

Characterization and Antibiotic Susceptibility Profile of *Vibrio cholerae* Isolated from Stool of Patients Attending Ahmadu Bello University Medical Centre, Samaru-Zaria, Kaduna State, Nigeria

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Abstract: Cholera remains a significant public health concern worldwide, particularly in developing countries like Nigeria. It has the potential to cause widespread fatalities, spread rapidly, and disrupt travel and trade. This study aimed to characterize *Vibrio cholerae* isolated from stool samples of patients attending Ahmadu Bello University Medical Centre, Samaru-Zaria, Nigeria and assess the antibiotic susceptibility profile of the identified strains. A total of 50 stool samples were collected from consenting patients with diarrhoea between July and August, 2023. These samples were screened for *Vibrio cholerae* using standard microbiological techniques. The isolates were identified based on colonial morphology, Gram staining, and biochemical tests. Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion method, with results interpreted according to Clinical and Laboratory Standards Institute (2018) guidelines. The study recorded a *Vibrio cholerae* prevalence of 12%. All isolates exhibited 100% susceptibility to sparfloracin, ciprofloxacin, and gentamicin. Susceptibility rates to other antibiotics were as follows: Trimethoprim-sulfamethoxazole (83.3%), Chloramphenicol (66.7%), and Tetracycline (66.7%). However, resistance was observed against Amoxicillin-clavulanate (50%) and Amoxicillin (66.7%). Statistical analysis revealed a significant association between *Vibrio cholerae* occurrence and socio-demographic factors such as age and education level. However, none of the analyzed risk factors showed a statistically significant correlation with the presence of the pathogen. This study has demonstrated that *Vibrio cholerae* is still endemic within the study area. The isolates were also resistant to some commonly used antibiotics within the study area. The most effective long-term strategy for cholera prevention is the implementation of sustainable water, sanitation, and hygiene systems. Ensuring access to safe water, basic sanitation, and proper hygiene practices will significantly reduce the risk of cholera outbreaks in the study area.

Key word: Antibiotics, characterization, stool, susceptibility, *Vibrio cholerae*

INTRODUCTION

Cholera is a severe diarrheal disease caused by *Vibrio cholerae* serogroups O1 and O139, primarily transmitted through contaminated water and poor hygiene (Ngwa *et al.*, 2021). It remains a major public health concern, particularly in regions with inadequate water, sanitation, and hygiene (WaSH) infrastructure. The disease is caused by the Gram-negative bacterium *Vibrio cholerae*, which colonizes the small intestine and produces cholera toxin, leading to acute watery diarrhea that can result in severe dehydration and death if untreated. The disease spreads rapidly, leading to high morbidity and mortality, with Africa, especially Nigeria, bearing a significant burden (Rebaudet *et al.*, 2013; Ngwa *et al.*, 2021; Al-Adham *et al.*, 2024).

Nigeria has experienced recurrent cholera outbreaks, exacerbated by factors such as population displacement, flooding, and limited access to clean water. The 2021 outbreak was one of the worst in recent history, affecting 33 states and resulting in over 111,000 cases and 3,600 deaths (Fagbamila *et al.*, 2022). Northern Nigeria remains a hotspot due to open defecation, unsafe drinking water sources, and poor sanitation, with Kaduna and Katsina states reporting high cases (Fagbamila *et al.*, 2022; NCDC, 2022; Elimian *et al.*, 2024).

Antibiotics are often used as adjunct therapy in severe cholera cases to reduce the duration of diarrhoea, decrease bacterial shedding, and shorten hospitalization. However, the emergence of antibiotic-resistant strains of *V. cholerae* has become a growing concern in many endemic regions

(Chisompola *et al.*, 2025). Continuous monitoring of antibiotic susceptibility patterns is therefore necessary to ensure the effectiveness of commonly used antimicrobial agents and to inform treatment guidelines.

This study aimed to investigate cholera outbreaks in a localized setting, focusing on key risk factors and effective control strategies. Given the proximity of the study area to high-burden regions, timely diagnosis, optimized treatment, and public health interventions are crucial. The research also emphasizes the need for enhanced surveillance, improved sanitation, and health education to mitigate the devastating impact of cholera.

