PHENOTYPIC AND PLASMID CHARACTERIZATION OF *VIBRIO CHOLERAE* ISOLATED FROM VENDED JOLLOF RICE SOLD IN BENIN CITY, NIGERIA

MORKA E.

Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria. E-mail: emorka96@gmail.com. Tel: 07038625892.

Accepted 21st May, 2020

Globally, the effects of food safety on consumers and those in food service sectors require serious attention for public health interest. This will help reduce the spread of several diseases and outbreak of cholera. The study aimed at assessing the phenotypic and plasmid characterization of *Vibrio cholerae* isolated from vended jollof rice samples sold in Benin City, Nigeria. A total of twenty jollof rice samples, four each were purchased from vended sites across five local government areas in Edo State, Nigeria between February to August 2015. The samples were analyzed using standard microbiological techniques for the presence of *V. cholerae* contamination. Antibiotic susceptibility pattern and plasmid profiling were carried out using Kirby bauer disc diffusion technique. The total viable count ranged from $1.4 \pm 0.18 \times 10^6$ to $6.4 \pm 0.23 \times 10^6$ CFU/g for samples obtained from Ovia North East and Oredo respectively. Percentage of pre-antibiogram showed multi-drug resistance by *V. cholerae* isolates except imipenem (50%). However, after curing was done, plasmid analysis of all twenty isolates using agarose gel electrophoresis showed that virtually all the isolates harbored plasmid which was responsible for their resistance. The findings therefore revealed that poor hygiene is a potential vehicle for transmitting food borne illnesses thus there is need to develop practical strategies geared towards ensuring food safety by the food vendors.

Key words: Vended jollof rice, *Vibrio* counts, antibiotic susceptibility patterns.

INTRODUCTION

Globally, cholera has been a public health threat resulting from possible lack of health awareness programs, surveillance studies and facilities. It is an epidemic disease caused by *Vibrio cholerae*, that spreads across the universe. Presently, the infection has become a reoccurring phenomenon in developing countries like Nigeria (Schaetti et al., 2013). Food can become contaminated by several pathogenic strains of bacteria when it does not contain the essential nutritive elements. Developing countries are highly affected due to economic meltdown, infrastructure and disaster awareness facilities (Sur, 2000). Outbreaks in recent areas may occur during wet or dry seasons and affect the entire age groups equally. In aquatic or estuarine environments, *V. cholerae* is expected to thrive more. People usually get infected by not having access to potable water for drinking and domestic purposes. Once infected the bacteria is passed out during stooling. Regions where human wastes are untreated, the infection is expected to be dominant. In the last quarter of 2009, it was reported that in Nigeria, over 260 people died of cholera outbreak in four Northern states with more than ninety-six people in Maidugari, Biu, Gwoza, Dikwa and Jere council areas of Bauchi State (WHO, 2011). Most parts of Nigeria, particularly the Northern region depend on hand dug wells as source of drinking water. Edo State also recorded 49 cases and 24 deaths of cholera outbreak far back April 1999 (WHO, 2011). Possible source of contamination is other cholera patients when their untreated diarrhoea discharge percolates into water supplies.

The World Health Organization (WHO) stated that food-borne diseases caused by pathogenic microbe are responsible for the high mortality rate globally. Poor sanitary practices by most of the food cook and vendors are possible source of

contamination (Rayza et al., 2016). Also observed was that the increasing level of antibiotic resistance by microorganisms and a number of plasmid DNA detection techniques have been developed to examine plasmid resistant genes (Birnboin and Doly, 1979).

MATERIALS AND METHODS

Sample collection

A total of twenty vended jollof rice food samples, four each were obtained from five local government areas in Edo State, Nigeria. Samples were collected between February to August 2015. They were randomly purchased and transferred to Lahor Research Laboratory, Benin City for microbiological analysis.

Bacteriology

The serially diluted jollof rice samples were inoculated into Thiosulphate Citrate Bile Salts Sucrose Agar plates and incubated aerobically at 37°C for 24 h. Morphological and biochemical tests were carried out to confirm the isolates by employing standard bacteriological methods (Cheesbrough, 2005).

