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Isolation and Characterization of Bacteria Culture Mimics of Vibrio Cholerae from Drinking Water Samples in Internally Displaced Persons (IDPS) Camps Within North Central Nigeria

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Abstract: Accurate identification of waterborne pathogens is critical for preventing outbreaks in humanitarian settings, particularly in internally displaced persons (IDP) camps where overcrowding and poor sanitation heighten disease risk. This study investigated the isolation and characterization of *Vibrio cholerae* culture mimics from drinking-water sources across IDP camps in Benue, Nasarawa, and Plateau States within North Central Nigeria. A total of 144 water samples were collected from wells, boreholes, streams, dams, water tanks, and rivers. Water samples were processed using membrane filtration, a 0.45 µm pore-size membrane filter was used to concentrate bacteria, after which the filters were placed into alkaline peptone water (APW) and incubated for 6–8 hours at 37 °C for enrichment. Following enrichment, the filter paper were aseptically picked and placed onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated at 37 °C for 24 hours. Yellow sucrose-fermenting colonies presumed to be *Vibrio*-like were further sub-cultured on nutrient agar to obtain pure cultures and were subjected to oxidase testing and phenotypic characterization. Thirteen isolates (9.0%) exhibited *Vibrio*-like phenotypes, including yellow TCBS colonies, oxidase positivity, and motility. These isolates were suspected to be *Vibrio cholerae* based on conventional and some biochemical reactions especially the negative reaction they gave with polyvalent typing sera for *Vibrio cholerae* 01 and 0139. However, whole genome sequencing (WGS) confirmed no *Vibrio cholerae* among the recovered organisms. Instead, six isolates were successfully sequenced and identified as *Aeromonas dhakensis* (n = 3), *Aeromonas hydrophila* (n = 1), and *Providencia alcalifaciens* (n = 2). The distribution of these bacteria varied across water sources, with Aeromonas species predominantly isolated from surface-related sources such as rivers, dams, and wells, while *Providencia alcalifaciens* was recovered from borehole and well water. These results demonstrate that reliance on culture-based identification alone can lead to misclassification of *Vibrio* species, potentially resulting in incorrect epidemiological interpretations. The presence of Aeromonas and *Providencia* species—both associated with gastrointestinal infections—indicates compromised water quality and underscores the public-health risks faced by displaced populations. The study highlights the importance of integrating molecular tools into water-surveillance systems to ensure accurate detection and guide effective interventions in resource-limited settings.

Keywords: *Culture, Mimics, Drinking, IDPs and Vibrio.*

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I. INTRODUCTION

Cholera, caused by the bacterium *Vibrio cholerae*, remains a major waterborne threat in settings with inadequate water, sanitation and hygiene (WASH) infrastructure. Unsafe drinking water is a primary driver of cholera transmission and of broader enteric disease burden in humanitarian and displaced-person settings; the World Health Organization emphasizes that maintaining safe drinking-water is fundamental to preventing waterborne outbreaks. [1] In Nigeria, recurrent and large cholera outbreaks over the last decade highlight the persistent vulnerability of many communities — including those in the north-central geopolitical zone — to contamination of drinking sources and ensuing epidemic spread. [2] Internally displaced persons (IDP) camps concentrate these risks: overcrowding, disrupted WASH services, and limited access to formal healthcare combine to raise both exposure and the probability of delayed detection and response to enteric disease outbreaks. [3]

Standard culture-based screening for *V. cholerae* typically relies on selective media such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar and simple biochemical tests; in low-resource settings these methods are often the front line of laboratory diagnosis. However, phenotypic overlap between *Vibrio* and other aquatic Gram-negative genera — notably *Aeromonas* spp. and certain enteric bacteria — may cause "culture mimics" that produce cholera-like colony morphologies and oxidase reactions, leading to potential misclassification of isolates from water and clinical specimens. Case reports and comparative studies have documented instances in which presumptive *V. cholerae* isolates were later shown by genomic or molecular methods to be *Aeromonas caviae* or other non-cholera organisms, with important implications for surveillance accuracy and outbreak management. [4][5]

Taxonomic and ecological studies indicate that *Aeromonas* and *Vibrio* co-exist in aquatic reservoirs and can share environmental hosts and niches, complicating environmental surveillance and necessitating robust confirmatory diagnostics. [6] In addition, reviews of laboratory practice in resource-limited contexts recommend multi-step workflows combining selective culture, biochemical testing, and molecular or genomic confirmation to reliably distinguish true *V. cholerae* from phenotypic mimics. [7]

Given the epidemiological importance of drinking-water as a transmission route in IDP contexts and the documented potential for laboratory misidentification, there is a clear need to systematically isolate, characterize and accurately identify bacteria that phenotypically mimic *V. cholerae* in drinking-water sources from IDP camps. The present study therefore aims to isolate presumptive *Vibrio*-like colonies from drinking water in selected IDP camps within north-central Nigeria, apply complementary phenotypic and genotypic identification methods, and characterize the isolates'.

