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Molecular Identification and Antibiogram of *Vibrio Cholerae* from Some Sources of Water in Tudun Wada and Malali Areas of Kaduna State

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Abstract

Vibrio cholerae, a Gram-negative bacterium, is the causative agent of cholera, a severe diarrheal disease associated with contaminated water and poor sanitation. This study aimed to isolate, identify, and determine the antibiotic susceptibility of V. cholerae from water sources in Tudun Wada and Malali areas of Kaduna State, Nigeria. A total of 18 water samples were collected from wells, taps, and rivers, with physicochemical analysis revealing pH values ranging from 5.8 to 7.4, indicating potential contamination, particularly in river sources. Presumptive V. cholerae isolates were identified using Thiosulphate Citrate Bile Salt (TCBS) agar and biochemical tests, followed by molecular confirmation via PCR targeting the ompW gene. Only one isolate from tap water in Tudun Wada was confirmed as V. cholerae, highlighting discrepancies between biochemical and molecular methods. Antibiotic susceptibility testing revealed multidrug-resistant (MDR) strains, with high resistance to ampicillin, nalidixic acid, and sulfamethoxazole/trimethoprim, while ciprofloxacin remained effective. The Multi-Antibiotic Resistance Index (MARI) indicated high-risk contamination in some isolates (MARI ≥ 0.2). These findings underscore the presence of V. cholerae in local water sources and the growing threat of antimicrobial resistance, emphasizing the need for improved water quality monitoring, sanitation infrastructure, and updated treatment protocols to mitigate public health risks.

Keywords: Cholera, *Vibrio cholerae*, ompW gene, Antibiotic susceptibility, water quality

Introduction

Water is fundamental to life, covering approximately 70% of Earth Surface. Access to clean, safe water is critical for public health. However, in many developing countries, water contamination and poor sanitation remain significant challenges, contributing to the spread of waterborne diseases such as cholera, diarrhoea, typhoid and hepatitis A (WHO, 2022). Cholera, caused by the gram-negative bacterium *Vibrio cholerae*, continues to pose a global health threat, especially in regions with inadequate water treatment and hygiene infrastructure. *V. cholerae* is typically found in aquatic environments and can cause severe diarrhoeal disease upon ingestion of contaminated food or water. While over 200 serovars have been identified, only O1 and O139 are linked to epidemic cholera (Islam *et al*., 2023). Cholera outbreaks often correlate with environmental factors such as water quality, temperature and rainfall patterns. Outbreaks are often linked to contamination in water or food sources, especially during seasons that favour bacterial proliferation (Faruque *et al*., 2018).

However, in recent years the emergence of antimicrobial-resistant strains of *V. cholerae* has complicated treatment strategies. Reports from Okeke *et al.* (2019) indicate resistance to commonly used antibiotics such as ampicillin, tetracycline and streptomycin, particularly in regions where cholera is endemic. The prevalence of multidrug-resistant (MDR) strains defined as those resistant to three or more classes of antibiotics is becoming an increasing serious concern (shakya *et al*., 2022). Conventional identification of *V. cholerae* includes isolation on selective media (e.g., TCBS agar) and biochemical tests (e.g., oxidase, indole tests). Molecular methods such as polymerase chain reaction (PCR) provide rapid and specific detection by targeting conserved genes like ompW, which plays a role in immune stimulation and bacterial adaptation (Zhou *et al*., 2021). This study is aimed to isolate, identify, and determine the antibiotic susceptibility of *Vibrio cholerae* from water sources in Tudun Wada and Malali areas of Kaduna State, Nigeria, providing insight into potential public health risk and the need for water quality monitoring.

2. Materials and Methods

2.1 Study Areas

This study was conducted in Kaduna North and Kaduna South local government area of Kaduna State. Two locations were randomly selected which are Malali and Tudun wada areas. Tudun Wada has an Latitude 10.529836 and a Longitude 7.410539 with elevation above sea level of 600 m while Malali has an Latitude 10.6363852009 and a Longitude 7.4824990846 with an elevation above sea level of 606 m. The Figure (1) below is a map of Kaduna State showing Kaduna North and Kaduna South areas of Kaduna state.

