ISOLATION, IDENTIFICATION AND ANTIBIOTICS SUSCEPTIBILITY PATTERNS OF *VIBRIO CHOLERAE* FROM DIARRHOEIC PATIENTS ATTENDING AHMADU BELLO UNIVERSITY MEDICAL CENTRE, SAMARU, ZARIA-NIGERIA

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Received: 13th November, 2023 **Accepted**: 23rd November, 2023 **Published:** 1st December, 2023

ABSTRACT

Background: Cholera is an acute watery diarrhoeal disease caused by *Vibrio cholerae*. Antibiotic resistance among *Vibrio cholerae* has the potential to pose a serious threat to public health since it could increase the prevalence of cholera.

Aim: This study was aimed at isolating, identifying and determining the antibiotic susceptibility pattern of *Vibrio cholerae* from diarrhoeic patients in Ahmadu Bello University Medical Center, Zaria-Nigeria.

Methods: Culture and biochemical tests were employed in isolating and identifying *Vibrio cholerae* from 100 stool samples. Structural questionnaires were used to collect data on socio-demographic and risk factors associated with *Vibrio cholerae*.

Results: The prevalence of *Vibrio cholerae* was 16%. The prevalence of *Vibrio cholerae* among female patients was 17%, while that of male patients was 15.1%. Also, *Vibrio cholerae* infection was not associated with gender ($p>0.05$). Age group 2-15 years had more *Vibrio cholerae* infection (37.5%) than other age groups. There was a significant association between *Vibrio cholerae* infection and age group ($p<0.05$). *Vibrio cholerae* infection was associated with education ($p<0.05$), with highest rate of infection among secondary patients (33.3%). There was no significant association between *Vibrio cholerae* and occupation ($p>0.05$), even though civil servants having the highest rate of infection (33.3%). Patients who used bottled water had the highest rate of *Vibrio cholerae* infection (25%). *Vibrio cholerae* isolates were highly resistant to amoxicillin (75%) and ceftriaxone (68.8%). However, they were 62.5% susceptible to each of gentamicin and levofloxacin. Among the isolated *Vibrio cholerae* 68.8% of them were multi-drug resistant.

Conclusion: This study demonstrated that Ahmadu Bello University and the surrounding area have a high prevalence of *Vibrio cholerae* infection. As a result, it's important to inform the locals about the need to enhance their hygiene. Gentamicin and levofloxacin are found to be drugs of choice in this study.

Keywords: Prevalence, *Vibrio cholerae*, Diarrhoea, Cholera, Multi-Drug Resistant

INTRODUCTION

Diarrhoea is defined as the abnormal passage of three or more watery or loose stools per day due to an infection in the gastrointestinal tract (WHO, 2017). Some of the most important causes of acute watery diarrhoea are *Vibrio cholerae*, *Salmonella* spp., and others like enterotoxigenic *Escherichia coli, Rotavirus,* etc. (Christaki *et al.*, 2020; WHO, 2021). *Vibrio cholerae* is a Gram-negative bacterium and the etiological agent for cholera (Cheesbrough, 2006). *Vibrio cholerae* produces a toxin called cholera toxin, which causes the symptoms of cholera (Sela *et al.*, 2023). Cholera is an extremely virulent disease that can cause severe acute diarrhea, and vomiting (WHO, 2023a). Cholera can be lethal if left untreated and is a major public health concern in developing countries where access to clean water and sanitation is limited (NCDC, 2017).

The growing problem of antibiotic resistance makes treating cholera more difficult (Tang *et al.,* 2023). In developing countries where the use of antibiotics is less regulated, the prevalence of antibiotic resistance among *Vibrio cholerae* infections is considerable and these rates are rising globally (Hossain *et al.,* 2023). Furthermore, the misuse of antibiotics to treat cholera might result in the development of resistant strains, which may render current treatments useless. (Bruzzese *et al.*, 2018).

