Susceptibility and Characterization of *Vibrio cholerae* Obtained from Aqua-based Samples in Selected Local Government Areas, Zamfara State, Nigeria

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Abstract*: *Vibrio cholerae* is causative agent of cholera and its impact has been associated with significant morbidity and mortality in health care institution and community settings globally. Studies have shown that two serotypes are associated with cholera outbreak globally, but an atypical strains with reduced resistance to antibiotics have been implicated in Nigeria. Hence, this study was designed to identify and determine the antibiogram of *Vibrio cholerae* obtained from water samples in Zamfara State with a view to help in antibiotic surveillance system. Thirty-eight (38) water samples comprising of well water, streams and rivers were randomly selected from nine Local Government Areas in Zamfara State; (Tsafe (3), Zurmi (2), Maradun (1), Talata Mafara (2), Gusau (3), Bungudu (13), Birnin Magaji/Kiyaw (1) and Shinkafi (12). *Vibrio cholerae* were isolated using TCBS agar by standard microbiological method. The sensitivity of the isolates was determined by disc diffusion method while the isolated was confirmed by polymerase chain reaction (PCR) using cholera toxin gene (*ctxA*) specific primers. A total of 15 isolates; well (1), rivers (11), streams (3), were obtained from 38 samples collected. The resistance profile of isolates showed that all isolates (100%) were resistant to ceftriaxone, cefixime, and ceftazidime. Also, 8%, 46% and 81% of the isolates were resistance to gentamicin, nitrofurantoin and amoxicillin/clavulanate respectively, but all the isolates were susceptible to ofloxacin with 88% susceptible to both gentamicin and ciprofloxacin. Likewise, 54% of isolates were susceptible to nitrofurantoin while 19% were susceptible to amoxicillin/clavulanate. The results obtained revealed presence of *Vibrio cholerae* in an environmental reservoir especially in river sources with high profile antibiotic resistance which could pose serious health risk to the community. Hence, antibiotic surveillance system in this region is advised.

***Keywords*: Antibiotic susceptibility, Characterization, PCR-specific primers, *Vibrio cholerae*, Water samples,**

I. INTRODUCTION

According to historical records, cholera originated in the Ganges Delta in India and spread over the world through seven succeeding pandemics that killed millions of people on all seven continents. The seventh pandemic began in South Asia in 1961 and spread to Africa in 1971 before reaching the Americas in 1991, with Africa suffering a disproportionately large share of the disease burden worldwide. Within 40 years, 3,221,050 probable cholera cases from African nations were reported to the World Health Organization, accounting for 46% of all cases reported globally (Mengel et al., 2014).

In 2011, the cholera pandemic was responsible for 99% of all deaths and 86% of all recorded cases worldwide. Only two strains, O1 and O139, of the cholera-causing *Vibrio cholerae*, which has several serogroups, generate epidemics. While *V. cholerae* O139 produced previous outbreaks, with the exception of recent rare cases, *V. cholerae* O1 was in the cause of all subsequent epidemics (WHO, 2018). The outbreak typically happens year-round during the rainy season, and its spread is strongly correlated with limited access to sanitary facilities and clean water. Slums in peri-urban areas and camps for internally displaced people or refugees are common examples of at-risk areas because they lack even the most basic amenities like clean water and sanitation (WHO, 2018). In Nigeria, a total of 13,009 suspected cases were reported in twelve states with 116 deaths between January to 22 June, 2018. Most affected age groups were 1-4years (29.2%) and 5-14years (24.8%) with overall Case Fatality Rate of 0.89% (NCDC, 2018). Zamfara State was second to record high number of cases of cholera outbreak in Nigeria (729) in 2018 with 9 deaths. This outbreak is usually sporadic and requires an urgent intervention. The well characterized strains of *Vibrio cholerae* responsible for cholera outbreak globally have been O1 and O139. However, earlier report on cholera outbreak in Nigeria showed that it was driven by atypical El Tor strains possessing virulent factors and resistance determinants (Marin et al., 2013) indicating that its epidemiology is changing. In Zamfara State, cholera outbreak has been occurring yearly and only preliminary investigations have been done. Hence, this study was designed to evaluate the antibiogram and characterize the *Vibrio cholerae* by PCR-specific primers.