MATERIALS AND METHODS

Study Area: The study area is a suburban University settlement. The University is located on latitude 11°15'N to 11°3'N and longitude 7°30'E to 7°45'E. The study was conducted in the Microbiology and Parasitology Laboratory of Ahmadu Bello University Medical Centre (ABUMC) Samaru-Zaria, Nigeria. The entire area is highly influenced by the presence of the University and this Medical Centre was selected because it serves as a major hotspot where the communities around the study area can afford standard and less expensive health check-ups and treatments. The population around the Medical Centre has increased from 20,000 in 1975 to about 100,000 in 1991 (1991 census) and even higher now. It is characterised by a tropical climate with two main seasons: a rainy season from May to October and a dry season from November to April. The monthly mean temperature records a range from 13.8°C to 36.7°C and an annual rainfall of 1092.8 mm. Communities of the study area comprise mainly Civil Servants, Peasant Farmers, and Traders (Abubakar *et al.*, 2024).

Ethical Approval: Ethical approval (number: UHS/ADM/S-8) was sought and obtained from ABUMC Samaru-Zaria, Nigeria, before the commencement of

sample collection. Informed consent was also sought and obtained from study participants before the administration of the questionnaire and sample collection.

Study Population: Study participants included patients of both male and female aged 2 years and above attending ABUMC Samaru-Zaria during the period of the research and who gave their consent to participate in the study.

Inclusion and Exclusion Criteria: The criteria as described by the Global Task Force for Cholera Control (GTFCC), (2019) for suspected cholera cases in areas where a cholera outbreak has not yet been declared was used. Patients of both sexes aged 2 years and above with reported symptoms of acute diarrhoea who gave their consent to participate in the study were included in the study. Patients without symptoms of diarrhoea and those with symptoms below 2 years of age were excluded from the study (GTFCC, 2019).

Questionnaire Administration: Interviewer-administered structured questionnaires were used to collect the following information from patients: socio-demographic characteristics such as age, sex, educational level, and occupation. Clinical information such as experienced symptoms, and possible exposure to risk factors. Data on behavioural habits collected included house characteristics, source of water, fruits and vegetables, management of waste, hygienic practices such as cleaning and disinfection procedures, types of toilet facilities, eating habits and travel history.

Collection of Samples: A total of 50 stool samples were collected for the study from participants with diarrhoeal symptoms from the Microbiology and Parasitology Laboratory unit, ABUMC, Samaru, Zaria, Nigeria. All the collected samples were labelled appropriately and stored in the refrigerator until they were analysed. Samples were processed in less than 2 hours from time of collection.

Media Preparation: All Media used for the study were prepared while adhering strictly to the manufacturer's instructions and basic

laboratory aseptic techniques. Media used were alkaline peptone water (APW) for enrichment of the desired isolates, Nutrient agar and blood agar, thiosulphate citrate bile-salt sucrose (TCBS) agar for selective isolation of *Vibrio cholerae*, SIM medium, Mueller-Hinton agar, and Kligler's iron agar (KIA).

Enrichment in Alkaline Peptone Water: About 3-4 loopfuls of the collected fresh stool samples were transferred into sterile alkaline peptone water (APWs) using a wooden applicator stick. The APW served as the enrichment medium for the target organism. *Vibrio cholerae* grows very rapidly in APW, and at 6-8 hours incubation time in an incubator at 37°C. This ensured that *Vibrio cholerae* is present in greater numbers than non-*Vibrio* organisms contained in the stool samples (Murray *et al.*, 2016).

Isolation and Identification of Isolates: After enrichment in APW, a loop full was aseptically transferred onto warm highly selective greenish thiosulphate citrate bile-salt sucrose (TCBS) agar. Using a sterilized wire loop, it was streaked onto the surface of the dried plates to yield discrete colonies and then incubated at 37°C for 24 hours. The TCBS cultures after overnight incubation were examined for large yellowish sucrose-fermenting colonies of *V. cholerae* (Uddin *et al.*, 2018). Cultures which showed these colonial features were sub-cultured onto non-selective agar to obtain pure cultures.

