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Brief Communication



# Retrospective Analysis of the Serovars and Antibiogram of *Vibrio cholerae* Isolates of the 2017 Ilorin Cholera Outbreak, Nigeria

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## ABSTRACT

In this retrospective study, we determined the incidence, serovars, and antibiogram of *Vibrio cholerae* isolated from 102 clinical stool samples collected from rice water diarrheic patients during an outbreak (May - July 2017) in Ilorin metropolis, Nigeria. The culture positive rate of the *V. cholerae* isolates was 41.2%, with 41 and 1 isolates from O1 (Inaba) and non-O1/O139 serogroups, respectively. The isolates were the most susceptible to ciprofloxacin (76.2%) followed by amoxicillin-clavulanate (71.4%). However, all isolates were resistant to ampicillin and tetracycline. In conclusion, *V. cholerae* O1 was the predominant circulating serogroup exhibiting multi-drug resistance during the outbreak.

**Keywords:** Waterborne; Cholera; Nigeria; Antibiogram; Serovars

Globally, about 1.3 - 4.0 million reported cholera cases and 21,000 - 143,000 cholera-associated mortalities occur per annum [1]. Although cholera has been used as an indicator of inequality and socio-infrastructural development [2], it has disproportionately affected low- and middle-income countries. Cholera outbreaks are frequently common in communities with inadequate sanitary conditions and poor water supply system [3]. In Nigeria, a cholera outbreak was first reported in 1970 [2]. Subsequently, cholera remained endemic in Nigeria with several reported outbreaks and high case-fatalities, almost annually [2, 3].

The most devastating cholera outbreak in Nigeria occurred in 1991, when the epidemic resulted in 59,478 cases and 7,654 deaths [3]. Throughout 2010, 41,787 cases and 1,716 cholera-associated deaths were reported in half of the states in Nigeria [4]. From January to September 2017, there were 5,138 cholera cases and 136 cholera-associated deaths [5]. In 2018, over 43,000 suspected cholera cases with a fatality rate of 1.95% were reported from 20

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**Conflict of Interest**
No conflicts of interest.

**Author Contributions**
Conceptualization: DAO, ES, IAN. Data curation: DAO, IAN. Formal analysis: IAN. Methodology: DOA, KK, MBA, AAA, CCO. Project administration: AF, AAA, CN. Writing - original draft: DAO, IAN, AAA, CCO, AF. Writing - review & editing: DAO, ES, IAN, AF, AAA, CCO, BMA, KK, CN.

out of 37 states (including the capital of Nigeria) [1, 3]. From January 1 to September 6, 2020, there were 1,115 suspected cholera cases and 61 cholera-associated deaths in Nigeria, wherein only 40 cases were confirmed through accurate laboratory tests [6]. During a similar period in 2019, 2,497 suspected cholera cases with 38 associated deaths were reported [6].

Over 200 serovars of *V. cholerae* have been identified till date. However, cholera epidemics are mainly caused by 2 serovars, *viz*, O1 and O139. Serovar O1 has 2 biotypes, classical and *El Tor*, and both these biotypes are further classified into 2 serovars, Ogawa and Inaba [7].

Antibiotics have been used as an adjunctive to rehydration therapy in the treatment of cholera to substantially reduce the duration of acute diarrhea and limit the spread of *V. cholerae* [8]. However, *V. cholerae* resistant to several clinically useful antibiotics have emerged, including resistance to most beta-lactams, trimethoprim-sulfamethoxazole, tetracycline, fluoroquinolones, and chloramphenicol [9]. In cognizance to the paucity of published data on cholera in recent time, this present study was instigated to determine the incidence, serogroups, and antibiogram of *V. cholerae* in clinical stool samples during an outbreak in Ilorin metropolis, North central Nigeria, in 2017.

This study was conducted at the University of Ilorin Teaching hospital (UITH), Ilorin. The hospital is located in the North central region of Nigeria at 8.4799° N, 4.5418° E. Purposive sampling technique was employed for the selected patients who had rice water diarrhea more than 4 times per day without any history of antimicrobial usage at the time of sample collection. Ethical approval was obtained from the Ethical Review Committee of UITH (IRB approval no.: UITH/HBT/RES/17/528).

All adult subjects and parents of children gave informed consent before enrollment into the study. One hundred and two stool samples were collected from acute diarrheic patients who attended the outpatient departments, as well as those were admitted in various inpatient units of the hospital, during a cholera outbreak (May - July 2017); the samples were investigated for the presence of *V. cholerae.* Fresh samples were collected from patients of all age groups at the first day of rice water diarrhea and immediately transported to the medical microbiology laboratory, where they were processed according to the standard of microbiological detection of diarrheagenic enterobacteria described by Bradford et al. [10].