In 2021, Nigeria lost about 3,604 people to cholera, and about 111,662 recorded suspected cases of cholera (Onyeji, 2022). Kaduna State in April to August, 2021 recorded 1,665 cholera cases, in which Zaria was identified as one of the most affected areas (Punch Newspaper, 2021). According to the NCDC, there have been a total of 583 cholera-related deaths reported, and 23,550 persons may have been exposed to the disease between January and November 27, 2022 (Ogundipe, 2023).

MATERIAL AND METHODS

Study Area

This research was carried out between June-November, 2023, in Ahmadu Bello University Medical Center, Samaru (Main Campus), Zaria.

Ethical Clearance and Consent

Ethical clearance was obtained from Ahmadu Bello University Medical Center (UHS/ADM/S-8). Patients consent was sought before sample collection.

Sample Size

A 5.06 per cent case of cholera reported by Sharma *et al.* (2017) in Tea Gardens of Assam, Iran, was used in calculating the sample size. The calculated size was round up to 100 for convenience in sample collection. Faeces (stool) 100 samples were collected from consented patients presenting with diarrheoa in Ahmadu Bello University Medical Center.

Questionnaire

Structured questionnaire was used to obtain information from the patients on socio-demographic and risks factors associated with *Vibrio cholerae* infection.

Collection of Sample

Faecal samples were collected in a sterile wide mouth universal container. The samples were labeled with dates, gender, and age of patients and enriched immediately. All the samples collected for each day were transported aseptically to the Department of Microbiology Laboratory, Ahmadu Bello University, Samaru, Zaria, for microbiological analysis.

Enrichment of Sample

A loop full of the stool sample collected was inoculated into the broth test tube containing 10ml of alkaline peptone water and incubated at 37°C for 6 hours.

Isolation of *Vibrio cholerae*

A loop full of the cultured alkaline peptone water broth was streaked on the surface of TCBS agar plate medium, a selective medium for the isolation of *Vibrio cholerae* and incubated at 37°C for 24hours. After incubation period, observation was made. Large, yellow, smooth and slightly flattened shinny colonies were suspected to be *Vibrio cholerae*. The isolates were aseptically sub cultured onto another prepared TCBS agar to obtain pure isolate.

Identification of *Vibrio cholerae*

Pure suspected isolates were subjected to Gram staining and biochemical tests; such as oxidase, Sulphur, Indole and Motility (SIM), Kilger Iron Agar (KIA), Simon Citrate and Methyl-Red and Voges-Proskauer (MRVP) tests. Once the isolates were confirmed, they were sub cultured onto nutrient agar and stored in refrigerator for further analysis.

Antibiotics Susceptibility Test

Antibiotics susceptibility testing was performed using the agar disc diffusion method. Antibiotic discs used includes: Amoxicillin (10µg), Chloramphenicol (30µg), Gentamycin (10µg), Trimethoprim/sulphamethoxazole (25µg), Ceftriaxone (30µg), and Levofloxacin (5µg). Three to five well isolated pure colonies were picked from overnight subculture nutrient agar plate using a sterilized wire loop and suspended in 4ml sterile normal saline before being adjusted to march 0.5 McFarland turbidity standards.

About 0.1ml of standardized bacterial suspension was transferred on to the surface of freshly prepared Mueller Hinton agar plate, sterilized swab stick was used to spread the inoculum evenly on the surface of plate. The suspension was allowed to diffuse for about 10 minutes. A sterilized forceps was used to place the antibiotic discs gently on the agar plates which were then incubated for 24hours at 37°C. After the incubation period, the zones of inhibition were measured using a transparent ruler. The zones were interpreted as susceptible, intermediate or resistance using Clinical and Laboratory Standards Institute guidelines (CLSI, 2021).

RESULTS

Out of a total of 100 diarrhoiec stool samples from patients attending Ahmadu Bello University Medical Center, Zaria, and sixteen (16) patients were infected with *Vibrio cholerae,* with a prevalence of 16.0%, as shown in figure 1.

Table 1 shows the prevalence of *Vibrio cholerae* among female patients (17%), while in male patients it was 15.1%. *Vibrio cholerae* infection was not associated with gender ($p > 0.05$).

