



Prevalence of *Chromobacterium* species in selected borehole waters in Awka Metropolis, Anambra State, Nigeria

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Abstract

Borehole water is an important source of domestic and drinking water in many Nigerian communities, including Awka metropolis, but its underground origin does not guarantee freedom from microbial contamination hence its microbiological quality may be compromised by environmental contamination. *Chromobacterium* species are free-living environmental bacteria associated principally with soil and freshwater environments and are of microbiological and public-health interest because some members can cause severe human infections. This study investigated the prevalence of *Chromobacterium* species in selected borehole waters in Awka metropolis, Anambra State, Nigeria. A cross-sectional study design was adopted. One hundred borehole water samples were collected from selected boreholes across ten villages in Awka metropolis and screened for the presence of *Chromobacterium* species. Samples were aseptically collected in sterile screw-capped containers and transported to the laboratory for microbiological analysis. The organisms were isolated using culture method and then identified using biochemical analysis. Selected isolates were characterized molecularly using 16S rRNA gene sequencing. Of the 100 borehole-water samples examined, nine were positive for *Chromobacterium* spp., giving an overall prevalence of 9.0%. Positive samples were obtained from six of the ten sampled villages, while four villages recorded no positive samples. PCR amplification of selected isolates produced approximately 1.5-kb products and molecular characterization demonstrated the presence of three species: *Chromobacterium phragmitis*, *Chromobacterium violaceum* and *Chromobacterium paludis*. The findings demonstrated that *Chromobacterium* occurs in selected borehole waters in Awka metropolis and that the recovered isolates exhibit species diversity. Regular microbiological monitoring, improved protection and management of boreholes, improved hygiene and appropriate treatment of drinking water are recommended.

Keywords: *Chromobacterium*, borehole water, prevalence, 16S rRNA, molecular identification, phylogeny, Awka metropolis, Nigeria

Introduction

Water is essential to life. An adequate, safe and accessible supply must be available to all (Tauxe *et al.*, 1997) [17]. Many people struggle to obtain access to safe water. In some countries, access to both clean water and sanitation is difficult, and waterborne infections are common. Waterborne bacterial infections remain a major public health concern, particularly in developing regions where access to safe drinking water is limited. Borehole water, commonly relied upon as a primary source of domestic water supply in many Nigerian communities including Awka metropolis, is relatively accessible and is often presumed safer than surface water but may harbor pathogenic microorganisms due to contamination from environmental pollution, poor sanitation, inadequate water handling practices and anthropogenic sources. Studies conducted in Nigeria have demonstrated that borehole water may contain bacterial contaminants, including potentially pathogenic organisms (Ibo *et al.*, 2020; Onuorah *et al.*, 2019). Enemor *et al.* (2020) [9, 10, 16] investigated selected residential borehole waters in Awka and recovered several bacterial species, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*. The authors concluded that some of the investigated water samples were unsuitable for human consumption. Similarly, investigations of borehole water by Nwaiwu *et al.* (2020) [12] reported bacterial and coliform

contamination in borehole water from a community in southeastern Nigeria.

Among environmental bacteria of potential public-health significance commonly associated with soil and water is the genus *Chromobacterium*. *Chromobacterium* species are Gram-negative bacteria associated predominantly with aquatic and soil environments. The genus contains several recognized species, including *C. violaceum*, *C. phragmitis*, *C. paludis*, *C. vaccinii*, *C. amazonense* and others. *C. violaceum*, the best-known member of the genus, is widely distributed in tropical and subtropical environments and is recognized as an environmental opportunistic pathogen (Batista and da Silva Neto, 2017) [3]. The organism is generally an environmental bacterium, but human infection can occur following environmental exposure, particularly through contaminated water or soil entering damaged skin. Although human infections are uncommon, they may progress rapidly and result in severe disease, including septicemia and metastatic abscess formation (Alisjahbana *et al.*, 2021; Lin *et al.*, 2016) [1, 19]. The presence of *C. violaceum* in water sources is therefore of environmental-health interest. An earlier Nigerian study by Uba and Eze (2004) [18] documented persistent occurrence of *C. violaceum* in borehole water in Port Harcourt and emphasized the need for further investigation of its occurrence in drinking-water sources. Importantly, the genus *Chromobacterium* is not restricted to *C. violaceum*.

Molecular and genomic studies have established several additional species. *Chromobacterium phragmitis* was described as a distinct species from isolates recovered from estuarine marshes, while *Chromobacterium paludis* was subsequently described from a Chesapeake Bay marsh (Blackburn *et al.*, 2019, 2020) ^[4, 5].

Despite the documented occurrence of *Chromobacterium* in Nigerian water environments, there is limited information regarding its occurrence in borehole-water sources in Awka metropolis. Consequently, there is a need to document the occurrence of *Chromobacterium* in selected borehole waters in Awka and to provide information that can support environmental surveillance, water-quality monitoring and public-health interventions. The present study was undertaken to determine the occurrence of *Chromobacterium* species in selected borehole waters in Awka metropolis as it will assist public-health authorities and communities in appreciating the importance of routine microbiological surveillance of borehole-water sources.