II. MATERIALS AND METHODS

Study area

The samples used in this study are water samples from wells, rivers and streams, obtained from different local government areas in Zamfara State.

Sample collection

Hundred millimeters (100 mL) of Samples were collected into

sterile universal bottles from sampling sites. Thirty-eight (38) samples were obtained from the following local government areas: Tsafe (3), Zurmi (2), Maradun (1), Talata Mafara (2), Gusau (3), Bungudu (13), Birnin Magaji/Kiyaw (1) and Shinkafi (12). The samples were transported to the Laboratory in cold pack and processed between 24-48h.

Sample processing and isolation

The workbench was disinfected using 90% alcohol. Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar (Hopebio, China) was used to isolate the microorganism of interest. The media was prepared according to the manufacturer's instructions. Serial dilution was done for the samples before inoculating by pour plate method.

Isolation and characterization of isolates

Colonies of interest were picked from the plates for further characterization. The bacterial colonies that developed on the nutrient agar plates were sub-cultured by streaking on freshly prepared nutrient agar plates until pure colonies were obtained according to the conventional procedure.

Isolation of chromosomal DNA

The pure bacterial isolates were identified on the basis of their morphological and polymerase chain reaction with specific primers. The genomic DNA was extracted using the Quick-DNA miniprep plus kit according to the instructions (Zymo research, Biolab, USA). Briefly, 200 µl an overnight broth culture the organisms were prepared in Eppendorff tubes and equal volume of the biofluid cell buffer were added. The contents of the tubes were vortexed for 10-15 seconds before being incubated for 10 minutes at 55ºC. Genomic binding buffer (420 µl) was added and vortexed for 10-15 seconds. The mixtures were transferred in a Zymo-Spin collection tube and centrifuged for one minute at 12000 rpm. The spin columns were filled with exactly 400 µl pre wash buffer and 700 µl of g-DNA wash buffer, spun at 12000 rpm for one minute, and the collecting tubes with the flow through were discarded in each step. The washing step was repeated but with 400 µl of g-DNA was buffer. The spin columns were transferred into clean Eppendorff tubes, and 50µl of DNA elution buffer was poured directly onto the matrix. It was incubated at room temperature for 5 minutes before being centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was immediately put to use in molecular applications.

Identification of bacterial isolates by PCR-specific primers

A PCR mixture consisting of 12.5µl of OneTaq Quck load 2X Master Mix Buffer (New England Biolabs), 0.5µl of 10 Mm of cholera toxin primers (forward 5'-ACAGAGTGAGTACTTTGACC-3' and reverse 5'-ATACCATCCATATATTTGGGAG-3'), and an aliquot of 2.0µl of DNA suspension was added to the mixture. With sterile water, the reaction mixture was rounded up to 25µl. The following thermal cycling profile was used for DNA amplification using miniPCR (USA): Initial denaturation at 95ºC for 2 minutes; 30 cycles of denaturation (30 seconds at 95ºC), annealing (1 minute at 55ºC), and extension (2 minutes at 72ºC) and a final extension at 68ºC for 5 minutes. The amplicons were electrophoresed and virtualized by geldoc system.

Antibiotic susceptibility testing

For the antibiogram, antimicrobial susceptibility discs containing different families: ceftazidime (CAZ), cefuroxime (CRX), gentamicin (GEN), cefixime (CXM), ofloxacin (OFL), amoxicillin/clavulanate (AUG), nitrofurantoin (NIT), ciprofloxacin (CPR) was be used according to Clinical Laboratory Standard Institute (2021). The antibiogram was interpreted using the same standard.