Age groups 2–15 years had more *Vibrio cholerae* infections (37.5%), and the least were among age groups 30-43 years (5.3%). There was a significant association between *Vibrio cholerae* infection and age group ($p<0.05$) (Table 1).

Based on level of education, patients with secondary education had the higher infection rate (57.1%); while the least infection was among patients with tertiary (9.1%). *Vibrio cholerae* infection was significantly associated with level of education (p-value <0.05), shown in Table 1. But the infection was not associated with occupation ($p > 0.05$), with civil servants having the highest rate of infection (33.3%) and unemployed patients having the least infection (5.6%) (Table1). Table 2 shows that a recent history of diarrhoea was not associated with *Vibrio cholerae* infection ($p > 0.05$). However, patients with a recent history had a higher rate of *Vibrio cholerae* (16.7%) infection. Patients who recently traveled had a higher rate of the infection (20.0%). There was no significant association between *Vibrio cholerae* and the risk factors ($p > 0.05$). Patients who used bottled water had the highest rate of infection with *Vibrio cholerae* (25%). Patients who used river as a source of drinking water had no *Vibrio cholerae* infection (0.0%), as shown in Table 3. Patients who do not practice safe food handling (22.2%) have a higher prevalence of *Vibrio cholerae* infection. There was a higher prevalence of *Vibrio cholerae* infection (23.1%) among those patients who did not wash their hands after using toilet (Table 3). Based on the type of toilet facility, there was a 0.0% prevalence of *Vibrio cholerae* infection among patients who used buckets. *Vibrio cholerae* infection was higher among other means (19.6%), as shown in Table 3. There was no significant association between the distributions of *Vibrio cholerae* infection and history of treatment of diarrhoea ($p > 0.05$) (Table 4). Table 5 shows the distribution of *Vibrio cholerae* infection based on symptoms. There was no significant association between *Vibrio cholerae* infection and symptoms ($p>0.05$). Table 6 shows antibiotic zones of inhibition against *Vibrio cholerae;* the results recorded were compared to resistant, intermediate, and susceptibility on the Clinical and Laboratory Standards Institute (CLSI, 2021). Table 7 shows percentage of antibiotic susceptible, intermediate and resistant *Vibrio cholerae* to Levofloxacin, Amoxicillin, Trimethoprim/sulphamethoxazole, Gentamicin, Ceftriaxone, and Chloramphenicol. Table 8 shows phenotypic of antibiotic susceptibility pattern of *Salmonella* species. Table 9 shows phenotypic of antibiotic susceptibility pattern of *Vibrio cholerae*. Figure 2 Shows occurrence of Multi-Drug Resistant bacteria isolated from diarrhoea patients. Among the 16 isolated *Vibrio cholerae,* 11 (68.8%) were multi-drug resistant.

