

A POTENTIAL CHOLERA EPIDEMIC SOURCE: SOME FRESH VEGETABLES IN GOMBE

Musa DAHIRU, Hafiz SULAIMAN

¹ Department of Biological Sciences, Faculty of Science, Federal University Kashere,
P.M.B. 0182, Gombe State, Nigeria

Corresponding author email: musahanifa@yahoo.com

Abstract

Most of the reported outbreaks of gastrointestinal disease are linked to the consumption of fresh products contaminated by bacteria. In view of these problems, this research wishes to determine the presence of *Vibrio cholerae*, in fresh vegetables sold in some Gombe markets, by isolating and identifying the biotypes. A total of 184 vegetable samples consisting of 3 vegetable types: Cabbage (*Brassica oleracea* L.), Lettuce (*Lactuca sativa* L.) and Tomato (*Solanum lycopersicum* Mill.) were collected and analyzed, during the month of August, 2016. Samples were inoculated on Thiosulfate Citrate Bile-salt Sucrose Agar and subjected to biochemical tests. Of the 184 cultured samples, 73.39% had yellow colonial growth, out of which 16.25% were confirmed to be *V. cholera*. Further screenings demonstrated that 23.08% are of each O139 and O1 Eltor biotypes, and other *Vibrios* were represented by 53.85%. Isolates from cabbage were 50% of each O139 and O1 Eltor biotypes. There were different biotypes observed among the sampled vegetables, thus indicating close association of contamination source to the vegetables, and collectively possess the risk of cholera not only at sporadic cases but of epidemics capacity to consumers.

Key words: biotypes, classical, Eltor, *Vibrio cholerae* O139.

INTRODUCTION

Cholera is one of the oldest and best understood of the epidemic-prone diseases. The ancestral home of cholera is thought to be the Ganges delta on the Indian subcontinent, where epidemic of cholera as disease was described as far back as the 16th century (Kindhauser, 2003). *V. cholerae*, is classified into two serotypes: O1 and non O1 (Bidinost et al., 2014).

The O1 serogroup of *V. cholerae* is further classified into two biotypes, namely, the classical and Eltor biotypes. The classical cause of epidemic cholera possesses the O1 antigen, and is known as *V. cholera* O1.

Strains of the *V. cholera* O1 are further subdivided on the basis of their O antigens into subtypes Ogawa and Inaba; some strains possess determinants of both of these subtypes and are known as subtype Hikojima.

Cholera is a diarrheal disease caused by infection of the intestine with the bacterium *V. cholerae*, either type O1 or O139. Both children and adults can be infected (WHO, 2004).

Most of the reported outbreaks of gastrointestinal disease linked to the fresh products

have been associated with bacterial contamination, the consumption of „four range” vegetables, a term that refers to packaged, cleaned, possibly chopped and mixed vegetables ready to be seasoned and eaten, have gained popularity among consumers (Falomir et al., 2010).

This type of meal is consumed by many individuals in Gombe metropolis where they are obtained in readymade form street hawkers and other vegetables traders. Okafo et al. (2003) reported the presence of *Escherichia coli*, *Vibrio* spp. and *Salmonella* spp. in raw vegetables harvested from soils irrigated with contaminated streams in Nigeria.

The introduction of pathogens into soils via these agricultural inputs can result in both environmental persistence and contamination of product growing in such environments (Steele and Odumeru, 2004).

Vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the vehicle for transmission of a range of microorganisms (Erdogrul and Sener, 2005). In view of these, the research wishes to determine the presence of *V. cholerae* in raw vegetables sold in Gombe metropolis, Nigeria.

MATERIALS AND METHODS

Description of area of study

The study area comprises of Gombe metropolis in Gombe State, which is located on longitude 110° 10'E and 100° 15'N and Kashere town, in Akko local government area of Gombe State Nigeria. Kashere is located between longitude 10° 55' 34" and latitude 9° 48' 50" of green witch meridian above sea level, with the Sudan savannah ecological zone of Nigeria. With a mean annual rainfall ranges of 600 mm-1200 mm and the maximum and minimum temperature of 22.7°C and 33.5°C, respectively. The vegetation cover is open savannah wood land with trees up to six meters or more. It is few kilometers drive from Pindiga district, the town occupies more than 20 kilometers square (Tanimu, 2014).

