5(62.5%) in-patients and 3(37.5%) out-patients.

All (100%) of the *Vibrio Species* isolated were Ogawa serotype (Table 3).

The antibiotic sensitivity pattern of the isolates to various antibiotics is as follows; all the 8 (100%) were sensitive to Cefuroxime Axetil, Ceftazidime, Gentamycin, Sparfloxacin and Tetracycline, Five (62.5%) were sensitive to Erythromycin and 4 (50%) to Chloramphenicol. All were resistant to Ampicillin, Cloxacillin, Streptomycin and Penicillin.

The relationship between isolation rate and the length of stay of the patients in the hospital showed that 7 (87.5%) of the patients stayed less than 2 days in the hospital, while only 1 (12.5%) had stayed up to 2 days in the hospital.

TABLE 1: Sources of sample/ Number of samples collected/sex of patient

Source of sample	Number of samples	Sex	
		Male	Female
Jos	36	30	6
Argungu	27	16	11
Kwali	43	40	3
Gwagwa	35	24	11
Suleja	15	13	2
Total	156	123	33

Table 2: Sources of Sample, Number Collected in Relation to Sex and Number/ Percentage of Isolates.

Source of sample	No. of samples	Male	Female	No./ % Yielding <i>Vibrio cholerae</i>
Jos	36	30	6	2(5.4)
Argungu	27	16	11	5(18.5)

Kwali	43	40	3	1(2.3)
Gwagwa	35	24	11	0(0)
Suleja	15	13	2	0(0)
Total	156	123	33	8(5.13)

Table 3: Age Group of Patients in Relation to the Sources of Samples and Number of Isolates.

Age group	Jos	Argungu	Kwali	Gwagwa	Suleja
≤ 9	8	3(1)	1	2	1
10-19	8(1)	9(1)	3	3	4
20-29	4(1)	12(3)	27(1)	9	2
30-39	3	3	3	16	2
40-49	10	0	9	5	3
50-59	3	0	0	0	3
60-69	0	0	0	0	0
≥70	0	0	0	0	0
Total	36	27	43	35	15

Discussion

In this study, one hundred and fifty six (156) stool samples were analyzed using selective cultural methods for the isolation of *Vibrio cholerae*. Only 8 (5.13%) of the 156 samples yielded *Vibrio cholerae*. This shows that *Vibrio cholerae* is associated with and a cause of some gastroenteritis outbreaks but not the sole causative agent of these outbreaks in Northern Nigeria in agreement with the work of Umoh, et al (2003) that cholera is one of the main causes of gastroenteritis in Northern Nigeria.

The common denominator in all the areas (Argungu, Jos, Suleja, Kwali and Gwagwa) is that they lack clean, portable water for consumption and generally rely on streams, ponds or wells that are exposed to sewage contamination. This situation spreads the disease rapidly.

The only limiting factor was that the survey was conducted in the dry season November 2010 – March 2011 when the communities had no rainfall and no overflowing streams that facilitates the spread of the disease.

Table 2 shows the sources of the samples and number collected in relation to the number of positive *Vibrio cholerae* isolated, Argungu with only 27 (17.3%) of the total samples collected had the highest number of positive samples which were 5 (18.5%), this is instructive showing that the number of samples collected does not determine the isolation of *Vibrio cholerae* but might be due to other factors which are reflected in other tables.

Table 3 depicts the age group of patients having gastroenteritis in relation to the location where the samples were collected. Most of the people that suffered from the outbreak based on the number of samples gotten were people from the age range of 20 – 29 (34.6%) and the least were people of the age range 50 – 59(1.9%). This could be explained with the fact that people at this age range (20- 29) are the most active, mobile and exposed of these age groups so their contact with contaminated food or water is likely to be more frequent thus reflecting in the number of cases gotten. All (100%) of the samples were Ogawa serotype which correlates with the findings of Linda *et al*, 2008 that the predominant serotype in this area (Sub-Saharan Africa) is Ogawa strain.

The Antibiotic sensitivity pattern of *Vibrio cholerae* isolated were as follows; all (100%) were sensitive to Ceftazidime, Cefuroxime Axetil, Gentamycin, Sparfloxacin and Tetracycline and all were resistant to Ampicillin, Cloxacillin, Streptomycin and Penicillin and thus is in consonance with the findings of Chandaret *et al*, 2008.

Most of the positive samples for *Vibrio cholerae* (7) were isolated from patients that had not been to the hospital or had spent less than 2 days in the hospital. It is evident that as the number of days spent in the hospital increased, the chances of isolating *Vibrio cholerae* from their stool samples decreased corresponding with the work of Umoh, *et al*, 2003.