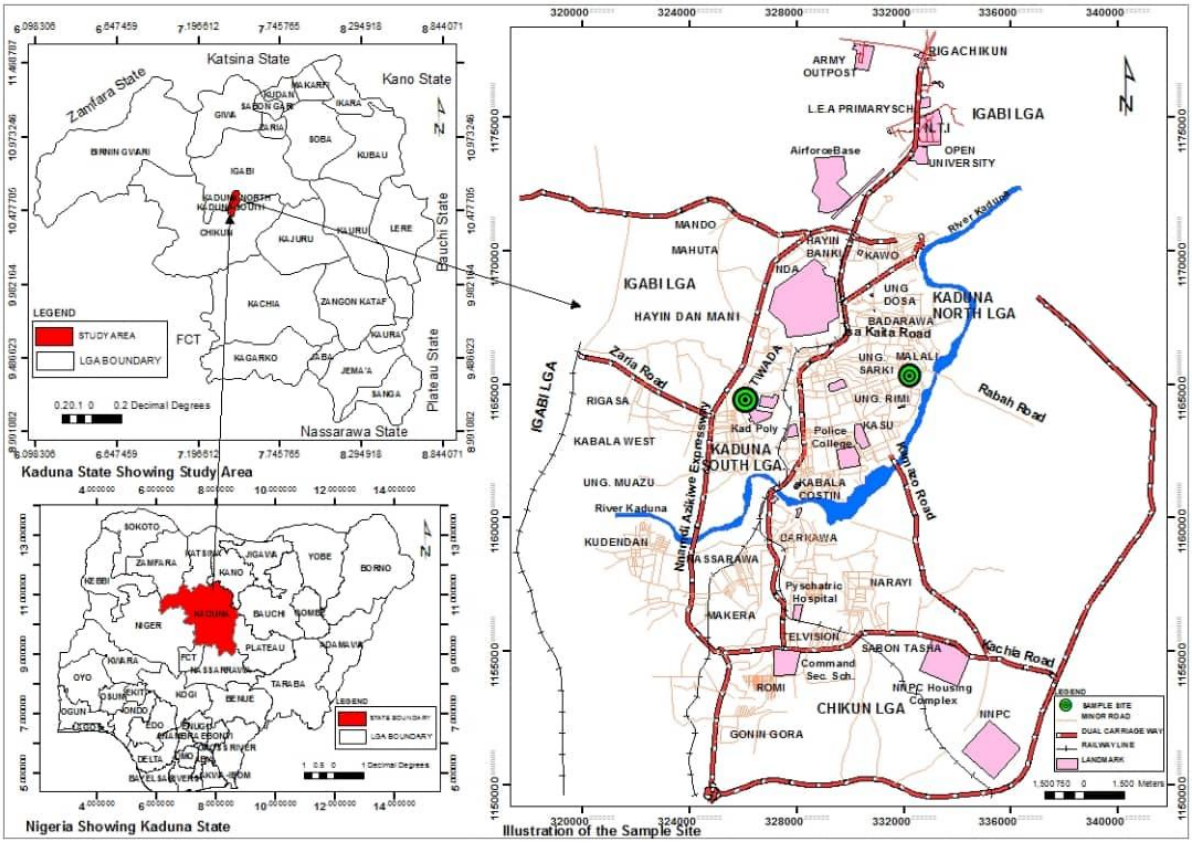

Figure 1: Map of Kaduna State showing Tudun Wada, Malali areas
Source: Department of Geography GIS unit KASU, 2023.

2.2 Sample Collection

A total of eighteen 1L water samples were collected aseptically from wells, tap and rivers using a convenience sampling method. The sample were gathered in sterile plastic bottles, transported in icebox, and processed immediately at the Biotechnology Laboratory, Kaduna State University.

2.3 Physiochemical Testing

The quality of the water was assessed based on the qualities that can be easily assessed by the consumer, these include; colour, turbidity, taste, odour, suspended solids (impurities), also pH test was carried out and observations were recorded.

2.4 Isolation of *Vibrio cholerae*

The culture media used was Thiosulphate Citrate Bile Salt (TCBS) agar. The media was prepared according to the manufacturer's instruction.