Sample collection

Three (3) vegetable types: Cabbage (*Brassica oleracea* L.), Lettuce (*Lactuca sativa* L.) and Tomato (*Solanum lycopersicum* Mill.) were collected for the study from three different markets (Shongo market, Gombe main market and Gombe old market) in August, 2016. These markets were selected because they serve as a major source of vegetables to Gombe metropolitan at retail and individual level. Total of 83 vegetable samples were purchased random from the three markets for this study (number of vegetable type depends on availability in the market during purchase). Each sample was placed in a separate polythene bag; samples were transported to laboratory and processed in less than 3 hours from collection.

Sample processing

Vegetable samples collected were processed based on the method described by Farjana and Rashed (2012) in which 1g of vegetable was homogenized in 9 ml of peptone water and incubated for 8 hrs at 37°C for resuscitation of weak *Vibrio* cells.

Isolation and identification

Thiosulfate Citrate Bile-salt Sucrose (TCBS) Agar was prepared according to the manufacturer (Oxoid, Hampshire, England). Gram staining was done according to the

method described by Nester et al. (2001). Other biochemical tests carried out include Triple Sugar Iron (TSI), which was prepared as recommended by the manufacturer and results were read after incubation at 37°C for 24 h. Cytochrome oxidase test was prepared using 0.5% tetramethyl-*p*-phenylenediamine hydrochloride (BBL Co.). The method used for the arginine dihydrolase, Lysine and ornithine decarboxylase assays were performed by using Moeller decarboxylase base medium (Difco) amended with an amino acid at a concentration of 1% (wt/vol) and adjusted to pH 6.8. String test was conducted to differentiate between *Vibrio* and *Aeromonas* species by preparing 0.5% solution of sodium deoxycholate by dissolving 0.5 g in 100 ml distilled water. After inoculation, the medium was covered with mineral oil and incubated at 37°C for 24 h. Cells grown in the presence of 0, 6, 8 and 10 % (wt/vol) NaCl in nutrient broth were used to determine the requirement for NaCl.

The medium was inoculated and incubated at 30°C or 37°C for up to 7 days, and positive results were determined by examining the turbidity, as described by Ottaviani et al., (2003) and Kaysner et al. (2004). Acid production from 1% Arabinose and Lactose fermentation were determined by using peptone water with bromocresol indicator (pH 6.8), and the results were read after 24 h of incubation at 37°C.

The methyl red reaction was tested by using MR-VP medium (Koneman et al., 1994) incubated at 37°C for 48 h after inoculation and Voges-Proskauer test assay was also performed by using a culture grown in MR-VP medium at 37°C for 48 h. *V. cholerae* O1 El-tor is differentiated from *V. cholerae* Classical and other *V. cholerae* members by its ability to give positive result to Voges-proskaeur biochemical test in accordance with the method described by Grace (2014).

Capsule stain was performed in accordance with the method describe by Roxana and Ann (2007) using milk broth culture. *V. cholerae* serogroups O139 are capsulated members as such detection of capsule in *V. cholerae* can be used to identify *V. cholerae* O139 in the absence of O139 polyvalent anti-sera. Anthony capsule stain protocol was conducted to observe encapsulated *V. cholerae* as describe by Roxana and Ann (2007).

Cholera red test and Citrate utilization were also performed in accordance with the method described by Koneman et al., 1994.

RESULTS AND DISCUSSION

From the 484 samples of vegetables cultured, 73.39% had yellow colonial growth typical of *V. cholerae*. Of the yellow colonies 73.39% were confirmed as *V. cholerae*, an equivalent to 7.06% of the total number sampled. The relatively low percentage occurrence of *Vibrio* could be attributed to the conclusion drawn by Falomir et al. (2010), that fresh vegetables normally carry natural non-pathogenic epiphytic microorganisms, but during growth, harvest, transportation and further handling the products can be contaminated with pathogens from animal and human sources (Falomir et al., 2010). From this result, it is obvious that vegetables like lettuce, tomatoes and cabbage could serve as a potential source or reservoir of *Vibrio cholerae*. Binsztein et al.