Pure Culture Preparation on Nutrient Agar: Colonies that showed sucrose fermentation on TCBS agar were sub-cultured on freshly prepared nutrient agar plates and incubated at 37°C for 24 hours.

Direct Motility Test in Distilled Water and Peptone Water: A loopful of growth from a nutrient agar subculture was mixed in a drop of sterile distilled water on one end of a slide. On the other end of the slide, another loopful of growth was mixed in a drop of peptone water. Each preparation was covered with a cover glass and examined microscopically using the 40X objective. *Vibrio* species are immobilized in distilled

water, but remain motile (characteristic darting motility) in peptone water.

Gram's Staining: Gram staining differential technique was done on the isolates from the pure cultures on nutrient agar as described by Cheesebrough (2006).

Biochemical Characterization and Screening Tests: A battery of biochemical tests were performed on the pure isolates adhering to standard procedure for further confirmation of *V. cholerae* (Tarh, 2020). These included the oxidase test, string test, sulphide production, indole utilization, and motility tests using sulphide, indole, and motility (SIM) medium, sugar fermentation test on Kligler's iron agar (KIA), the methyl-red reaction test, and Voges-Proskauer assay were conducted using the culture grown in MR-VP medium. These biochemical characterization tests were carried out according to the method of Cheesebrough (2006).

Haemolysis on Blood Agar: Prepared blood agar plates containing 10% sheep blood were pre-warmed inside the hot air oven for 5 minutes. This was then inoculated with bacterial colonies from a 24-hour growth culture, and incubated at 37°C for 24 hours to observe for haemolysis (clear zones) around the colonies of *V. cholerae* (Tarh, 2020).

Antibiotic Susceptibility of *Vibrio cholera* Isolates: Susceptibilities of pure isolates of *V. cholerae* were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Bauer *et al.*, 1966). Selected antibiotic used included (Sparfloxacin, ciprofloxacin, tetracycline, augmentin, gentamycin, chloramphenicol, septrin, and amoxicillin). Results were interpreted using Clinical and Laboratory Standard Institute (CLSI) guideline. Breakpoints and susceptibilities to each antibiotic disc were categorized as susceptible, intermediate or resistant (CLSI, 2018).

Data Analyses: Data obtained were analysed using SPSS version 21. P values of <0.05 were considered significant. Results were

presented in Tables and charts as appropriate.

RESULTS

Vibrio cholerae was successfully isolated from 13 of the 50 samples collected. The cultural characteristics of the 13 suspected *Vibrio cholerae* isolates are summarized in Table 1. All 13 isolates demonstrated turbidity and growth in alkaline peptone water. On thiosulfate-citrate-bile salts-sucrose (TCBS) agar, large to medium-sized shiny yellow colonies were observed. On nutrient agar, the colonies appeared translucent, smooth, and shiny.

A summary of the microscopic characteristics of the suspected *Vibrio cholerae* isolates is presented in Table 2. Gram staining of the 13 culture-positive isolates revealed Gram-negative, pink, small, curved to straight rod-shaped bacteria. Of these, 6 isolates exhibited motility, displaying darting movement in peptone water, but were immobile in sterile distilled water when observed under the microscope. The biochemical properties of the isolates are summarized in Table 3. In Kligler iron agar (KIA) cultures, all six motile isolates produced a red slant over a yellow butt reaction (K/A), with no gas production or hydrogen sulfide (H₂S) formation. Additionally, zones of clearing were observed around discrete colonies on blood agar. All isolates tested positive for indole production, oxidase activity, and the string test. However, all isolates recorded negative result for the methyl-red test, while 4 isolates tested positive for Voges-Proskauer (VP).

The antibiotic susceptibility profiles of the confirmed *Vibrio cholerae* isolates obtained from enrolled participants are summarized in Table 4. All isolates (100%) were susceptible to gentamicin, sparflaxacin, and ciprofloxacin. The susceptibility to trimethoprim-sulfamethoxazole was 83.3% (5/6), while 66.7% (4/6) were susceptible to chloramphenicol and tetracycline. Amoxicillin-clavulanate showed the lowest susceptibility at 50%, followed by

amoxicillin with 33.3%. Two samples, M6 and M23, were resistant to three of the four antibiotic classes tested.