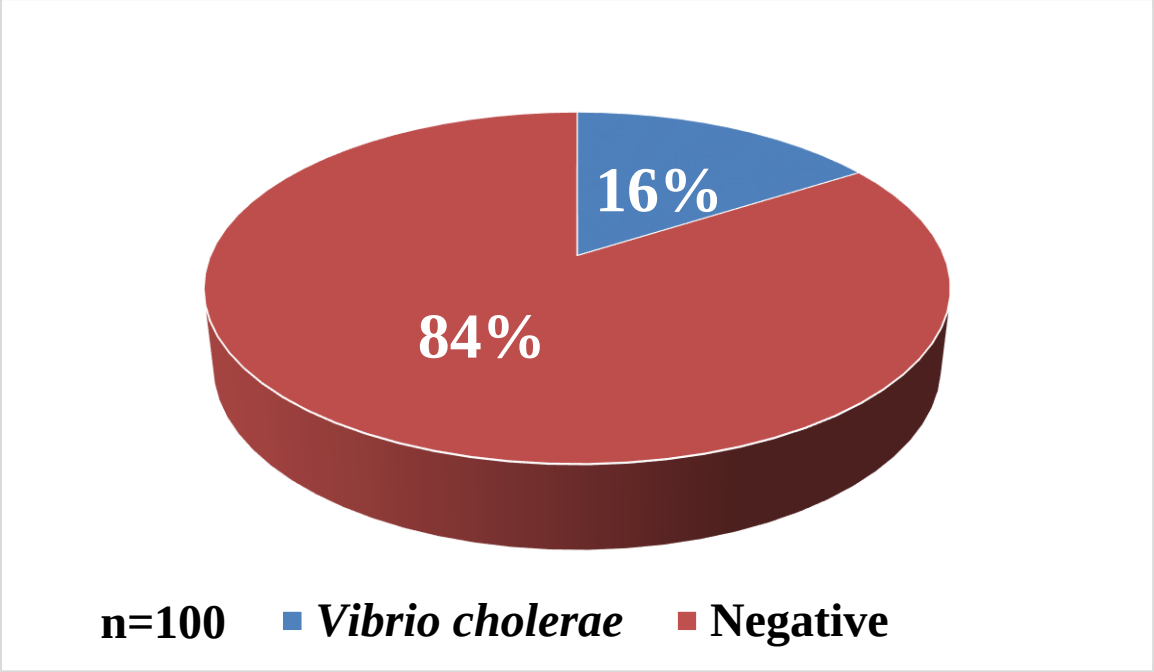

Figure 1: Prevalence of *Vibrio cholerae* infection among Diarrhoiec Patients
Key: n= number of samples collected

Table 1: Distribution of *Vibrio cholerae* infection based on Social Demographic Factors

Socio-demographic factor	Number examined	Number positive (%)	χ^2	*p-value*
Gender			0.069	0.793
Female	47	8(17.0)		
Male	53	8(15.1)		
Age-group (years)			7.357	0.025
2-15	16	6(37.5)		
16-29	65	9(13.8)		
30-43	19	1(5.3)		
Level of education			13.913	0.003
Informal	19	3(15.8)		
Primary	8	3(37.5)		
Secondary	7	4(57.1)		
Tertiary	66	6(9.1)		
Occupation			2.871	0.238
Civil servant	6	2(33.3)		
Student	76	13(17.1)		
Unemployed	18	1(5.6)		

Table 2: Prevalence of *Vibrio cholerae* Infection based on Potential Risk Factors

Potential Risk factor	Category	Number examined	Number positive (%)	χ^2	*p-value*
Recent history of diarrhoea	No	28	4(14.3)	0.085	0.771
	Yes	72	12(16.7)		
Type of Disease Diagnosed earlier	Cholera	4	0(0.0)	1.467	0.690
	Dysentery	11	1(9.1)		
	Typhoid	59	11(18.6)		
	No response	26	4(15.4)		
Travelled recently	No	60	8(13.3)	0.794	0.373
	Yes	40	8(20.0)		

Table 3: Distribution of *Vibrio cholerae* infection based on Risk factors

Risk factors	Category	Number examined	Number positive (%)	χ^2	*p-value*
Drinking water	Bottle	8	2(25.0)		
	River	6	0(0.0)		
	Sachet	52	7(13.5)	3.077	0.545
	Tap	32	7(21.9)		
	Well	2	0(0.0)		
Safe food handling practice	Always	67	12(17.9)		
	No	9	2(22.2)	1.491	0.475
	Sometimes	24	2(8.3)		
Hand Washing after use of toilet	Always	82	13(15,9)		
	No	13	3(23.1)	1.438	0.487
	Sometimes	5	0(0.0)		
Type of toilet facility	Bucket	3	0(0.0)		
	Pit latrine	8	1(12.5)		
	Water closet	33	4(12.1)	1.567	0.667
	Other means	56	11(19.6)		

Table 4: Distribution of *Vibrio cholerae* based on History of Treatment of Diarrhoea

Treatment history	Number examined	Number positive (%)	χ^2	*p-valve*
Currently on herbs				
No	77	12(15.6)		
Yes	23	4(17.4)	0.043	0.836
Taken antibiotics recently				
No	37	8(21.6)		
Yes	63	8(12.7)	1.381	0.240