(2004) suggested that in the interim period between cholera epidemics, *V. cholerae* is still present in the environment in a Viable But Non Culturable (VBNC) state, thus, the occurrence of culturable *V. cholerae* of 16.25% is of public health concern, since there could still be viable but non-culturable (VBNC) once still presence in these samples as a result of adverse environmental conditions.

The high percentage of isolation observed in Gombe new market relative to other sources, could be attributed to a possibility of on-farm contamination due to the use of manure from animal or human faeces.

Other possible sources may include contamination from birds' faeces, since no peculiar environmental condition was observed at the time of collection.

Further screening revealed 23.08% of each O139 and O1 Eltor biotypes respectively, while other *Vibrio* were 53.85% (non O139 and non O1 Eltor) biotypes as shown in Table 1.

Table 1: Level of microbial contamination of *V. cholerae* in some selected vegetables in Gombe Metropolis

Sample type	Total no. of samples	No. of growth on TCBS (%)	Yellow colonies (%)	No. of <i>V. cholerae</i> identified (%)	No. of <i>V. cholerae</i> O139 (%)	No. of <i>V. cholerae</i> O1 Eltor (%)	No. of other <i>V. cholerae</i> (%)
Lettuce	61	46 (75.4)	41 (89.13)	6 (14.63)	1 (16.67)	1 (16.67)	4 (66.67)
Tomatoes	75	36 (48.00)	22 (61.11)	5 (22.73)	1 (20.00)	1 (20.00)	3 (60.00)
Cabbages	48	27 (56.25)	17 (62.96)	2 (11.76)	1 (50.00)	1 (50.00)	0 (0.00)
	184	109 (59.24)	80 (73.39)	13 (16.25)	3 (23.18)	3 (23.08)	7 (53.85)

Table 2: Summary of total number of *Vibrio* spp. isolated from different sources

Samples type	Total no.	No. of growth on TCBS(%)	Yellow colonies (%)	No. of <i>V. cholerae</i> identified (%)	No. of <i>V. cholerae</i> O139(%)	No. of <i>V. cholerae</i> O1 Eltor (%)	No. of other <i>V. cholerae</i> (%)	Sources
Lettuce	20	18 (90.00)	18 (90.00)	4 (22.22)	1 (25.00)	1 (25.00)	2 (50.00)	Gombe
Tomatoes	25	20 (80.00)	13(65.00)	4 (30.78)	1 (25.00)	1 (25.00)	2 (50.00)	New
Cabbages	18	16 (18.89)	15 (93.75)	2 (13.33)	1 (50.00)	1 (50.00)	0 (0.00)	Market
1 st Total	63	54 (85.71)	46 (85.19)	10 (21.74)	3 (30.00)	3 (30.00)	4 (40.00)	
Lettuce	22	19 (86.36)	14 (73.70)	1 (7.14)	0 (0.00)	0 (0.00)	1 (100)	Gombe
Tomatoes	25	11 (44.00)	9 (81.81)	1 (11.11)	0 (0.00)	0 (0.00)	1 (100)	Old
Cabbages	20	9 (45.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	Market
2 nd Total	67	39 (58.21)	23 (58.97)	2 (8.69)	0 (0.00)	0 (0.00)	2 (100)	
Lettuce	19	9 (4.74)	9 (100)	1 (11.11)	0 (0.00)	0 (0.00)	1 (100)	Shongo
Tomatoes	25	5 (20.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	Market
Cabbages	10	2 (20.00)	2 (100)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
3 rd Total	54	16 (29.63)	11 (68.75)	1 (9.09)	0 (0.00)	0 (0.00)	1 (100)	

The presence of *V. cholerae* O139, O1 Eltor biotypes and other biotypes in this research is in agreement with WHO report, that *V. cholerae*

O1 El Tor has gradually spread to most of the continent (WHO, 2010). Similarly, the isolation of three different serogroups is also another

threat, which needs public health surveillance and strategies in anticipation of possible cholera epidemic in Gombe state.

Among the sample types, tomato samples harbor about 22.73% *V. cholerae*, while lettuce and cabbage had 14.63% and 11.76%, respectively, of *V. cholerae*. In the serotypes identified, cabbage had 50% of each O139 and O1 Eltor biotypes respectively, while isolates from lettuce and tomatoes had 66.67% and 60.00% as non O139 and non O1 Eltor biotypes as indicated in Table 1.