Freshly collected fecal samples were enriched in alkaline peptone water and selenite-F broth at 37°C for 6 h, and then sub-cultured onto thiosulfate-citrate-bile salts-sucrose deoxycholate agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) and incubated at 36°C for 24 h. All *V. cholerae*-like colonies were subjected to conventional biochemical tests, and the bacterial isolates that showed results corresponding to *V. cholerae* were identified serologically by slide agglutination test using specific antisera (polyvalent O1, O139, and monospecific Ogawa and Inaba antisera) obtained from Denka Seiken Company Limited, Tokyo, Japan.

All *V. cholerae* isolates were tested for their susceptibility to 6 different antimicrobial agents by agar disk diffusion technique in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [11]. The following antimicrobial agents were used: ampicillin (10 μg), nitrofurantoin (30 μg), ciprofloxacin (5 μg), tetracycline (10 μg), amoxicillin-clavulanate (10 μg), and ceftriaxone (30 μg). Antimicrobial susceptibility results were reported as recommended by the CLSI guidelines. *Escherichia coli* ATCC 25922 was used as the control strain for the disc diffusion test.

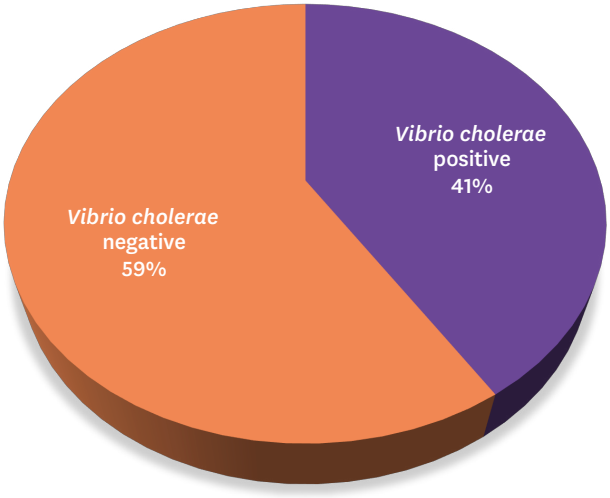


**Figure 1.** *Vibrio cholerae* culture positive cases among acute diarrheal patients in Ilorin, Nigeria.

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp, Armonk, NY, USA). Descriptive statistics are presented as frequencies and percentages in figures. Binary logistic regression was used to determine the association of biodata and *V. cholerae* isolates. *P*-values <0.05 at 95% confidence intervals (CI) were considered statistically significant.

The culture positive rate of *V. cholerae* isolated from rice water diarrheic patients was 41.2% (**Fig. 1**). Forty-one (97.6%) *V. cholerae* isolates were identified as *V. cholerae* O1 (Inaba) serogroup, whereas 1 isolate failed to agglutinate with either O1 or O139 antisera, and thus, belonged to a non-O1/O139 serovar. The highest prevalence of *V. cholerae* isolates was observed among diarrheic patients aged between 31 and 40 years (83.3%), and the least prevalence was found among those aged between 41 and 50 years (22.2%). Furthermore, their prevalence was relatively higher in females (56.1%) than in males (29.5%). After bivariate logistic regression, sex (odds ratio [OR] = 0.33 [95% CI, 0.14 - 0.75], $P = 0.008$) was found to be associated with *V. cholerae* infection among the patients (**Table 1**). The *V. cholerae* isolates were susceptible mostly to ciprofloxacin (76.2%) followed by amoxicillin-clavulanate (71.4%). However, all isolates were resistant to ampicillin and tetracycline (**Table 2**).

**Table 1.** Distribution of *Vibrio cholerae* isolates by age and sex of diarrheal patients

| Demography | No. of Subjects | No. of *Vibrio cholera* positive patients (%) | OR (95% CI) | *P*-value |
|---|---|---|---|---|
| Age | | | | |
| 0 - 10 | 19 | 7 (36.8) | Referent | |
| 11 - 20 | 14 | 5 (35.7) | 1.05 (0.25 - 4.42) | 0.947 |
| 21 - 30 | 21 | 11 (52.4) | 0.53 (0.15 - 1.88) | 0.326 |
| 31 - 40 | 12 | 10 (83.3) | 0.12 (0.02 - 0.69) | 0.018[a] |
| 41 - 50 | 18 | 4 (22.2) | 2.04 (0.48 - 8.71) | 0.335 |
| >50 | 18 | 5 (27.8) | 1.52 (0.38 - 7.09) | 0.557 |
| Sex | | | | |
| Male | 61 | 18 (29.5) | Referent | |
| Female | 41 | 23 (56.1) | 0.33 (0.14 - 0.75) | 0.008[a] |

[a]Significant association determined by Bivariate Logistic Regression.
OR, odds ratio; CI, confidence intervals.