Table 5: Distribution of *Vibrio cholerae* based on Symptoms

Symptom	Category	Number examined	Number positive (%)	χ^2	*p-valve*
Abdominal pain	No	75	12(16.0)	0.000	1.000
	Yes	25	4(16.0)		
Fever	No	68	11(16.2)	0.005	0.944
	Yes	32	5(15.6)		
Body weakness	No	62	10(16.1)	0.002	0.964
	Yes	38	6(15.8)		
Vomiting	No	84	14(16.7)	0.174	0.677
	Yes	16	2(12.5)		
Loss of appetite	No	79	12(15.2)	0.184	0.668
	Yes	21	4(19.0)		

Table 6: Susceptibility Profile of the Isolated *Vibrio cholerae*

	Zone of Inhibitions (mm)					
ID	**LEV(5µg)**	**AX(10µg)**	**SXT(25µg)**	**CN(10µg)**	**CRO(30µg)**	**C(30µg)**
V006	16I	12R	12I	13I	18R	16I
V008	21S	13R	9R	15S	17R	19S
V013	22S	15I	15I	20S	21I	13I
V024	22S	18S	17S	17S	24S	19S
V025	24S	14I	11I	19S	23S	11R
V032	22S	10R	11I	19S	23S	23S
V033	22S	8R	16S	18S	24S	24S
V041	181	6R	8R	13I	19R	11R
V045	23S	6R	6R	16S	18R	14I
V046	14I	9R	6R	10R	10R	15I
V052	16I	15I	8R	12I	17R	21S
V063	6R	13R	8R	6R	12R	6R
V069	24S	11R	6R	18S	9R	9R
V073	23S	6R	9R	15S	9R	10R
V094	15I	12R	6R	14I	8R	6R
V099	21S	6R	6R	20S	10R	6R

Key; LEV- Levofloxacin, AX-Amoxicillin SXT- Trimethoprim/sulphamethoxazole
CN- Gentamicin, CRO- Ceftriaxone, C- Chloramphenicol, ID- Isolate number
S- Susceptible, I-Intermediate, R-Resistant

Table 7: Percentage of Antibiotic Susceptibility, Intermediate and Resistance of *Vibrio cholerae*

Antibiotics	**n=16**	**S (%)**	**I (%)**	**R (%)**
LEV **(**5µg**)**		10(62.5)	5(31.3)	1(6.2)
AX **(**10µg**)**		1(6.2)	3(18.8)	12(75.0)
SXT **(**25µg**)**		2(12.5)	4(25)	10(62.5)
CN **(**10µg**)**		10(62.5)	4(25)	2(12.5)
CRO **(**30µg**)**		4(25)	1(6.2)	11(68.8)
C **(**30µg**)**		5(31.3)	4(25)	7(43.7)

Key; LEV- Levofloxacin, AX-Amoxicillin, SXT- Trimethoprim/sulphamethoxazole
CN- Gentamicin, CRO- Ceftriaxone, C- Chloramphenicol
S- Susceptible, I-Intermediate, R-Resistance
n- Number of Isolates

Table 8: Resistance Patterns of *Vibrio cholerae*

ID	Susceptible	Intermediate	Resistance	MDR
V006		LEV,SXT,CN,C	AX,CRO	+
V008	LEV,CN,C		AX,SXT,CRO	+
V013	LEV,CN	AX,SXT,CRO,C		-
V024	LEV,AX,SXT,CN,CRO,C			-
V025	LEV,CN,CRO	AX,SXT	C	-
V032	LEV,CN,CRO,C	AX, SXT		-
V033	LEV,SXT,CN,CRO,C		AX	-
V041		LEV,CN	AX,SXT,CRO,C	+
V045	LEV,CN	C	AX,SXT,CRO	+
V046		LEV,C	AX,SXT,CN,CRO	+
V052	C	LEV,AX,CN	SXT,CRO	+
V063			LEV,AX,SXT,CN,CRO,C	+
V069	LEV,CN		AX,SXT,CRO,C	+
V073	LEV,CN		AX,SXT,CRO,C	+
V094		CN, LEV	AX,SXT,CRO,C	+
V099	LEV,CN		AX,SXT,CRO,C	+