Most of the vegetables that are brought to Gombe Metropolis Markets are usually from nearby irrigation sites, whose main source of water for irrigation is from contaminated river that carries wastewater from domestic sources. It is also a usual practice to use sheep or cow dung and the addition of poultry litter is cheap, easy and increases the soil fertility, as observed by local farmers, thus most prefers it. This practice could lead to contamination as Hamilton et al. (2006) reported, that contamination can arise as a consequence of treating soil with organic fertilizers, such as sewage sludge and manure, and from the irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables (Hamilton et al., 2006). Norma and his colleagues reported isolation of tomatine and tomatidine, flavonoids, chlorophyll, carotenoids, and phenolics antioxidant and inhibited the growth of pathogens such as *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria ivanovii* (Norma et al., 2015).

Similar observations were reported in the antimicrobial activity of red cabbage whose constituent includes phenolic substances, flavonoids and glucosinolates. Dorantes et al., (2000) reported antimicrobial activity of *Capsicum* due to the phenolic compound and 3-hydroxycinnamic acid (coumaric acid). Lee et al., (2003) reported antibacterial activity of a group of fruits and vegetables including: bell pepper, carrot, cucumber, garlic, ginger, grape, red onion, red cabbage, spinach and strawberry and suggested that all green vegetables have no antibacterial activity on *Staphylococcus epidermidis* and *Klebsiella pneumoniae* whereas all purple and red vegetable and fruit juices have showed antibacterial activities, like

tomatoes and red cabbage. Glucosinolates are secondary metabolites that occur in many species of plants including cabbage (Kusznierewicz et al., 2012), and it was reported to exhibit significant antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi with direct or synergistic effect in combination with other compounds. These could probably explain the low isolation rate that was observed among cabbage samples. It is however important to note that, though cabbage was reported to have contained some constituents that were found to exhibit antimicrobial properties, *V. cholerae* of epidemic potential was still observed in this work, as colonizers on cabbage.

CONCLUSIONS

Cholera is primarily known as a water-borne disease in the endemic regions, contamination of food can also be an imperative mode for cholera transmission (Glass et al., 1992). Samples collected from Gombe new market (GNM) had the highest *Vibrio* occurrence (21%) compared to Gombe Old market (GOM) and Shongo market (SM) (8.69% and 9.09% respectively). It is also pertinent to note, although the percentage distribution of *V. cholerae* was observed to be higher in samples collected from SM, yet the number is higher in GOM compared to SM. There were 30.00% of each O139 and O1 Eltor biotypes observed in samples collected from Gombe new market, while no isolate was identified from GOM and SM as shown in Table 2. Isolation of *V. cholerae* O139 and other serogroups in this study signifies a risk of cholera epidemics and other enteric diseases in Gombe which was not recorded since 2010.

REFERENCES

- Bidinost, C., Saka, H. A., Aliendro, A., Sola, C., Panzetta-duttari, G., Carrenza, P., Echanique, J., Patrito, E. and Bacco, J. L. (2014). Virulence Factors of non-O1 and non O139 isolated from Cordoba Argentina. *Revista Argentina de Microbiologia*: 30: 158 – 163.
- Binsztein, N., Costagliola, M. C., Pichel, M., Vera, C., Akselman, R. Vacchino, M. and Huq, A. (2004). Viable but non culturable *V. cholerae* O1 in the Aquatic environment of Argentina. *Appl. Environ. Microbiol.* 70 (12): 7481-7486.