Membrane filtration was employed; were 100ml of each water sample was filtered through a standard membrane MF-Millipore filter. (47 mm, 0.45 µm pore size) using sterile forceps the membrane filter was placed aseptically in 100ml of alkaline peptone water (APW) pH 8.6 and enriched for 6-8 hours at 35-37°C (Alkaline peptone water has been described as enrichment media that is effective for culturing *V. cholerae* due to the fact that the alkaline nature does not allow the growth of other bacteria (Huq *et al*., 2013)). Two loopfuls of broth was streaked on already prepared sterile Thiosulphate citrate bile salt sucrose (TCBS) agar. The inoculated TCBS agar was incubated at 35- 37°C for 18- 24 hours to determine the presumptive microbial load. After incubation, a distinct yellow and green colony observed on the plates was counted as presumptive Vibrio isolates.

2.5 Biochemical Characterization

Isolates were characterized using standard biochemical tests such as Indole, Methyl red, Citrate, Kligler Iron Agar and Oxidase test (Chesbrough, 2006; Sylvia, 2013).

2.6 Antimicrobial Susceptibility Test

The Kirby–Bauer disc diffusion assay was used to determine the antimicrobial resistance patterns of isolates in accordance with the guidelines of the Clinical Laboratory Standards Institute (2021). Isolate were screened against Ampicillin (AMP, 30µg), Ciprofloxacin (CPX, 10µg), Nalidixic acid (NA, 30µg), Sulfamethoxazole/Trimethoprim (SXT, 30µg), Streptomycin (S, 30µg), 30 µg), Ofloxacin (OFX, 30µg), Pefloxacin(PEF, 30µg), Amoxicillin/Clavulanate (AU, 30µg), Cephalexin (CEP, 30µg), and Gentamicin (CN, 10µg). The zones were interpreted as susceptible, intermediate or resistant using Clinical and Laboratory Standards Institute guidelines (CLSI, 2021).

2.7 DNA Extraction and Polymerase chain reaction (PCR) confirmation

DNA was extracted using phenol-chloroform method and amplified via PCR targeting the ompW gene (588 bp). PCR products were analysed via 1% agarose gel electrophoresis stained with ethidium bromide and visualized under UV light.

Targeted specific primer ompW gene:
Forward primer: CACCAAGAAGGTGACTTTATTGTG
Reverse primer: GAACTTATAACCACCCGCG (Nandi *et al*., 2000).

STASTICAL ANALYSIS

Data obtained was subjected to one-way ANOVA test using a probability level of *P*

>0.0 5 as the threshold for statistical significance.

3. Results

3.1 Physicochemical Characteristics of Water Samples

Table 1 represents the physicochemical characteristics (colour, turbidity, impurities, pH) of 18 water samples collected from tap, well and river sources in Tudun-wada amd Malali areas.

Table 1: Physicochemical Properties of Water Samples From the Study Areas

Sample	Location	Source	Turbidity	impurity	Colour	pH
T1	Tudun-wada	Tap	Clear	None	Colourless	7.3
T2	Tudun-wada	Tap	Clear	None	Colourless	7.4
T3	Tudun-wada	Tap	Clear	None	Colourless	7.3
T4	Tudun-wada	Tap	Clear	None	Colourless	7.2
W1	Tudun-wada	Well	Mildly turbid	Small debris	Brownish	6.3
W2	Tudun-wada	Well	Mildly turbid	Small debris	Mildly clear	6.8
W3	Tudun-wada	Well	clear	Clear	Colourless	6.8
W4	Tudun-wada	Well	Mildly turbid	Small debris	Brownish	6.9
R1	Tudun-wada	River	Turbid	Suspended particles	Brownish	7.4
T1	Malali	Tap	Clear	None	Colourless	6.2
T2	Malali	Tap	Clear	None	Colourless	6.9
T3	Malali	Tap	Clear	None	Colourless	6.7
T4	Malali	Tap	Clear	None	Colourless	6.8
R1	Malali	River	Turbid	Suspended particles	Brownish	6.1
R2	Malali	River	Turbid	Suspended particles	Brownish	5.8
W1	Malali	Well	Mildly turbid	Small debris	Mildly clear	6.1
W2	Malali	Well	Clear	Small debris	Colourless	6.8
W3	Malali	Well	Clear	Small debris	Colourless	6.0