III. RESULTS

Colony morphology

Vibrio colonies on TCBS agar appeared either yellow (showing that they are sucrose fermenters). Typical Vibrio colonies which appeared yellow.

Isolate distribution

A total of 15 isolates; well (1), rivers (11), streams (3), were obtained from 38 samples collected; solar water (2), well water (6), river water (20) and stream water (3). The number of isolates obtained from sampled local government include: Tsafe (3), Zurmi (2), Maradun (0), Talata Mafara (0), Gusau (3), Bungudu (6), Birnin Magaji/Kiyaw (0) and Shinkafi (1). The Figure 1 shows the gel electrophoresis of PCR product of The cholera toxin gene (*ctxA*) for the confirmation of *Vibrio cholerae*

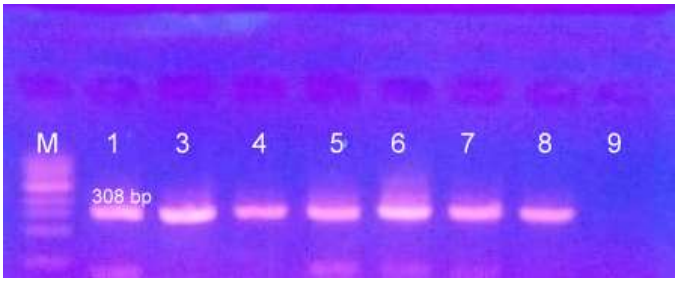

Figure 1: The PCR product of The cholera toxin gene (*ctxA*) for the confirmation of *Vibrio cholerae*. M: 100bp ladder, *ctxA*: 1-8 and Negative control (PCR mix with no DNA).

Antibiotic resistance profile

The resistance profile in zamfara state showed that all isolates were 100% resistant to ceftriaxone (CRX), cefixime (CXM), and ceftazidime (CAZ). Isolates showed 8%, 46% and 81% resistance to gentamicin (GEN), nitrofurantoin (NIT) and amoxicillin/clavulanate (AUG) respectively. It was also observed that all the isolates were susceptible to ofloxacin (OFL), 88% were susceptible to both gentamicin (GEN) and ciprofloxacin (CPR), 54% of isolates were susceptible to nitrofurantoin (NIT) and 19% were susceptible to amoxicillin/clavulanate (AUG). The susceptibility pattern of isolates is represented below (Figure 2).

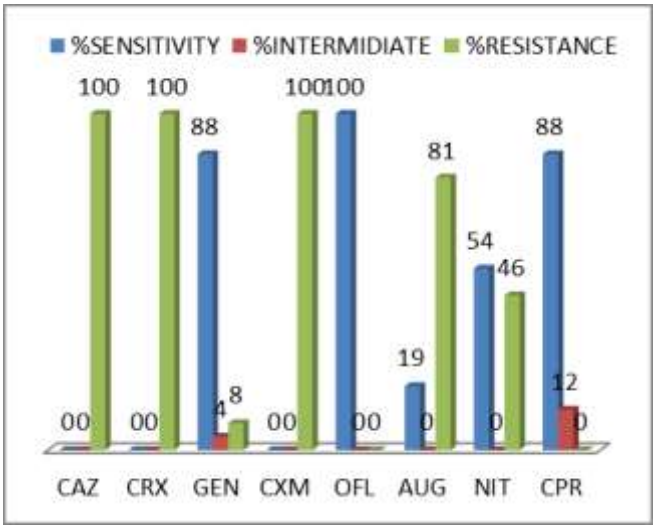

Figure 2: Antibiotic susceptibility profile across zamfara state.

CAZ: ceftazidime, CRX: ceftriaxone, OFL: ofloxacin, GEN: gentamicin, CXM: cefixime, AUG: amoxicillin/clavulanate, NIT: nitrofurantoin, CPR: ciprofloxacin.