Tables 5 through 7 provide an analysis of the socio-demographic factors and potential risk factors for cholera infection among the enrolled participants. Table 5 shows that the majority (80%, 40/50) of patients attending the ABUMC during the study were aged between 21 and 40 years, with the 21-30 age group being the most represented (68%, 34/50). The incidence of *V. cholerae* infection was highest in the 21-30 age group (8.8%, 3/34), although the 41+ age group had a 100% case occurrence (2/2). No cases were recorded in the 31-40 and 1-10 age groups.

As shown in Table 6, 68% (34/50) of the enrolled patients were male, while 32% (16/50) were female. Despite the higher proportion of male patients, *V. cholerae* was detected at a higher rate in females (12.5%, 2/16) compared to males (11.8%, 4/34).

In terms of education, 70% (35/50) of the patients had attained tertiary education (Table 6). However, the infection rate was lowest in this group, with only 5.7% (2/35) of cases. The "Non-formal" education group had the second-highest infection rate, at 42.9% (3/7). When considering occupation, the majority of patients were unemployed (mostly students, 80%, 40/50), and this group had a relatively low infection rate (7.1%, 3/42). Conversely, the self-employed group, which included only five individuals, had the highest case occurrence at 40% (2/5).

Table 7 presents an analysis of potential risk factors associated with cholera infection among the enrolled patients. Among the six confirmed *V. cholerae* cases, 66.7% (4/6) were from Samaru-Zaria, with an infection rate of 16%, while 33.3% (2/6) were students residing on the Main Campus of ABU, Zaria, Nigeria. Regarding hand hygiene, all six cholera-positive patients reported regular hand washing with water before meals and after using the toilet. Half of these patients (3/6, 50%) used sachet water as their primary source of drinking

water, and 33.3% (2/6) had a history of recent travel.

Table 1: Cultural Characteristics of the Isolated Bacteria

Media	Cultural characteristics	Inference
APW	Turbid and cloudy, pellicle growth observed on the surface	<i>V. cholerae</i> suspected
TCBS agar	Large to medium sized yellow and shiny colonies	“”
Nutrient agar	Translucent, smooth, shiny colonies	<i>V. cholerae</i> suspected

Keys: APW-Alkaline peptone water, TCBS-Thiosulphate citrate bile-salt sucrose agar

Table 2: Microscopic Characteristics of Suspected *Vibrio cholerae* Isolates

Parameters	Microscopic characteristics	Inference
Shape	Curved to straight rods observed	<i>Vibrio cholerae</i> suspected
Arrangement	Single and paired	<i>Vibrio cholerae</i> suspected
Gram's staining reaction	Gram-negative, pink-stained cells	<i>Vibrio cholerae</i> suspected
Motility in peptone water	Motile with darting motility	<i>Vibrio cholerae</i> suspected
Motility in sterile distilled water	Non-motile	<i>Vibrio cholerae</i> suspected

Table 3: Biochemical Characteristics of the Bacterial Isolates from Stool

Isolates	ST	OX	IN	VP	MR	KIA	Haemolysis on BA	Inference
M12	+	+	+	+	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>
M6	+	+	+	-	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>
M16	+	+	+	+	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>
M23	+	+	+	+	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>
M50	+	+	+	-	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>
M39	+	+	+	+	-	K/A; -G, -H ₂ S	β-haemolysis	<i>V. cholerae</i>

Keys: (+)-Positive, (-)-Negative, -H₂S-Negative for Hydrogen Sulphide, K-Alkaline, A-Acid, -G-Negative for Gas production, VP-Voges-Proskauer, BA-Blood Agar, MR-Methyl-Red, KIA-Kligler's Iron Agar, ST-String, IN-Indole, and OX-Oxidase

Table 4: Antibiotic Susceptibility Profile of *V. cholerae* Isolates

Antibiotic/ Isolates	SP (10µg)	CPX (30µg)	AM (30µg)	AMC (10µg)	CN (30µg)	T (30µg)	CH (30µg)	SXT (30µg)
M12	S	S	R	S	S	S	R	S
M6	S	S	R	R	S	R	R	S
M16	S	S	S	S	S	S	S	S
M23	S	S	R	R	S	R	S	R
M50	S	S	S	S	S	S	S	S
M39	S	S	R	R	S	S	S	S
Sensitivity (%)	6 (100)	6 (100)	2 (33.3)	3 (50)	6 (100)	4 (66.7)	4 (66.7)	5 (83.3)
Resistance (%)	0 (0)	0 (0)	4 (66.7)	3 (50)	0 (0)	2 (33.3)	2 (33.3)	1 (16.7)