Key; w= Well, T= Tap, R= River

From the above Table (1) river water had the lowest pH values, particularly in Malali having as low as 5.8 suggesting possible contamination with suspended particles which could be as a result of industrial waste, heavy metal contaminations like aluminum and cadmium or acid producing bacteria could lead to a decrease in the pH

of the river water . Out of 18 samples obtained, 16 samples yielded smooth yellow circular and slightly flattened colonies on TCBS agar which showed that they are presumptive *Vibrio cholerae.*

3.2 Biochemical Characterization of Presumptive *V. cholerae* Isolates

The biochemical profiles of suspected *V. cholerae* isolates from Tudun-wada and Malali water sources are presented in Tables 2a and 2b, respectively. Five biochemical tests were performed: oxidase, indole, citrate utilization, Triple Sugar Iron (TSI), and methyl-red tests. Isolates demonstrating oxidase positivity, indole production, and citrate utilization, while testing negative for methyl red, were considered presumptive *V. cholerae*.

Table 2a: Biochemical test of suspected *V. cholerae* isolated from some water sources from Tudun wada

Isolate ID	Oxidase	Indole	Citrate	Methyl red	Gas	H_2S	TSI Test		
							G	L	S
W1	-	-	-	+	-	-	+	-	+
W2	-	-	-	+	-	-	+	-	+
W3	-	-	-	+	-	-	+	-	+
R1	+	-	+	-	-	-	+	-	+
T1	-	-	-	+	-	-	+	-	+
T2	-	-	-	+	-	-	+	-	+
T3	+	+	-	-	-	-	+	-	+
T4	-	+	+	-	-	-	+	-	+

Key: W= Well, T= Tap, R= River, TSI= Triple Sugar Iron, G=glucose, L=lactose, S=sucrose, H_2S=Hydrogen Sulphide, (+) = positive result, (-) = negative result

Table 2b: Biochemical test of suspected *V. cholerae* Isolated from Some Sources of Water from Malali

Isolate ID	Oxidase	Indole	Citrate	Methyl red	Gas	H_2S	TSI Test		
							G	L	S
W1	+	-	+	-	-	-	+	-	+
W2	-	-	-	-	-	-	+	-	+
W1	-	-	-	+	-	-	+	-	+
R1	+	+	+	-	-	-	+	-	+
R2	+	+	+	-	-	-	+	-	+
T1	-	-	-	+	-	-	+	-	+
T2	-	-	-	+	-	-	+	-	+
T3	+	-	+	-	-	-	+	-	+

Key: W= Well, T= Tap, R= River, TSI= Triple Sugar Iron, G=glucose, L=lactose, S=sucrose, H_2S=Hydrogen Sulphide, (+) = positive result, (-) = negative result

3.3 Antibiotic Susceptibility Patterns

The antibiotic susceptibility profiles of presumptive *Vibrio cholerae* isolates from Tudun-wada and Malali are presented in Tables 3a and 3b.

Table 3a. Antibiotic Susceptibility of *V. cholerae* Isolates from Tudun-wada Water Sources

Isolate ID	OFX 10mcg	PEF 10mcg	CPX 10mcg	AU 30mcg	CN 10mcg	S 10mcg	CEP 10mcg	NA 30mcg	SXT 30mcg	PN 30mcg
W1	I	R	I	R	S	R	I	I	R	R
W2	S	S	S	S	S	S	S	S	S	S
W3	S	S	S	S	S	S	S	S	S	I
R1	S	I	S	I	S	I	R	R	I	R
T1	I	S	S	S	S	S	I	S	S	R
T2	S	S	S	I	S	S	I	S	S	R
T3	S	S	S	S	S	S	S	S	S	R
T4	S	S	S	S	S	S	I	S	S	R

Key: W= Well, T= Tap, R= River, S= susceptible, I= intermediate, R= Resistance OFX= Ofloxacin, PEF= Pefloxacin, CPX= Ciprofloxacin, AU= Amoxicillin/Clavulanate, CN= Gentamicin, S= Streptomycin, CEP= Cephalexin, NA= Nalidixic Acid, SXT=Sulfamethoxazole/Trimethoprim, PN= Ampicillin