Key; LEV- Levofloxacin, AX-Amoxicillin, SXT- Trimethoprim/sulphamethoxazole CN- Gentamicin, CRO- Ceftriaxone, C- Chloramphenicol, ID- Isolate number
S- Susceptible, I-Intermediate, R-Resistance, MDR-Multi-Drug Resistance, +; multi-drug isolate, -; non-multi-drug isolate

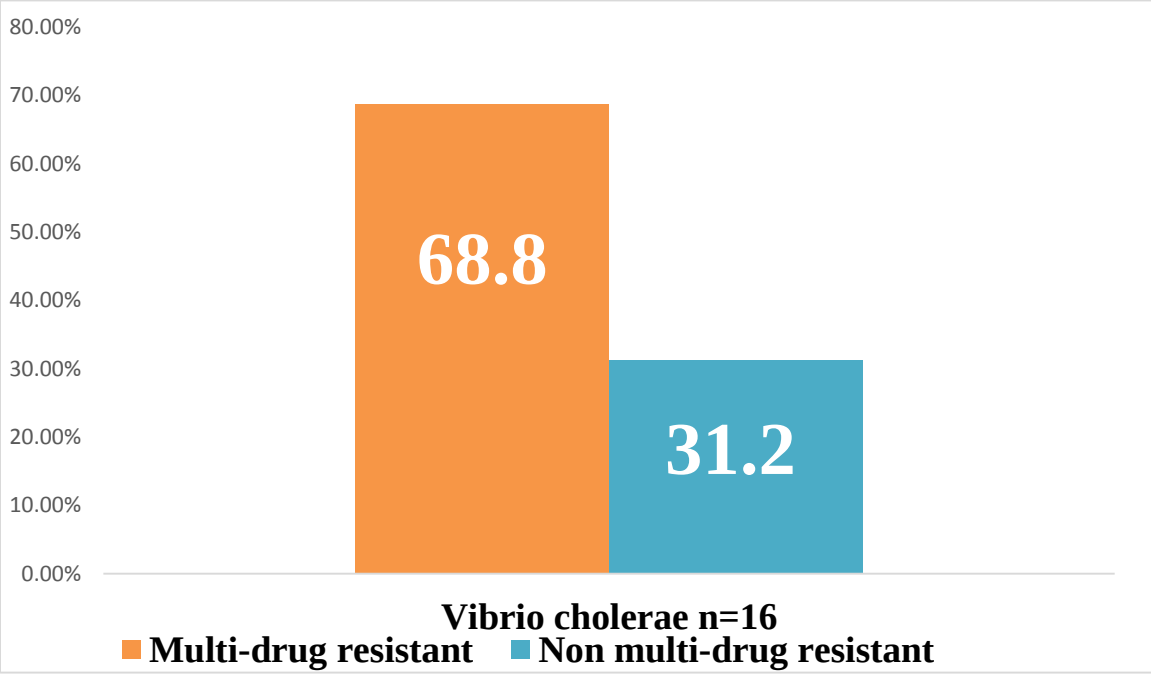

Figure 2: Occurrence of multi-drug and non-multi-drug isolates
Key; n- number of isolates

DISCUSSION

The prevalence of *Vibrio cholerae* infection (16%) in this study was in agreement with the findings of Azike *et al*. (2023), who reported 16% in Kaa, Rivers State, Nigeria. However, it was lower than the one reported in Gwagwalada, Abuja, Nigeria, with a prevalence of 35.5% (Okoroiwu and Onah, 2023).

The infection rate was slightly higher among female patients (17.0%) than their male (15.1%) counterpart. This is not surprising, because women and girls have a heightened risk of coming into contact with a high infectious dose of cholera through their domestic roles, including taking care of sick family members, cleaning latrines, fetching and handling water, and preparing contaminated raw food. Individuals in the age group 2–15 had the highest prevalence of *Vibrio cholerae* infection (37.5%) than other groups, and this may not be unconnected to their weak immune systems, which makes them more susceptible. Additionally, children in this age group may have a higher risk of exposure to contaminated water due to factors such as playing in contaminated areas or not having access to clean drinking water.