II. MATERIALS AND METHODS

➢ *Description of the Study Area*

The study was conducted in selected internally displaced persons (IDP) camps located in Benue, Nasarawa, and Plateau States within North Central Nigeria. The region lies between latitudes 7°10'–10°10' N and longitudes 7°10'–11°10' E and forms part of the Middle Belt, characterized by ethnic diversity and predominantly agrarian livelihoods. Benue State has an estimated population of 5.7 million, while Nasarawa and Plateau States host substantial rural settlements with active agriculture and mining activities. Recurrent farmer–herder conflicts and communal violence have displaced thousands of residents across the three states, with Benue alone recording nearly 500,000 displaced persons as of 2023. These camps experience persistent challenges including overcrowding, inadequate sanitation, and irregular access to potable water. Such conditions create a high-risk environment for waterborne diseases, making the assessment of drinking-water safety and bacterial contamination critically important.

➢ *Study Design*

A prospective, event-driven environmental surveillance design was implemented to isolate and characterize bacteria that phenotypically mimic *Vibrio cholerae* from drinking-water sources within IDP camps.

➢ *Study Sites and Sampling Frame*

Sampling sites included major drinking-water sources used by camp residents, such as boreholes, River, wells, and surface-water collection points. Each site was sampled monthly to capture temporal variations in microbial contamination.

➢ *Water Sample Collection*

Drinking-water samples were aseptically collected into sterile one-liter bottles following standard WHO guidelines for water sampling. Each sample was transported to the Benue State University Teaching Hospital (BSUTH) microbiology laboratory for processing in an ice packed cooler.

➢ *Culture and Isolation*

Water samples were processed using membrane filtration. A 0.45 µm pore-size membrane filter was used to concentrate bacteria, after which the filters were placed into alkaline peptone water (APW) and incubated for 6–8 hours at 37 °C for enrichment. Following enrichment, the filter paper were aseptically were picked and placed onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated at 37 °C for 24 hours. Yellow sucrose-fermenting colonies presumed to be *Vibrio*-like were further sub-cultured on nutrient agar to obtain pure cultures.

➢ *Phenotypic Characterization of Presumptive Isolates*

Presumptive isolates were subjected to standard microbiological identification techniques. Oxidase testing was carried out using commercial oxidase strips, with a purple color within 10 seconds interpreted as a positive oxidase reaction. Typing sera for polyvalent *vibro chorea* 01

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and 0139 used for all the isolate turns out Negative. These phenotypic characteristics serological reactionss were used to identify bacteria with cholera-like features that warranted further confirmatory testing in line with documented reports of *Vibrio cholerae* mimics.

➢ *DNA Extraction*

Frozen bacterial isolates were revived on Nutrient Agar and incubated at 37°C for 18–24 hours. Single colonies were inoculated into 5 mL LB broth and incubated overnight with shaking. Genomic DNA was extracted using the Qiagen QIamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions for Gram-negative bacteria. DNA concentration and purity were evaluated using a NanoDrop one spectrophotometer (Thermo Fisher Scientific, USA), while DNA quantity was confirmed using invitrogen Qubit 4.0 (Thermo Fisher Scientific, USA). Only samples with A260/A280 ratios between 1.8–2.0 and above 5ng/ul were selected for sequencing.

➢ *Library Preparation and Sequencing*

Whole genome sequencing libraries were prepared using the Illumina DNA prep Kit (Illumina Inc., USA) according to the manufacturer's instructions. Library fragment size distribution was determine using Agarose gel electrophoresis. Sequencing was carried out on the Illumina MiSeq platform (2 × 150 bp paired-end reads) at National Reference Laboratory of the Nigeria Center Disease Control (NCDC).