Table 3b. Antibiotic Susceptibility of *V. Cholerae* Isolates from Malali Water Sources

Isolate ID	OFX 10mcg	PEF 10mcg	CPX 10mcg	AU 30mcg	CN 10mcg	S 10mcg	CEP 10mcg	NA 30mcg	SXT 30mcg	PN 30mcg
R1	S	S	I	R	I	S	I	I	I	I
R2	S	S	I	I	S	S	I	S	S	S
W1	S	S	S	S	S	S	I	I	R	S
W2	S	S	S	S	S	S	S	S	S	S
W3	I	I	I	R	R	R	I	R	I	R
T1	I	I	S	I	I	I	R	I	I	I
T2	S	S	S	I	S	S	S	I	S	I
T3	I	I	I	I	R	R	I	R	I	R

Key: S= susceptible, I= intermediate, R= Resistance OFX= Ofloxacin, PEF= Pefloxacin, CPX= Ciprofloxacin, AU= Amoxicillin/Clavulanate, CN= Gentamicin, S= Streptomycin, CEP= Cephalexin, NA= Nalidixic Acid, SXT=Sulfamethoxazole/Trimethoprim, PN= Ampicillin

The antibiotic susceptibility testing revealed distinct patterns between the two study areas: in Tudun-wada, isolate W2 showed complete susceptibility to all antibiotics while ampicillin (PN) demonstrated universal resistance in other isolates, gentamicin (CN) maintained full effectiveness, while cephalexin (CEP) showed the lowest susceptibility; similarly in Malali, well isolate W2 was fully

susceptible to all the classes of Antibiotics used. Figure (2) represents the comparative antibiotic susceptibility patterns between Tudun-wada (2a) and Malali (2b) isolates, displaying mean susceptibility percentages with standard error bars. The analysis revealed that fluoroquinolones (OFX, PEF, CPX) maintained consistently high efficacy in both regions, with susceptibility rates exceeding 75%. In contrast, β-lactam antibiotics, particularly ampicillin (PN), showed the most pronounced resistance patterns.

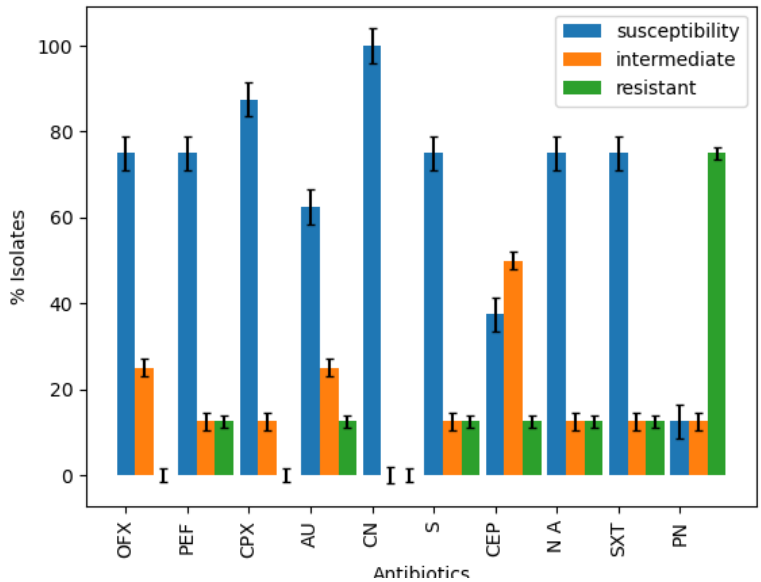

Figure 2a: Antibiotic susceptibility profile of *V. cholerae* isolates from Tudun-wada.