Sensitivity test

Kirby Bauer disk diffusion technique was employed for the antibiotic susceptibility test (Bauer, 1996). Three to five colonies were inoculated into a tube containing tryptic soy broth, and incubated overnight at 37°C. Standardization of the inoculums was done by diluting the broth cultures until turbidity matched the 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20 ml of Muller-Hinton agar in a 100-mm disposable plate (Sterilin, UK). The inoculated plates were air-dried, and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed down to ensure contacts. The following discs were used: Imipenem (10 µg), erythromycin (15 µg), Amoxicillin (25 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Cefixime (5 µg), Cephalexin (30 µg), and Ciprofloxacin (5 µg). The zones of inhibition were observed, measured and results were compared and reported as sensitive (S) or resistance (R) based on CLSI guidelines (2016).

Plasmid analysis

Plasmid DNA was isolated from cultured cells using alkaline lysis method as described by Birnboin and Doly (1979). Plasmids were separated by gel electrophoresis in 0.8% agarose gel. Positive plasmid bands were observed under the U.V trans illuminator.

Statistical analysis

The statistical package (SPSS) was used. One-Way ANOVA was used to check for significant difference. *P* values less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the total *V. cholerae* count of vended jollof rice samples (CFU/g) which ranged from $1.4\pm0.18 \times 10^6$ to $6.4\pm 0.23 \times 10^6$ for Ovia Norh-East and Oredo Local Government Area, respectively. The isolates were subjected to morphological and biochemical test as shown in Table 2. Table 3 represents the antibiotic susceptibility pattern of the isolated *V. cholerae* (Pre-Antibiogram). The isolates were multi- drug resistant. The percentage of pre-antibiogram of isolated *V. cholerae* is shown in Table 4. It was observed that the isolates were multi -drug resistant. Figures 1 and 2 also reveal the plasmid profile of multiple drug resistant *Vibrio* specie analyzed on a 0.8% agarose gel electrophoresis. Cured isolates showed a decrease in resistance as shown in Table 5. Table 6 represents the percentage

Table 1. Total mean *Vibrio cholerae* count of vended jollof rice sample (CFU/g). ($\times10^6$).

Local government area	Mean count
Egor	2.4 ± 0.32
Ovia North East	1.4 ± 0.18
Ikpoba okha	5.4±0.15
Oredo	6.4 ± 0.23
Ovia South West	4.4 ± 0.22

Table 2. Biochemical reaction of isolates.

Parameter	Result
Motility	+
Swarming surface growth	_
Citrate	+
Indole	_
Oxidase	+

Table 3. Antibiotics susceptibility test of *V. cholera*.

Pre	Pre-antibiogram							
	IMP	E	AMC	CXM	CAZ	CFM	CN	CIP
1	R(8)	R(0)	R(8)	S(24)	R(7)	R(8)	R(8)	R(9)
2	R(8)	R(8)	R(0)	R(11)	R(5)	R(8)	S(25)	R(6)
3	R(10)	R(8)	R(6)	R(6)	R(6)	R(8)	R(5)	R(8)
4	S(20)	R(0)	R(9)	S(25)	S(22)	R(8)	S(20)	S(23)
5	R(9)	R(6)	R(5)	R(8)	R(6)	R(0)	R(9)	R(6)
6	R(10)	R(0)	R(7)	R(13)	R(7)	R(8)	R(5)	R(8)
7	R(5)	R(0)	R(6)	R(9)	R(6)	R(6)	R(8)	R(7)
8	S(25)	R(0)	R(0)	R(10)	S(20)	R(10)	R(9)	R(5)
9	S(20)	R(6)	R(11)	R(5)	R(8)	R(5)	R(6)	R(7)
10	S(20)	R(0)	R(6)	R(8)	R(0)	R(13)	S(20)	S(20)
11	S(20)	R(0)	R(8)	R(8)	S(24)	R(0)	S(20)	S(25)
12	S(22)	R(0)	R(5)	R(8)	R(6)	R(6)	S(20)	S(20)
13	S(25)	R(0)	R(10)	R(13)	S(24)	R(6)	S(22)	S(20)
14	S(23)	R(0)	R(8)	R(8)	R(6)	R(10)	S(20)	R(8)
15	R(8)	R(0)	R(6)	R(2)	R(8)	R(5)	R(9)	R(9)
16	R(8)	R(6)	R(12)	R(8)	R(9)	R(12)	R(7)	S(25)
17	R(8)	R(0)	R(10)	R(8)	R(4)	R(9)	R(5)	R(6)
18	S(19)	R(0)	R(6)	R(4)	S(20)	R(7)	S(22)	S(20)
19	R(9)	R(0)	S(20)	S(25)	R(8)	R(0)	R(8)	R(7)
20	S(24)	R(0)	R(6)	R(6)	S(20)	R(8)	S(25)	S(25)

Source: CLSI, (2016).
Key: IMP= Imipenem (10 µg), E= Erythromycin (15 µg), AMX= Amoxicillin (25 µg), CXM= Cefuroxime (30 µg), CAZ= Ceftazidime (30 µg), CFM= Cefixime (5 µg), CN= Cephalexin (30 µg), CIP= Ciprofloxacin (5 µg), S = sensitive, and R = resistant.