➢ *Bioinformatics Workflow Summary*

All analyses were performed on a Linux-based Ubuntu 22.04 LTS system. Software tools were managed in Conda environments, and command-line scripts were used for workflow automation. Bactopia V3.0 was used in analysis. (Bactopia is a flexible pipeline for bacteria analysis which incorporates AMRfinder)

➢ *Plasmid and Mobile Element Analysis*

Plasmid replicon types were identified using PlasmidFinder (v2.1), while mobile genetic elements such as insertion sequences, transposons, and integrons were identified with MobileElementFinder . Detected plasmid incompatibility groups (e.g., IncQ1, IncHI1B, Col3M) were correlated with the AMR genes they carried to determine possible plasmid-mediated resistance.

➢ *Data Submission to NCBI*

All high-quality genome assemblies and raw sequencing reads were submitted to the National Center for Biotechnology Information (NCBI) under an assigned BioProject accession number. Metadata and assembled genomes were deposited through the NCBI Submission Portal (https://submit.ncbi.nlm.nih.gov/).

➢ *Data Analysis*

Descriptive statistics were used to summarize the distribution of isolates across sampling sites.

➢ *Ethical Considerations*

Ethical approval for environmental sampling was obtained from the Benue State University Teaching Hospital (BSUTH) Ethics Committee and relevant State Emergency Management Agencies. Permission was granted by camp coordinators and community gatekeepers prior to sample collection.

III. RESULTS

Table 1. Distribution of Water Samples and Vibrio-Like Culture Outcomes Across Sampling Locations.

State	Water Source	Total Samples (N)	TCBS Yellow Colonies n (%)	Oxidase Positive n (%)	Isolates Selected for WGS
Benue	Well	9	0 (0)	0 (0)	0
Benue	Borehole	54	2 (3.7)	2 (3.7)	2
Benue	Dam	9	1 (11.1)	1 (11.1)	1
Benue	Water Tank	9	0 (0)	0 (0)	0
Benue	River	9	3 (33.3)	3 (33.3)	3
Nasarawa	Stream	18	2 (11.1)	2 (11.1)	2
Plateau	Well	27	4 (14.8)	4 (14.8)	4
Plateau	Borehole	9	1 (11.1)	1 (11.1)	1
Total		**144**	**13 (9.0)**	**13 (9.0)**	**13**

Table 2. Phenotypic Characterization of Vibrio-Like Isolates Before Molecular Identification.

Isolate Code	TCBS Colony Colour	Oxidase Test	Motility	Presumptive isolate
BAR-OCT	Yellow	Positive	Motile	*Vibrio spp.*
BD1-FEB	Yellow	Positive	Motile	*Vibrio* spp.
BGBD- OCT	Yellow	Positive	Motile	*Vibrio* spp.
BAR-SEPT	Yellow	Positive	Motile	*Vibrio* spp.
NIS-OCT	Yellow	Positive	Motile	*Vibrio* spp.
NIS-AUG	Yellow	Positive	Motile	*Vibrio* spp.
PGBH-AUG	Yellow	Positive	Motile	*Vibrio* spp.

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PJW1-SEPT	Yellow	Positive	Motile	*Vibrio* spp.
BAR-JULY	Yellow	Positive	Motile	*Vibrio* spp.
BUBH- JULY	Yellow	Positive	Motile	*Vibrio* spp.
PJW1-DEC	Yellow	Positive	Motile	*Vibrio* spp.
PJW1 JULY	Yellow	Positive	Motile	*Vibrio* spp.
PGW-NOV	Yellow	Positive	Motile	*Vibrio* spp.

Table 3. Whole Genome Sequencing (WGS) Identification of Isolates Initially Presumed to be Vibrio Cholerae.

Isolate Code	Initial Presumptive ID	Final Species Identity (WGS)
BAR-OCT	*Vibrio* spp.	*Aeromonas dhakensis*
BD1-FEB	*Vibrio* spp.	*Aeromonas dhakensis*
BGBD- OCT	*Vibrio* spp.	*Aeromonas dhakensis*
BUBH- JULY	*Vibrio* spp.	*Providencia alcalifaciens*
PJW1-DEC	*Vibrio* spp.	*Aeromonas hydrophila*
PGW-NOV	*Vibrio* spp.	*Providencia alcalifaciens*

Table 4. Distribution of WGS-Confirmed Aeromonas and Providencia Species Across Water Sources.