Keys: CH-Chloramphenicol, CN-Gentamicin, SP-Sparfloxacin, T-Tetracycline, CPX-Ciprofloxacin, AM-Amoxicillin, AMC-Amoxicillin-clavulanate, SXT-Trimethoprim-sulfamethoxazole, S-Sensitive, and R-Resistant, µg-Microgram

Table 5: Samples Collected in Relation to Age, Number Examined, and Cholera Occurrence among Patients at the ABUMC, Main Campus, Samaru-Zaria

Age Group	Number Examined (%)	Number Positive (%)	χ^2	p-value
1-10	4(8)	0(0.0)	16.995	0.002
11-20	4(8)	1(25.0)		
21-30	34(68)	3(8.8)		
31-40	6(12)	0(0.0)		
41 and above	2(4)	2(100.0)		
Total	50(100)	6(12.0)		

Table 6: Occurrence of *V. cholerae* Cases in Relation to Socio-demographic Factors

Socio-demographic Factors	Number Examined (%)	Number Positive (%)	χ^2	p-value
Sex				
Male	34(68)	4(11.8)	0.006	0.941
Female	16(32)	2(12.5)		
Education				
Primary	6(12)	0(0.0)	11.174	0.011
Secondary	2(4)	1(50.0)		
Tertiary	35(70)	2(5.7)		
Non-formal	7(14)	3(42.9)		
Occupation				
Unemployed	40(80)	3(7.5)	4.782	0.092
Employed	5(10)	1(20.0)		
Self-employed	5(10)	2(40.0)		

Table 7: Occurrence of *V. cholerae* Cases in Relation to Possible Risk Factors

Risk Factors	Number Examined	Number Positive (%)	χ^2	p-value
Recent Travel				
Yes	16(32)	2(12.5)	0.006	0.941
No	34(68)	4(11.8)		
Water Source				
Tap	15(30)	2(13.3)	0.824	0.662
Sachet	31(62)	3(9.7)		
Bottle	4(8)	1(25.0)		
Food Handling Practice				
Yes	34(68)	4(11.8)	0.006	0.941
Sometimes	16(32)	2(12.5)		
Hand Hygiene				
Water Only	47(94)	6(12.8)	0.435	0.509
Soap and Water	3(6)	0(0.0)		
Residence				
Campus	25(50)	2(8.0)	0.758	0.384
Off Campus	25(50)	4(16.0)		

DISCUSSION

Cholera is a severe and potentially life-threatening diarrheal disease caused by the toxigenic strains of *Vibrio cholerae*. It is primarily transmitted through the consumption of contaminated water or food and unhygienic practices (Muzembo *et al.*, 2022). Clinical manifestations range from mild to severe acute diarrhoea, voluminous stool output, hypovolemic shock, and acidosis (Putri *et al.*, 2023). *Vibrio cholerae* represents a significant global public health burden, contributing to considerable morbidity and mortality worldwide (Igere and Ekundayo, 2020; Chowdhury *et al.*, 2022). Cholera outbreaks remain a pressing issue in certain regions, including Nigeria, where sporadic cases are reported, particularly in the northern part of the country, albeit at relatively low incidence

rates (Gulumbe *et al.*, 2023). The presumptive characterization of *Vibrio cholerae* isolates in this study relied on culture media and colonial morphology. Samples were enriched overnight in sterile alkaline peptone water (APW) to promote the growth of *Vibrio cholerae*. Turbidity observed in APW cultures, coupled with the appearance of large to medium-sized shiny yellow colonies on thiosulfate-citrate-bile salts-sucrose (TCBS) agar (which is originally green), indicated the presence of *Vibrio cholerae* (Tarh, 2020). These observations were consistent with the description of *V. cholerae* by Tarh (2020), who noted that shiny yellow colonies on TCBS agar derived from APW-enriched cultures are characteristic features of the bacterium. Gram staining revealed that the isolates lost the primary stain (crystal violet)