Table 4. Percentage sensitivity test of *V. cholera.*

Pre	Pre-antibiogram							
	IMP	E	AMC	CXM	CAZ	CFM	CN	CIP
1-20	50%	0%	5%	15%	35%	0%	45%	40%

of post antibiogram after curing. Results of post sensitivity test showed that resistance of the isolates to the various tested drugs was plasmid mediated.

DISCUSSION

Documented reports have shown that lack of good drinking water facilities, unavailability of sanitary facilities and unhygienic condition, especially during food processing have been the major causes of infections among youths particularly the under aged (WHO, 2017; Tambekar et al., 2008). The mean *Vibrio* counts of Jollof rice samples ranged from $1.4\pm 0.18 \times 10^6$ to $6.4 \pm 0.23 \times 10^6$ for Ovia Norh-East and Oredo Local Government Area respectively. The counts were higher than 10^4 permissible limits. The report, however, is not in agreement with the

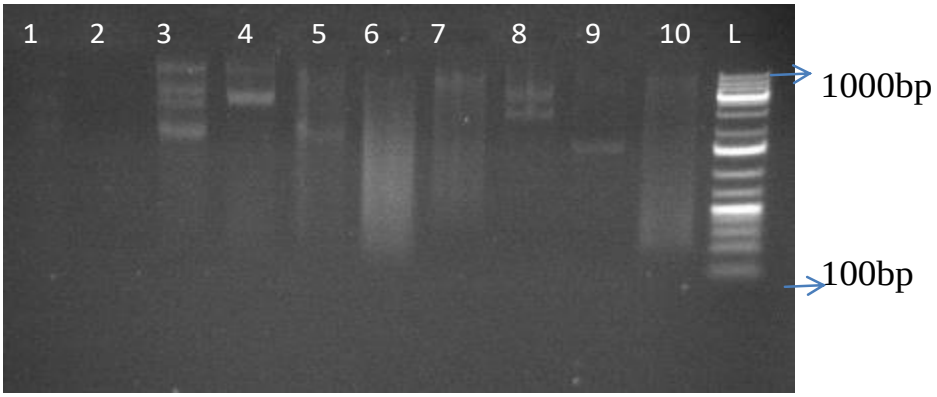

Figure 1. Plasmid profile of multiple drug resistant *V.* isolates.

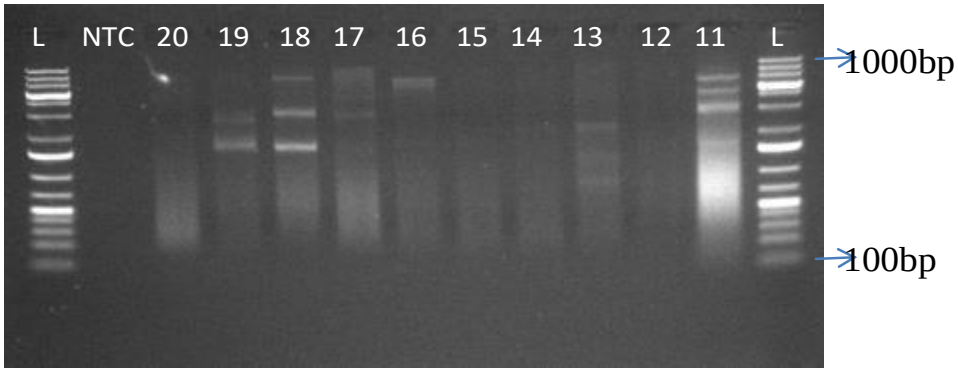

Figure 2. Plasmid profile of multiple drug resistant *Vibrio* isolates.