- Dorantes, L., Colmenero, R., Hernandez, H., Mota, L., Jaramillo, M. E., Fernandez, E., Solano, C. (2000). Inhibition of growth of some foodborne pathogenic bacteria by *Capsicum annum* extracts. *International Journal of Food Microbiology*. 2000; 57(1-2): 125-128.
- Erdogru, C. and Sener, F. (2005). Modern Food Microbiology. (4th edition) CBS Publication New Dalshi Indian 413 -417.
- Falimir, M. P., Gozalbo, D. and Rico, H. (2010). Coliform bacteria in fresh vegetables: from cultivated lands to consumers. Fomartex press, Burjassot, Spain.
- Farjana, R. and Rasheed, N. (2012). Prevalence of Pathogenic Bacteria in Common Salad Vegetables of Dhaka Metropolis. *Bangladesh J. Bot.* 159-166.
- Glass, R. I. and Black, R. E. (1992). The epidemiology of cholera. In Barua, D. and Greenough, W. B. (Eds.). *Cholera*, p. 129-154. New York: Plenum.
- Grace, O. A. (2014). Hazards associated with enteric agents on vegetables grown in irrigated fields treated with manure in parts of Kano and Plateau states, Nigeria. A Dissertation Submitted to the School of Post Graduate Studies, Ahmadu Bello University, Zaria in Partial Fulfilment of the Requirements for the Award of Doctor of Philosophy in Microbiology, Department of Microbiology, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria.
- Hamilton, A. J., Stagnitti, F., Premier, R., Boland, A. M. and Hale, G. (2006). Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and Environmental Microbiology*. 72:3284-3290.
- Kaysner, C. A. and DePaola, J. A. (2004). *Vibrio*. In: Food and Drug Administration Bacteriological Analytical Manual. <http://www.cfsan.fda.gov/~ebam/bam-9.html> 3rd October, 2006.
- Kindhauser, M. K. (2003). Global defense against the infectious disease threat. *World Health Organization*, Geneva., pp. 74- 79.
- Koneman, W. E., Allen, D. S., Janda, M. W., Schreckenberger, C. P, and Winn, C. J. W. (1994). Introduction to Diagnostic Microbiology. J.B. Lippincott Company, East Washington Square, J.B. Lippincott Company, Philadelphia, USA.
- Lee Y-L, Cesario T, Wang Y, Shanbrom E, Thrupp L. (2003). Antibacterial activity of vegetables and juices. *Nutrition* 2003; 19:994.
- Nester, E. W., Anderson, D. G., Roberts, C. E., Roberts, C. E., Pearsall, N. N. and Nester, M. T. (2001). *Microbiology: A human Perspective* (3rd ed). Mc Geaw-Hill.
- Norma, P. S., Saul, R., Luis, A. C., Alvarado, M. I. E., José de Jesús, O., Marco, A. L., Carmen, L. D., Fernando, J. A. and Enrique, M. (2015). Total Phenolic, Flavonoid, Tomatine, and Tomatidine Contents and Antioxidant and Antimicrobial Activities of Extracts of Tomato Plant. *International Journal of Analytical Chemistry*. <http://dx.doi.org/10.1155/2015/284071>
- Okafo, C. N., Umoh, V. and Galadima, J. (2003). Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. *Sci. of the Total Environ.* 311: 49-56.
- Ottaviani, D., Masini, L. and Bacchiocchi, S. (2003). A biochemical protocol for the isolation and identification of current species of *Vibrio* in seafood. *Journal of Applied Microbiology*, 95, 1277-1284 doi:10.1046/j.1365-2672.2003.02105.x
- Kusznierewicz, B., Renata B., Agnieszka, B., Piekarska, A., Huk, A., Manikowska, A., Antonkiewicz, J., Namies'nik, J., and Konieczka, P. (2012). The Dose-Dependent Influence of Zinc and Cadmium Contamination of Soil on Their Uptake and Glucosinolate Content in White Cabbage (*Brassica oleracea* var. *capitata* f. *alba*). *Environmental Toxicology and Chemistry*, Vol. 31, No. 11, pp. 2482-2489. DOI: 10.1002/etc.1977
- Roxana, B.H and Ann, C.S (2007). Capsule Stain Protocols. American Society for Microbiology, USA. www.asmscience.org
- Steele, M. and Odumeru, J. (2003). Irrigation Water as Source of Foodborne Pathogens on Fruit and Vegetables (Review). *Journal of Food Protection*, 67:2839-2849.
- Tanimu, F. (2014). Demographic and Socio-economic Characteristics of Akko Local Government. Un Published Dissection.
- World Health Organization (2004). Cholera in 2004. *Weekly Epidemiological Record*, vol. 80, pp. 157-164.
- World Health Organization (2010). Cholera 2009. *Weekly Epidemiological Records*, 85:293-308.