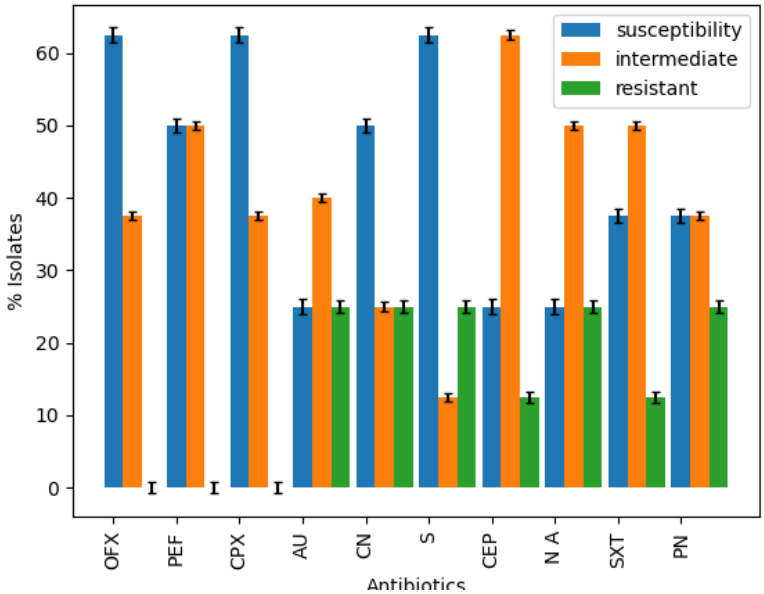

Figure 2b: Antibiotic susceptibility profile of *V. cholerae* isolates from Malali

3.4 Multi-Antibiotic Resistance Index (MARI)

The MARI analysis revealed concerning resistance patterns among the *V. cholerae* isolates (Table 4). Values ranged from 0-0.5, with several isolates exceeding the 0.2 threshold that indicate high-risk contamination. In Tudun-wada, well water isolate W1 showed the highest resistance (MARI=0.5), followed by river isolate R1 (MARI=0.3). Similarly, Malali isolates demonstrated elevated resistance in well W3 (MARI=0.5) and tap T3 (MARI=0.4).

Table 4: Multi Antibiotics Resistant Index of suspected *V. cholerae* from some sources of water from Tudun-wada and Malali area.

Isolate ID	Location	a	b	MARI
W1	Tudun-wada	5	10	0.5
W2	Tudun-wada	0	10	0
W3	Tudun-wada	0	10	0
R1	Tudun-wada	3	10	0.3
T1	Tudun-wada	1	10	0.1
T2	Tudun-wada	1	10	0.1
T3	Tudun-wada	1	10	0.1
T4	Tudun-wada	1	10	0.1
R1	Malali	1	10	0.1
R2	Malali	0	10	0
W1	Malali	1	10	0.1
W2	Malali	0	10	0
W3	Malali	5	10	0.5
T1	Malali	1	10	0.1
T2	Malali	0	10	0
T3	Malali	4	10	0.4

Key: a= number of antibiotic resisted, b= number of antibiotic used, MARI = multi antibiotic resistant index, R = river source, W= well source, T = taps source.

3.5 Molecular Confirmation of *V. cholerae*

PCR analysis targeting the species-specific ompW gene was performed on four biochemically presumptive *V. cholerae* isolates (R1 and T3 from Tudun-wada; W1 and R1 from Malali). As demonstrated in Figure 3, only isolate T3 - obtained from tap water in Tudun-wada - showed amplification of the expected 588bp fragment, confirming its identity as *V. cholerae*. The remaining three isolates failed to produce this diagnostic band, suggesting they may represent other Vibrio species or strains lacking the targeted ompW sequence.