Although a systemic review and meta-analysis indicated that education, particularly less than secondary education, was linked to cholera risk (Richterman, 2018). In this study, *Vibrio cholerae* infection was more common in patients with secondary education (57.1%) than in patients with other levels of education. Patients with secondary education may be more susceptible to contracting cholera than patients with other levels of education because they are more prone to partake in risky behaviors including swimming in tainted water, consuming raw seafood, and being in close proximity to animals.

Civil servants (33.3%) had the highest risk of *Vibrio cholerae* infection because they were more likely to work in areas with limited access to clean water and better access to healthcare, which may lead to an early diagnosis. Patients with a recent history of diarrhoea are at a higher risk of *Vibrio cholerae* infection. This may be due to an imbalance in the gut microbiome by destroying good bacteria, which can allow bad bacteria like *Vibrio cholerae* to thrive (Ararsa *et al.,* 2023).

Patients who using bottle water as source of drinking water had higher rate of the infection (25.0%) than those who use other sources of water for drinking, this finding was in contrary to Lui *et al.* (2018) who reported 1.33% infection rate. According to Ararsa *et al.* (2023) patients who did not wash their hands after using toilet were more likely to be tested positive for *Vibrio cholerae* infection from their stool sample which was in agreement with this study where 23.1% infection rate was recorded against them compared to those who always wash hands (15.9%).

There was no significant association between *Vibrio cholerae* infection and symptoms ($p > 0.05$). It's worth noting that not all patients will have the same symptoms; even if they have the same infection, this may be due to factors such as age, overall health, and underlying medical conditions (Rosso *et al.,* 2023).

Reports on increased drug resistance toward commonly used antibiotics against *Vibrio cholerae* is causing serious problem in management of cholera cases. In this study, the isolated *Vibrio cholerae* were highly resistance to amoxicillin (75%) and ceftriaxone (68.8%) compared to the ones earlier reported by Nateghizad *et al.* (2023) where 42% and 9% of the *Vibrio cholerae* were resistance amoxicillin and ceftriaxone respectively. Also, 12.5% of the isolates were resistance to gentamicin and this was higher to the 4.35% reported by Budiman *et al* (2022). Occurrence of multi-drug resistant isolates in this study was 68.8%. This was higher to the on reported by Gupta *et al.* (2016) where 6.45% of *Vibrio cholerae* isolated were multi-drug resistant.

Changing antibiogram profile of *Vibrio cholerae*, developing MDR strains over years may be attributed to the spontaneous mutation due to indiscriminate use of antibiotics or horizontal transfer of resistance genes (Shrestha, *et al.,* 2015).

CONCLUSION

The prevalence of *Vibrio cholerae* infections among diarrhoea patients attending Ahmadu Bello University Medical Centre was 16%. There was significant association ($p<0.05$) between *Vibrio cholerae* infection and age group and level of education. There was no significant association ($p>0.05$) between *Vibrio cholerae* infection and gender, occupation, potential risk factors, risks factors, history of treatment and symptoms
Among the isolated *Vibrio cholerae,* 68.8% were multi-drug resistant. *Vibrio cholerae* isolates were highly resistance to amoxicillin and ceftriaxone but highly susceptible to gentamicin and levofloxacin.

RECOMMENDATION

i. Raise public awareness about proper hand washing, food hygiene and other prevention measures.
ii. Encourage appropriate use of antibiotics by healthcare providers and the public.
iii. Governments must invest in the infrastructure and resources necessary to support robust research and development of new antibiotics.
iv. Public health officials, healthcare providers, and the pharmaceutical industry must work together to address the growing threat of multi-drug resistant bacterial infections.
v. Health care facilities must prioritize infection prevention and control practices to limit the spread of these infections.
vi. The need for increased surveillance of antibiotic resistance, to improve communication between healthcare providers and public.

Acknowledgement

We acknowledge the Management of Ahmadu Bello University Medical Center, for providing ethical clearance towards sample collection.

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