upon decolorization with alcohol, confirming their classification as Gram-negative. Microscopically, the isolates appeared as small, curved to straight rod-shaped (bacilli) bacteria, consistent with observations by Martin *et al.* (2021). Among the 13 culture-positive isolates, six exhibited characteristic darting motility in peptone water, which was absent when the isolates were inoculated into distilled water. This finding aligns with Cheesebrough (2006), who noted that darting motility distinguishes *Vibrio cholerae* from *Aeromonas* species.

This study also documented the growth of tiny yellow colonies of other bacterial species on the *Vibrio*-selective TCBS medium, as reported by Tarh (2020) and Morgado *et al.* (2024). Seven out of the 13 isolates presumptively identified on TCBS medium exhibited biochemical reactions inconsistent with those specific to *Vibrio cholerae*. These isolates produced hydrogen sulfide on Kligler's iron agar (KIA) and were positive for the methyl-red test. This observation is in agreement with Cheesebrough (2006), who highlighted that small colonies of other bacteria, such as *Enterococcus* and *Aeromonas*, can occasionally grow on TCBS medium, but differ from the large colonies typical of *Vibrio cholerae*. The detection and characterization of *Vibrio cholerae* isolates, with a prevalence of 12% (6 out of 50 stool samples) from patients at ABUMC, confirm that *V. cholerae* is a significant contributor to gastroenteritis outbreaks. However, it is not the sole causative agent in the study area. This finding aligns with Morgado *et al.* (2024), who identified cholera as one of the leading causes of gastroenteritis in Northern Nigeria.

Notably, oral rehydration therapy (ORS) remains the primary treatment for cholera; antibiotics are crucial in severe cases to reduce the duration of diarrhoea and the excretion of pathogenic bacteria (Pietroni, 2020). However, antibiotic use may contribute to the emergence of multidrug-resistant (MDR) strains. In this study, isolates from samples M6 and M22

exhibited resistance to three of the four classes of antibiotics tested. The emergence of MDR *V. cholerae* strains has been reported in several studies (Siriphap *et al.*, 2022; Shah *et al.*, 2023; Saha *et al.*, 2024). All *V. cholerae* isolates in this study demonstrated 100% susceptibility to sparfloxacin and ciprofloxacin, consistent with findings by Urassa *et al.* (2000). Similarly, 100% susceptibility to gentamicin was observed, corroborating findings by Awuor *et al.* (2020) and Eashmen *et al.* (2021). Most isolates also showed sensitivity to trimethoprim-sulfamethoxazole (83.3%) and chloramphenicol (66.7%). Tetracycline, endorsed by WHO as the drug of choice for cholera outbreaks, exhibited 66.7% sensitivity. These findings align partially with Awuor *et al.* (2020), suggesting that gentamicin, sparfloxacin, ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole remain effective options for managing *V. cholerae* infections.

The study revealed that tetracycline, the most commonly available and locally used antibiotic for treating bacterial-induced diarrheal diseases, including cholera, remains effective in the study area. However, the growing resistance to this antibiotic is of public health concern. This resistance could be attributed to its indiscriminate use (Elimian *et al.*, 2022) and the acquisition of resistant genes via plasmids. Temporary discontinuation of tetracycline use may help restore susceptibility. The bacterial isolates also exhibited resistance to amoxicillin-clavulanate (50%) and amoxicillin (66.7%), consistent with findings by Abioye *et al.* (2023). The high resistance to these antibiotics, which target bacterial cell wall synthesis, can be explained by the Gram-negative bacterial physiology of *Vibrio cholerae*, including its cell wall structure and reduced antibiotic permeability (Elimian *et al.*, 2022).