Table 5. Antibiotics sensitivity test of *V. cholera.*

Pre	Post-antibiogram							
	IMP	**E**	**AMC**	**CXM**	**CAZ**	**CFM**	**CN**	**CIP**
1	S(22)	S(20)	S(22)	S(24)	R(10)	R(10)	S(21)	S(19)
2	S(20)	S(22)	S(27)	S(20)	R(8)	S(22)	S(25)	S(22)
3	S(18)	S(18)	R(26)	S(28)	S(21)	S(21)	S(26)	S(25)
4	S(20)	S(27)	S(29)	S(25)	S(22)	S(18)	S(20)	S(2S)
5	S(19)	S(25)	S(20)	S(26)	S(26)	R(3)	S(25)	S(21)
6	S(26)	S(28)	S(19)	S(24)	R(9)	S(25)	R(8)	S(18)
7	S(23)	R(2)	R(8)	R(11)	S(23)	R(11)	R(10)	S(23)
8	S(25)	S(20)	S(23)	S(18)	S(20)	S(24)	R(11)	S(28)
9	S(20)	S(25)	R(8)	R(8)	S(20)	S(18)	S(24)	S(24)
10	S(20)	S(28)	S(22)	R(9)	R(7)	S(25)	S(20)	S(20)
11	S(20)	S(24)	R(8)	R(11)	S(24)	R(2)	S(20)	S(25)
12	S(22)	R(0)	R(6)	S(22)	R(7)	R(8)	S(20)	S(20)
13	S(25)	R(0)	S(25)	S(25)	S(24)	R(9)	S(22)	S(20)
14	S(23)	S(24)	R(11)	S(23)	R(8)	S(20)	S(20)	S(29)
15	S(28)	S(19)	S(22)	R(7)	R(9)	R(8)	R(10)	S(21)
16	S(23)	S(29)	S(19)	R(10)	S(21)	S(24)	R(8)	S(25)
17	S(25)	R(0)	S(24)	R(11)	R(6)	R(11)	R(9)	S(19)
18	S(19)	R(0)	S(28)	R(6)	S(20)	R(11)	S(22)	S(20)
19	S(25)	S(26)	S(20)	S(25)	S(18)	R(4)	S(20)	S(28)
20	S(24)	R(0)	R(10)	S(28)	S(20)	S(25)	S(25)	S(25)

Source: CLSI (2016).
Key: IMP= Imipenem (10 µg), E= Erythromycin (15 µg), AMX= Amoxicillin (25 µg), CXM= Cefuroxime (30 µg), CAZ= Ceftazidime (30 µg), CFM= Cefixime (5 µg), CN= Cephalexin (30 µg), CIP= Ciprofloxacin (5 µg), S = sensitive, and R = resistant.

Table 6. Percentage sensitivity test *V. cholera.*

Post	Post-antibiogram							
	IMP	E	AMC	CXM	CAZ	CFM	CN	CIP
1-20	100%	70%	65%	60%	60%	50%	70%	100%

work done by Oje et al. (2018) who reported that most of the sampled foods in his study were found to contain total aerobic bacterial count of less than the permissible limit thereby rendering the food fit for consumption. High counts observed in this study could be a result of the level of exposure to the dirty environment of the various sampling sites (Oje et al., 2016). *V. cholerae* counts differed significantly ($p<0.05$) across local government areas. The risk factors associated with the high prevalence of *Vibrios* in food samples may pose impact on the cholera disease outbreak Tambekar et al., 2008).

V. cholerae isolates were confirmed by their morphology and biochemical reactions. According to the survey of antibiotics susceptibility test, the percentage of post antibiogram showed that imipenem and ciprofloxacin with 100% were more susceptible when compared to other antibiotics used in the study. They are in accordance with the work of Okoh (2012) who made a similar observation by reporting that ciprofloxacin and ofloxacin were mostly sensible compared to other antibiotics during the research. It is important to note that frequent surveillance programs are significant ways of identifying changes in the spectrum of pathogenic microorganisms causing severe infections and to properly checkmate the trends in antibiotic resistance patterns (Amare et al., 2019). The study clearly revealed that resistance in the *Vibrio* isolates to the various tested drugs was plasmid mediated not chromosomal. This is in accordance with the work done by Thavasi et al. (2007). This is also supported by Obi et al. (2007) who reported that plasmid mediated mechanism may increase the possibility of horizontal spread. Regular food safety enhancement programs all through the numerous food supply chain must be observed by food cooks in order to enhance food safety level in the entire stages of the food production processes (Amare et al., 2019). The study therefore has provided useful information in the search for safe and efficient antibiotics. In addition, it also gave us some insight into the problems and created awareness to the consumers towards the antibiotic resistance level in vended jollof rice food sold in Benin City, Nigeria.