IV. DISCUSSION

The presence of *Vibrio cholerae* in water is not surprising as it has been repeatedly named as a major waterborne pathogen of medical interest. In Nigeria, in 2018, there were a total of 43,996 cholera cases and 836 deaths across 20 states (Elimian et al., 2019). The occurrence of *Vibrio cholerae* from the samples in this study could be attributed to the fecal contamination of communal streams or rivers, hence, the high incidence in river/stream samples which is observed in the pattern of results from this study. The absence of *Vibrio cholerae* in some samples is assumed to be as result of the good quality of the water, but other factors such as stressful conditions could be responsible. There is a suggestion that *Vibrio cholerae* can exist as viable but non culturable under stressful conditions, but can be triggered as soon as it encounters the right environmental factors and go on to cause outbreaks (Abakpa et al., 2013).

The local government with positivity included Tsafe, Zurmi, Gusau, Bungudu and Shinkafi. These specific local governments have been implicated in various cholera incidences. In 2021, a report showed that 30 people died as a result of cholera in zamfara state with an influx of treatment going on at Bakura, Bugundu, Tsafe, Gusau, Zurmi, Kaura Namoda and Birnin Magaji (Are, 2021).

This study showed a significant level of susceptibility to ofloxacin (100%) and ciprofloxacin (88%) which is slightly similar to study by Chijioke et al., (2014) where there was 100% susceptibility to ciprofloxacin and ofloxacin. Isolates in this study were 88% susceptible to gentamicin. High level susceptibility to gentamicin has been recorded in various studies as explained by Uppal et al., (2017). The previously mentioned author explained that the *Vibrio cholerae* strains responsible Odisha and West Bengal outbreaks were gentamicin sensitive. All isolates were resistant to ceftriaxone as opposed to findings by Mandal et al. (2012) who recorded high level susceptibility of isolates to ceftriaxone. This variation could be attributed to porous surveillance protocols in Nigeria and a lacking drug regulation policy.

The occurrence of *Vibrio cholerae* across samples collected showed a high level exposure of consumers to unsafe polluted water and this exposure could contribute to an already high incidence rate of cholera outbreak in the state and the country at large. The growing problems with antimicrobial drug resistance are beginning to wear down our antibiotic abilities to fight antibiotic resistance, and thus limiting therapeutic options to present-day clinicians (Igbinosa, 2010). The epidemiological observation of antimicrobial resistance is crucial for treatment of infections and in preventing the spread of antimicrobial-resistant micro-organisms (Oramadike & Ogunbanwo, 2015). With pathogenic *Vibrio* species becoming a major public health concern, a rapid and effective detection method would be needed in order to monitor its presence in the environment and the knowledge of its drug sensitivity (Frans et al., 2011; Oramadike & Ogunbanwo, 2015).

The virulence elements in the *Vibrio cholerae* arsenal are numerous. Some of the primary determinants of *V. cholerae* pathogenecity include serotype switching, toxin expression, biofilm development, numerous transcriptional circuits, genome flexibility, adhesion and invasions, cytolytic proteins, secretion systems, and the capacity to adapt to various stimuli (Ramamurthy et al., 2020). There are many serogroups of *V. cholerae* which ubiquitously inhabits aquatic environments, but *V. cholerae* which cause outbreaks of cholera disease leading to endemic and pandemic outbreaks is limited to only *V. cholerae* serogroups O1 and O139 producing cholera toxin (Ceccarelli et al., 2019). *Vibrio cholerae* serogroups non-O1/non-O139 are non-agglutinable vibrios and have not caused endemic and pandemic outbreaks, although these bacteria have caused sporadic infections (Morita et al., 2020; Vezzulli et al., 2020). Toxigenic non-O1/non-O139 *Vibrio cholerae* strains appear to be frequently isolated from both clinical and environmental samples worldwide and are reported to be highly diverse (Ceccarelli et al., 2015).

The results obtained revealed presence of *Vibrio cholerae* in an environmental reservoir especially in river sources with high profile antibiotic resistance which could pose serious health risk to the community. Hence, antibiotic surveillance system in this region is advised.

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