State	Water Source	Species Identified	Number of Isolates
Benue	River	*Aeromonas dhakensis*	1
Benue	Dam	*Aeromonas dhakensis*	1
Benue	Borehole	*Aeromonas dhakensis*	1
Benue	Borehole	*Providencia alcalifaciens*	1
Plateau	Well	*Aeromonas hydrophila,*	1
Plateau	Well	*Providencia alcalifaciens*	1
Total			**6**

IV. DISCUSSION

The environmental surveillance detected 13 (9.0%) TCBS yellow, oxidase-positive "*Vibrio*-like" colonies from 144 drinking-water samples, but whole-genome sequencing (WGS) confirmed only six isolates as bacterial mimics: four *Aeromonas dhakensis/hydrophila* and two *Providencia alcalifaciens* (Tables 1–4). This pattern — a modest overall yield of TCBS-positive colonies with a smaller subset confirmed as non-*V. cholerae* by molecular methods — is consistent with the literature showing that (i) Aeromonas spp. frequently grow on Vibrio-selective media and produce *Vibrio*-like phenotypes, and (ii) environmental culture alone overestimates *V. cholerae* presence without molecular confirmation [8,4].

Ecologically, the higher culture yields from surface-impacted sources (river, dam, some wells) in our study mirror known habitat preferences of Aeromonas and occasional recovery of Providencia from aquatic and sewage-impacted environments. Aeromonads are ubiquitous in fresh and surface waters and are repeatedly reported to colonize drinking-water systems, biofilms and aquatic invertebrate hosts — niches that can produce transient culturable populations recoverable on TCBS and other non-selective media [9,5]. Our finding of *Aeromonas dhakensis* and *A. hydrophila* aligns with multiple surveys and reviews that identify these species as common environmental isolates and occasional human opportunists [8,9]. Similarly, *Providencia alcalifaciens* has been described from water, sewage and food and is recognised as an occasional enteric pathogen; its detection here is plausible where sanitary conditions are compromised [10].

Where our results diverge from some prior reports is in the proportion of presumptive colonies that were ultimately confirmed as Aeromonas/Providencia. Several studies report a high frequency of Aeromonas among non-*Vibrio* isolates from environmental TCBS plates, sometimes explaining the majority of presumptives [8,11]. In our dataset only ~46% (6/13) of presumptive colonies produced WGS confirmations — the remainder may reflect (a) sequencing failures or low-quality reads (a common practical limitation), (b) other genera yielding similar phenotypes (e.g., non-cholera *Vibrio* spp., other Enterobacterales), or (c) mixed or damaged cells that produce ambiguous biochemical signals. The WHO wastewater and environmental surveillance guidance highlights exactly this methodological challenge and explicitly recommends molecular confirmation and sequencing where resources allow, because culture alone can both under- and overestimate public-health-relevant *V. cholerae* presence [11]. Aeromonas spp. and some *P. alcalifaciens* strains carry virulence determinants and have been linked to diarrhoeal illness and extra-intestinal infections, particularly where WASH is poor [3,10]. Thus, while these bacteria are not pandemic *V. cholerae* O1/O139, their presence signals compromised water safety and a non-negligible risk for enteric disease in IDP settings. Practically, our data support routine use of multi-step workflows (selective culture → phenotypic screening → targeted PCR/WGS) and argue for integrating environmental sequencing into surveillance to avoid misclassification and to better prioritise public-health responses [11,4].

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V. CONCLUSION

The findings from this study demonstrate that reliance solely on phenotypic identification of *Vibrio*-like colonies from drinking-water sources can lead to significant misclassification, as only six of the 13 presumptive isolates were confirmed by WGS and none were *Vibrio cholerae*. Instead, *Aeromonas dhakensis*, *Aeromonas hydrophila*, and *Providencia alcalifaciens* were identified—organisms known to mimic *Vibrio* on selective media and capable of causing enteric infections in vulnerable populations. The predominance of Aeromonas spp., particularly in surface-related water sources, aligns with global evidence that these organisms are widespread in aquatic environments and frequently recovered from drinking-water systems. Although not cholera-causing, their presence signals compromised water quality and an associated health risk for residents of IDP camps. These results underscore the need for integrated surveillance that combines selective culture with molecular tools such as PCR or WGS to ensure accurate detection, guide interventions, and strengthen water safety monitoring in resource-limited settings.

RECOMMENDATIONS

- Strengthen water-quality monitoring in IDP camps by integrating culture-based methods with molecular confirmation (PCR or WGS) to avoid misidentifying Vibrio cholerae and related pathogens.
- Improve water-treatment infrastructure—such as chlorination points, protected boreholes, and regular maintenance—to limit contamination by Aeromonas and Providencia species.
- Implement routine sanitation and hygiene (WASH) interventions, including community education on safe water handling and storage.
- Establish national and state-level surveillance frameworks for non-cholera waterborne pathogens to better inform public-health responses.

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