Conclusion and recommendations

Vibrio cholerae is much feared because it is a killer disease; proper enlightenment campaign among the populace is of paramount importance.

It is not frequently sought for in bacteriology laboratories in most government hospitals. Government should assist in the control of cholera not only in the cities but also in the rural areas where people are more prone to infection.

During an epidemic, the critical elements of cholera control are early identification of cases through surveillance and case-finding, notification to health authorities and WHO, establishment of treatment centres, health education and proper disposal of human waste. Travellers to epidemic areas should be extremely careful about what they eat or drink and scrupulous about personal hygiene. Under the international health regulations, notification of WHO about cases of cholera is mandatory. National Health Authorities should report the first suspected cases to WHO at the earliest possible moment: laboratory confirmed cases should also be reported immediately. Thereafter, health authorities should report confirmed cases on a weekly basis.

National risk management strategies should be planned and implemented by a national coordinating committee. This committee should be responsible for cholera preparedness, intersectorial cooperation, regional and interregional collaboration, collection and reporting of information on cholera cases and deaths, organization of any special training that may be required, procurement, storage and distribution of essential supplies and implementation, supervision, monitoring and evaluation of control activities.

Through the course of this research it was found out that the microbiology laboratories of most of the government hospitals in the epidemic areas were not equipped with materials necessary for the isolation of *Vibrio cholera* even in epidemic periods.

However, since cholera does not give notice of its arrival, laboratories should be prepared. At least alkaline peptone water and Thiosulphate Citrate Bile-salt Sucrose Agar should be available.

Cholera is usually transmitted through the faecal-oral route, with the infective dose being around 10^8 . Individuals with reduced gastric acidity and blood group O are more susceptible to the infection. In situations where poor environmental sanitation, poor domestic and personal hygiene are rampant together with unavailability of clean, portable drinking water; transmission of cholera is more likely.

It has been suggested that control of cholera epidemics is too big a task for a national control programme, although this may be true to some extent in cases of extensive epidemics but a properly organized national control programme will provide the necessary framework for quick detection and prompt containment. This is also the best means for ensuring preparedness of cholera control.

Safe and effective cholera vaccines are available and constitute important tools for cholera control.

This study showed that *Vibrio cholerae* is one of the causes of gastroenteritis outbreak in Northern Nigeria.

References

- [1.] Arora DR, Arora B (2008): Textbook of Microbiology, 3rd Edition
- [2.] Chander, Kaitsha J. Gupta N, Melita M, Singla Anitarksh N, Sarkar BL. (2001): Epidemiology and antibiograms of *Vibrio cholera* isolates from a tertiary care hospital in Chandigarh, North. *India, Indian J Med* 613-617.
- [3.] Chow KH, Yuen KY, Yam WC. (2008): Detection of RTX Toxin Gene in *Vibrocholerae* by PCR. *Journal of Clinical Microbiology* P.2594-2597.
- [4.] Constantinde M, Murtugudde GCR, Sapino MRP, Nizan A, Brown CW, Busalacchi AJ, Yunus M, Nair GB, Lanata CF, Calkins J, Manna B. Rajendran K, Bhattacharya MK, Huq A, Sack RB, Colwell. (2008): Environmental Signatures associated with Cholera Epidemics. *Proc. Natl AcadSci, USA* 105:17676-17681.
- [5.] Cruickshank R, Duguid JP, Marmion BP, Swan RHA. (1977): Medical Microbiology 20th Edition.