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**Table 2.** Antimicrobial susceptibility pattern of *Vibrio cholerae* isolates from acute diarrheal patients

| Antimicrobial | Susceptible (%) | Resistant (%) |
|---|---|---|
| Ciprofloxacin | 32 (76.2) | 10 (23.8) |
| Tetracycline | 0 (0.0) | 42 (100.0) |
| Ampicillin | 0 (0.0) | 42 (100.0) |
| Nitrofurantoin | 19 (45.2) | 23 (54.8) |
| Ceftriaxone | 28 (66.7) | 14 (33.3) |
| Amoxicillin-clavulanate | 30 (71.4) | 12 (28.6) |

Despite several efforts to control cholera, it is a major public health problem in Nigeria. In the present study, we found that the incidence of *V. cholerae*-associated diarrhea was 41.2%. Although fewer cases in endemic areas have been reported by other studies [12, 13], a previous study has reported higher isolation rates of *V. cholera* during outbreaks [14]. The difference in detection rate could be due to the level of endemicity and stage of the outbreak in the study locations. Furthermore, difference in sample processing and laboratory methods could also affect the detection rate of *V. cholerae*.

Although cholera affects individuals of all age groups and sex, high infection rates have consistently been reported among younger children [13]. However, our data was not in conformity with these findings. In our study, 83.3% cholera cases were found in patients aged between 31 and 40 years, thus, contributing significantly to the burden of the rice water diarrhea. A similar finding was reported by Garbati et al. [15], wherein the highest number of cholera cases were reported in patients between 36 and 45 years of age. This could be due to poor sanitary condition of most parts of our study area, where most people reside in overpopulated slums. Despite this observation, the proportion of pediatric cases was much higher than what has been previously reported in Maiduguri city of Nigeria [15].

The distribution pattern of cholera cases by age and sex is largely dynamic in Nigeria, as there are mixed available reports. A study reported higher number of cholera cases in adults than in children [16], whereas more cholera cases were reported in children than adults by another study [17]. Furthermore, some studies have reported higher number of cholera cases in females than in males [16] and *vice-versa* [18]. In conformity with the findings of Nnaji et al. [18], our study showed significant association between the number of cholera cases and the sex of patients.

This study revealed *V. cholerae* O1 as the predominant circulating serovar and highlighted the absence of the O139 strain. The predominance of serovar O1, particularly the serotype Indawa, has been reported in previous outbreaks from Nigeria where it has been the main serotype responsible for cholera infections [19]. Furthermore, none of the isolates in our study were non-O1 and non-O139 *V. cholerae*. In Nigeria, non-O1 and non-O139 serovars have been rare in the last decade [20].

Varying antimicrobial susceptibility patterns have been reported among *V. cholerae* strains isolated from different parts of Nigeria. Our study demonstrated high antimicrobial resistance to ampicillin and tetracycline. Circulation of ampicillin resistant *V. cholerae* isolates has also been documented by Uppal et al. [13]. This suggests that these drugs may not be suitable for therapeutic management of cholera cases.

In our study, most isolates were susceptible to ciprofloxacin (76.2%) and amoxicillin-clavulanate (71.4%). In contrast to our findings, Mohammed et al. [19] reported a 44%

fluoroquinolones resistance rate of *V. cholerae* in sub-Saharan Africa. The high level of antimicrobial resistance against tetracycline, a drug previously considered the choice of treatment for cholera confirms the indiscriminate use of this drug [20]. Other studies have also reported increasing tetracycline resistance among *V. cholerae* O1 strains involved in major epidemics in Africa [19]. Unfortunately, the retrospective nature of our study design did not allow us to explore more determinants of the cholera cases.

This study revealed that *V. cholerae* O1 (Inaba) was the predominant circulating serogroup exhibiting multi-drug resistance during the outbreak. Hence, there is a need for active surveillance of cholera in all sporadic diarrhea cases in the community.

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