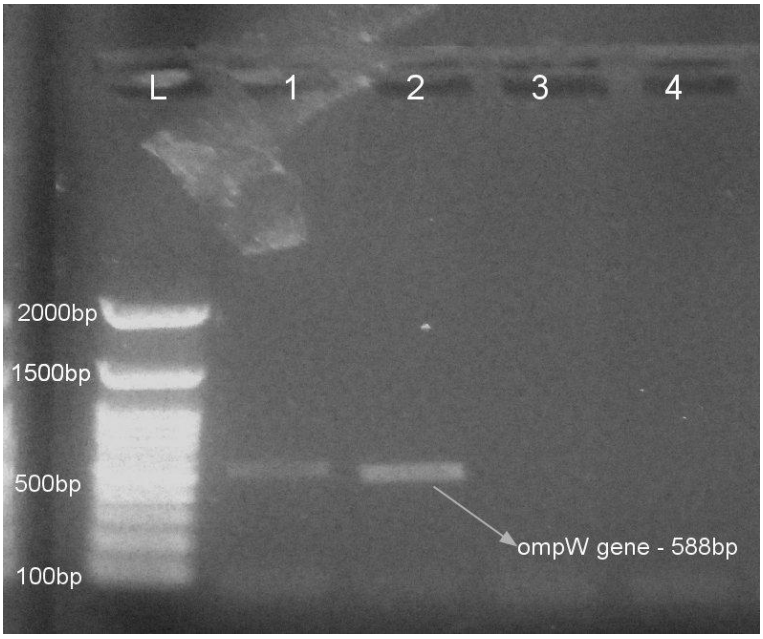

Figure 3: Agarose Gel Electrophoresis of PCR product showing ompW gene of *V. cholerae*

Key:
Lane L: represent Molecular ladder,
Lane 1 represents W1 (well source) from Malali Area location
Lane 2 represents T3 (tap source) from Tudun-wada Area
Lane 3 represents R1 (river source) from Tudun-wada Area
Lane 4 represents R1 (river source) from Malali Area.

4. Discussion

Safe drinking water access remains crucial for preventing waterborne diseases like cholera. Our study recorded pH values ranging from 5.8 to 7.4 across water sources, with river samples showing the most acidic conditions (pH 5.8), likely due to suspended particulate matter affecting water quality (Godfrey et al., 2020). While tap and well water maintained near-neutral pH levels meeting WHO standards (WHO, 2022), the detection of *Vibrio cholerae* in

these sources raises public health concerns. Previous studies confirm *V. cholerae's* optimal growth between pH 6.0-9.0, with reduced survival in highly acidic environments (Adeolu et al., 2021; Tonye et al., 2022).

Among 18 water samples, 16 produced presumptive *V. cholerae* colonies on TCBS agar, yet only one isolate (T3 from Tudun Wada tap water) was confirmed through ompW gene PCR amplification (Shrestha et al., 2022). This aligns with Bulus et al. (2015), who similarly reported molecular confirmation of waterborne *V. cholerae*. Biochemical testing revealed inconsistencies, including oxidase-negative results contradicting typical *V. cholerae* profiles (Parimal et al., 2017; Bulus et al., 2015), underscoring the necessity of molecular confirmation over biochemical methods alone (Ahmed et al., 2019).

Antibiotic resistance patterns showed concerning trends, with high resistance to ampicillin, nalidixic acid, and sulfamethoxazole/trimethoprim, while ciprofloxacin remained effective. The antibiotic susceptibility test results revealed varying resistance patterns amongst isolates however statistical analysis using one-way ANOVA test showed no significant difference among the antibiotic resistance levels ($p > 0.05$) . These findings mirror global reports of increasing antimicrobial resistance in environmental *V. cholerae* strains (Parvin et al., 2020). Multidrug resistance (MARI 0.3-0.5) suggests contamination from high-risk sources (Tahoun et al., 2021), consistent with previous characterizations of *V. cholerae* using biochemical and molecular markers (Rahman et al., 2019; Gupta et al., 2021). These results highlight the organism's persistence in aquatic environments, particularly where sanitation infrastructure is inadequate.

5. Conclusion

This study confirms the presence of *Vibrio cholerae* in water sources from Tudun-wada and Malali, with molecular identification verifying its occurrence in tap water—a concerning finding given this source's presumed safety. The organism's detection, coupled with observed multidrug resistance patterns, highlights significant public health risks, particularly in communities relying on untreated or poorly maintained water systems. While ciprofloxacin remains effective against local strains, widespread resistance to ampicillin and other first-line antibiotics underscores the growing threat of antimicrobial resistance in waterborne pathogens. These findings emphasize the urgent need for strengthened water quality monitoring, improved sanitation infrastructure, and evidence-based updates to cholera treatment protocols. The persistence of *V. cholerae* in these

environments calls for integrated interventions combining enhanced water treatment, community education, and antimicrobial stewardship to mitigate outbreak risks and combat the emergence of resistant strains. Continued surveillance using both culture-based and molecular methods will be essential for tracking epidemiological trends and guiding preventive measures in vulnerable regions.

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Conflicts of Interest:

The authors declare no competing interests.

Ethical Approval:

Not applicable.

Data Availability:

Data are available upon reasonable request.

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