A notable observation in this study was the demographic pattern of *V. cholerae* infections. Patients aged 21–30 years

accounted for 50% (3/6) of all positive cases, making this (21–30 years) the most affected age group. This trend may be linked to poor hand hygiene, as all six cases in this age group reported washing their hands with water alone. This observation aligns with Ngwa *et al.* (2021), who identified ineffective hand hygiene as a significant factor in cholera outbreaks, such as the 1995–1996 outbreak in Kano State, Nigeria. Additionally, individuals in this age range are typically more mobile, socially active, and frequently exposed to contaminated food or water, increasing their risk of infection by the bacterial isolate (Brockett *et al.*, 2020). Gender-wise, the study found that most infected patients were male (66.7%, 4/6), while females accounted for 33.3% (2/6). Although males constituted a larger proportion of study participants (68%, 34/50) compared to females (32%, 16/50), females showed a slightly higher infection rate (12.5%, 2/16) compared to males (11.8%, 4/34). However, the infection rate difference of 0.7% was statistically insignificant. This slight disparity could be due to the sample size or gender roles in the study area, where women are more likely to engage in activities such as caregiving, washing, and handling food, which may increase their exposure to cholera. In terms of age distribution, the highest infection rates were observed among patients aged 41 years and above (100%, 2/2) and those aged 11–20 years (25%, 1/4). These groups represented only 12% of the total study population, they are among the most vulnerable due to reduced stomach acid production and weaker immune systems, as noted by Adagbada *et al.* (2012).

Occupational status ($\chi^2 = 4.782$, $p = 0.092$) was not statistically significantly associated with cholera cases. However, self-employed individuals, particularly traders at the Samaru community market, were at higher risk of infection. The daily activities of the people in this area such as handling potentially contaminated items and consuming food or water from local vendors, led to a higher cholera occurrence

rate (40%) compared to other occupational groups.

The study also highlighted the role of education in cholera prevention. Patients with secondary education showed the highest prevalence of infection (50%), followed by those with non-formal education (42.9%, 3/7). Statistical analysis ($\chi^2 = 11.2$, $p = 0.011$) indicated that education significantly influenced cholera occurrence, underscoring the importance of awareness and health education in mitigating the disease's spread. These findings align with Akel *et al.* (2023), who observed that lower levels of education were associated with increased cholera risk of infection.

Notably, none of the analyzed risk factors showed statistically significant associations with cholera ($p > 0.05$) infection, certain trends emerged. Residents living off-campus had a higher risk of infection (16%), likely due to limited access to safe water and poor sanitation in these areas. In addition, individuals who practiced poor food handling (12.5%) and inadequate hand hygiene (12.8%) were at greater risk of *V. cholerae* infection. These observations are consistent with findings of Ngwa *et al.* (2021), who identified poor hand hygiene and unsafe drinking water as key contributors to cholera outbreaks in Kano State, Nigeria.

CONCLUSION

Cultural and microscopic characteristics revealed 13 bacterial isolates as presumptive *Vibrio cholerae*. Characterization by biochemical tests confirmed 6 out of the 13 presumptive isolates as of *Vibrio cholerae* with a prevalence of 12% recorded. Antibiotic susceptibility profile of the characterized *V. cholerae* isolates showed all 6 isolates to be 100% susceptible to ciprofloxacin, gentamicin, and sparflaxacin. Isolates were also susceptible to trimethoprim-sulfamethoxazole (84.6%), chloramphenicol (76.9%), and tetracycline (69.2%). Much resistance was observed against antibiotics of the penicillin class namely amoxicillin-clavulanate (50%) and

amoxicillin (66.7%). Isolates of samples M6 and M22 were multidrug-resistant, showing resistance to three of the four classes of antibiotics used. Questionnaires administered to enrolled patients at the ABUMC revealed that although, there was no association of cholera with sex. The cholera occurrence rate was slightly higher among females than males attending the Medical Centre. Individuals in the age group 41-above had a higher infection rate in this study. Patients at the secondary level of education and those with non-formal education had the highest rate of occurrence

in this study. To mitigate potential outbreaks, periodic cholera surveillance should be conducted in regions surrounding known hotspots, such as Samaru, which is in close proximity to Funtua LGA in Katsina State, Nigeria, a location with consistently high reported cases. Such surveys would enhance local capacity in monitoring and responding to cholera occurrences. Also, regular antibiotic susceptibility testing of *V. cholerae* isolates is crucial to monitor the efficacy of locally available antibiotics and prevent the emergence of resistant strains.

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