- [6.] David BM. (2008): CTX Prophages in classical biotype *Vibrio cholera* Functional phage genes but dysfunctional phage genomes. *Journal of Bacteriology* 182:6992-6998.
- [7.] Honda T, Finkelstein RA. (2000): Purification and Characterization of a haemolysin produced by *Vibrio cholerae*.
- [8.] Janda JM. (1998): Microbiology and Microbial infection by Topley and Wilson, volume 2 published by Arnold Great Britain.
- [9.] John A, Ndon, SundeMilldo, William Wherenberg B (1992): *Journal of clinical Microbiology*.P.2730-2732.
- [10.] Kiiiru JN, Saiduum, Goddens BM, Wamae NC, Butaye, P, Kariuki SM. (2009). Characterization of *Vibrio cholerae*01 strain carrying an SXT/R391-like element from Cholera outbreaks in Kenya. *BMC Microbiology* P.1278.
- [11.] Linda B, Adams and Ronald J Seibeling . (2008): Baton Rouge, Louisiana. 70803.
- [12.] Lopez AL, Clement JD, Dean J, Jodar I. (2008): Cholera vaccines for the developing world. *Human Vaccine* 4(1): 165-169.
- [13.] LizarragaPariida ML, Quilci M. (2009): Molecular Analysis of *Vibrio cholera* 01 and Clinical strain, including new non Toxigenic variant isolated in Mexico during the Cholera epidemic years. *J Clinical Microbiology* 47 (33): 1364-1371.
- [14.] McCathy SA. (2009): Toxigenic *Vibrio cholera* and Cargo ships entering Gulf of Mexico *Lancet* 339:624-625.
- [15.] Mohammed HF, Mhatu FS, Lyamuya EF. (2005): Susceptibility of *Salmonella*, *shigella* and *Vibrio cholera* 01 to Antimicrobial Agents. *Medical Journal* 14-17.
- [16.] MSNBC, 16 Nov. 2010; Cholera Epidemic death toll rises to 352.
- [17.] Muhammad Alid Akond, Saidul A, Hassan SMR, Sarder Nasir Uddin, Momena Shirin. (2008): Antibiotic Resistance of *Vibrio cholera* from poultry sources of Dhaka, Bangladesh; *Advances in Biological Research*. 2(3-4): 60-67. ISSN 1992-0062@Dosi Publications.
- [18.] News 24, 24, Jan. 2009; 381 new Cholera cases in Mpumalanga.
- [19.] Nguyen BM. (2009): Cholera outbreak caused by an altered *Vibrio cholera* 01 EI Tor Biotype strain producing classical cholera Toxin B in Vietnam. *J. Clinical Microbiology*. 47 (5): 1568-1571.
- [20.] Osei FB, Duker A.A (2008): Shifting Prevalence of Major Diarrhoeal pathogens in patients seeking Hospital care during floods in Dhaka, Bangladesh. *I Am J.Trop Med Hyg*.79(95): 708-714.
- [21.] Roy J.Almeida, Hickman-Breinner FW, Evangeline G. Sowers, Nancy D. Pulu Farmer III, Wacksmith IK. (2008): Comparisons of a latex agglutination assay and an enzyme linked immunosorbent assay for detecting Cholera Toxin. *Journal of Clinical Microbiology*.P128-130.
- [22.] Sack DA, Tacker CO, Cohem MB. (2008): Validation of a volunteer model of Cholera with Frozen Bacterias the challenge. *Infect Immun* 66:1968-72.
- [23.] Sack DA, Sack RB. Cholera.*Lancet*. (2004): 363:223-233.
- [24.] SaiduAlam, Sarder Nasir, Uddin Momena Shirin, Hassan SMR. (2008): Antibiotic Resistance of *Vibrio cholera* from poultry sources of Dhaka, Bangladesh. *Advances in BiologicalResearch* 2 (3-4): 60-67; 753-851.
- [25.] Saka Zaki R, Albert MJ. (2008): Bacteriology of *Vibrio* and related organisms in cholera. Medial book company, p. 37-54.
- [26.] Shukla Das, RumpaSaha, Iqbal R Kaur. Trends of Antibiotic Resistance of *Vibrio cholera* strains. *J.Med* 127:478-482.
- [27.] Siddique AK, Nur GB, Sack RB. (2004): cholera *Lancet*. 365:223-225.
- [28.] Smith AM, Sooka A, Ismail H, Nadan S, Crisp N, Weewink E, Keedy KH, (2007): Analysis of *Vibrio cholera* isolates from Northern province of South Africa. P 151-154.
- [29.] Teruyo Ito, Shogo Kuwahara, Takeshi Yokota. (2009): Automatic and Manual latex Agglutination Tests for measurement of cholera Toxin and Heat Labile.
- [30.] Urassa WK, Mhardo YB, Mhalu FS, Mhonda SJ. (2000): Antimicrobial susceptibility pattern of *Vibrio cholera* 01 strain during two cholera outbreaks in Dares Salaam, Tanzania.
- [31.] World Health Organization, Cholera in Zimbabwe: Epidemiological Bulletin Number 16, Week 13 (22-28 March, 2009). WHO *Zimbabwe Daily Cholera Update*, 16 April 2009.

[32.] Yechezkel Kashi, Yael Darun Poleg, Lyora A. Cohen, HananGanez, Yoav Y. Broza, HanohGoldsmidt, Elinor Malul, Lea Valinsky, Larisa Lerner, MeiraBroza. (2006): *Vibrio cholera* strain typing and phylogeny study based on simple sequence repeats.