CONCLUSION AND RECOMMENDATION

Plasmid is an extra chromosomal DNA; its role in antibiotics resistance cannot be overemphasized. However, the results of the pre and post plasmid curing of the *Vibrio* isolates in this study have shown that the initial resistance to the various tested antibiotics was plasmid mediated not chromosomal. This can be linked to poor hygienic practices and other environmental factors. Consequently, the antibiotic resistance observed is a major threat to the successful treatment of secondary infection in cholera patients. However, from this study, imipenem and ciprofloxacin appeared to be the best drug of choice for the treatment of cholera infection with 100% sensitivity. Hence, public health education must be strengthened by food regulatory agencies in Nigeria to enhance food safety due to little or no knowledge of hygiene acquired by some of these food cooks and vendors operating in the studied localities.

CONFLICT OF INTERESTS

The author have not declared any conflict of interests.

REFERENCES

Amare, A., Worku, T., Birukitait, A., Ashagirie, B., Adugna, M., Getaneh, A. and Mulat, D. (2019). Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: a cross sectional study. *BMC Microbiology,* 19-120

Bauer, A. H., Kirby, W.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disc method. *American journal of clinical pathology*,45(4): 493-496

Brinborn, H.C and Dolly, H.C. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Journal of Clinical Microbiology* (**5**): 1513-1523

Cheesbrough, M. (2005). District laboratory practice in Tropical countries 2. London Cambridge University Press.pp 112-115.

Clinical and Laboratory Standard Institute (CLSI). (2016). Performance Standard for Antimicrobial Susceptibility Testing, Twenty-Fouth informational supplement, CLSI document M100-524, Wayne, P.A. United States. Pp 50-57

Obi, C.L., Ramalivhana, J., Momba, M.B., Onabolu, B., Igumbor, J.O., Lukoto M., Mulaudzi, T.B., Bessong, P.O., Jasen van Rensburg, E.L., Green, E., Ndou, S. (2007). Antibiotics resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS Patients with or without diarrhoea and their household drinking water in rural communities in Limpopo Province South Africa. *Africa Journal of biotechnology*. 6(8): 1035-1047.

Oje, O.J., David, O.M., Adeosun, O.M., Adebayor, A.A. and Famurewa, O. (2016). Multi antibiotic resistant *Escherichia coli* in ready-to-eat foods from food outlets in Ekiti State University and its environ. *British Microbiology Research Journal*, **3**(1): 1-11

Oje, O.J., Ajibade, V.A., Fajilade, O.T. and Ajenifuja, O.A. (2018). Microbiological analysis of ready-to-eat (RTE) foods vended in mobile outlet catering units from Nigeria. *Journal of Advances in Food Science and Technology*, 5(1): 15-19

Okoh, E.B. (2012). Antibiotic susceptibility patterns and plasmid prophile of *Vibrio cholera* from water samples in Ebele Community, Rivers State. Nigeria. *Journal of Applied Science and Environmental Management* 16(1)135-140.

Rayza, D.M., Marcela, B.V., Charles, F. and Suzi, B.C. (2016). Food safety and hygiene practices of vendors during the chain of street food production in florianopolis, Brazil: A cross-sectional study. *Food control*, 62:178-186

Schaeti, C., Sundaram, N., Merten, S., Ali, S.M., Nyambedha, E.O. and Lapika, B. (2013). Comparing socio-cultural features of cholera in three endemic African settings. *BMC Medicine*, 11(1): 206

Sur, D. (2000). Severe cholera outbreak following floods in a northern district of West Bengal. *Indian Journal Medical Research*. 112:178-182.

Tambekar, D., Jaiswal, V., Dhanorkar, D., Gulhane, P. and Dudhane, M. (2008). Identification of microbiological hazards and safety of ready-to-eat food vended streets of Amravati City, India. *Journal of Applied Biosciences* **7**: 195-103

Thavasi, R., Aparmadevi, K., Jayalaksmi, S., Balasubraman, T. (2007). Plasmid mediated antibiotic resistance in marine bacteria. *Journal of enviromental biology* 28(3):617-621.

World Health Organisation (WHO) (2011). Guidelines for Drinking Water Quality (4^{th} ed.) World Health Organisation, Geneva, 564pp.

WHO (2017). Guidelines for Drinking water quality, Volume 1: Recommendations. World Health Organization, Geneva.