Materials and methods

Study area

The study was carried out in Awka town in Awka South LGA, Anambra State, which is the capital city of the State located in the South-East geopolitical zone of Nigeria. Based on 2006 population census, the population of Awka is about 301,657 (three hundred and one thousand six hundred and fifty-seven) people (Okezie and Igbokwe, 2015) ^[14] and as of 2022, it has a population of 430,200 (Wikipedia, 2026) ^[22].

Study population

The population targeted is ten borehole waters each in ten villages in Awka, Anambra State making it a total of 100 bore hole waters. The villages included; Umuzocha, Umuayom, Umuogwali, Umudioka, Amudo, Amachalla, Umunoke, Umukwa, Nkwelle and Umuoramma,

Study design

A cross-sectional study design was employed to determine the prevalence and distribution of *Chromobacterium* species in selected borehole-water sources.

Sampling techniques

In this study, random sampling technique was used. Ten samples were collected from ten selected boreholes in each of ten villages in Awka town of Anambra State, Nigeria in the month of November.

Sample Collection

One hundred (100) water samples were collected using sterile containers with screw caps from ten bore holes each from ten villages in Awka. Accidental contamination during collection was avoided by allowing the water to run for 1 minute before it was collected in the sterile containers to reduce the possibility of collecting stagnant water at the outlet. Each of the sterile containers was labelled with full details of the source of water, date and time of collection. The water samples were delivered to the laboratory and tested within 6 hours of collection (Ochei and Kolhatkar, 2004) ^[13].

Isolation and identification of *Chromobacterium*

Isolation of the organism was carried out using appropriate microbiological culture procedures employing enrichment

followed by selective and differential media and selection of colonies exhibiting characteristics suggestive of *Chromobacterium* (Diaz-Torres *et al.*, 2020) ^[5]. Suspected colonies were subsequently subjected to macroscopic, microscopic, biochemical and molecular identification by methods described by Kanika (2011), Olutiola, *et al.*, (2000) ^[11, 15], Chattopadhyay *et al.*, (2002), Ochei and Kolhatkar (2004), Whitman, (2015) and Cheesbrough, (2006) ^[7, 8, 13, 21].

Data Analysis

The prevalence of *Chromobacterium* species was calculated as the proportion of positive samples among the total number of borehole-water samples examined, expressed as a percentage (Centers for Disease Control and Prevention [CDC], 2012) ^[6].

$$\text{Prevalence (\%)} = \frac{\text{Number of positive samples}}{\text{Total number of samples examined}} \times 100$$

Descriptive statistics were used to summarize the distribution of positive and negative samples among the selected villages. Data were summarized using frequencies and percentages.

Results

The presumptive isolates exhibited characteristics compatible with *Chromobacterium*. The organisms were Gram-negative bacilli, catalase positive, oxidase positive, motile and citrate positive. They were negative for indole, methyl red, Voges–Proskauer, urease and they demonstrated nitrate reduction as seen in Table 1.

Table 1: Biochemical characteristics of bacterial isolates

Test	Observation	Result
Gram stain	Gram-negative bacilli	Positive
Catalase test	Bubble production	Positive
Oxidase test	Purple colour within 30 secs	Positive
Motility	Motile	Positive
Citrate	Blue colour with growth	Positive
Indole test	No red ring after Kovac's reagent	Negative
Methyl Red (MR)	No red colour	Negative
Voges–Proskauer (VP)	No pink/red colour	Negative
Urease test	No pink colour	Negative
Nitrate reduction	Nitrate reduced to nitrite	Positive
Glucose fermentation	Acid produced	Positive
Sucrose fermentation	No acid production	Negative
Lactose fermentation	No acid production	Negative
Mannitol fermentation	No acid production	Negative
Fructose	Acid produced	Negative
Hydrogen sulfide (H ₂ S) production	No black precipitate	Negative
Gas production	No gas bubbles or cracks	Negative

The bacterial isolates obtained from borehole water was identified and out of the 100 borehole water samples analysed, 9 were positive for *Chromobacterium spp.*, while 91 were negative, giving an overall prevalence of 9.0 % (Table 2).

Table 2: Overall prevalence of *Chromobacterium* species in selected borehole waters

Result	Number of samples	Percentage (%)
Positive	9	9.0
Negative	91	91.0
Total	100	100.0

The frequency of *Chromobacterium spp* isolated as regards the ten villages is presented in Table 2. The distribution was not uniform among the ten villages. Six out of the ten villages have water sample/s positive for *Chromobacterium spp* while four villages were negative for all their water samples. The highest within-village positivity was observed in Umunoke, Nkwelle and Umuoramma, where two of ten samples were positive (20% each). Umuzocha, Umuayom and Umuogwali each recorded one positive sample (10%). Four villages—Umudioka, Amudo, Amachalla and Umukwa—recorded no positive sample.

Table 3: Distribution of *Chromobacterium spp.* among the villages

Villages	Negative	Positive	Total	Village positivity (%)
Umuzocha (UZ)	9	1	10	10
Umuayom (UA)	9	1	10	10
Umuogwali (UO)	9	1	10	10
Umudioka (UD)	10	0	10	0
Amudo (AM)	10	0	10	0
Amachalla (AC)	10	0	10	0
Umunoke (UN)	8	2	10	20
Umukwa (UU)	10	0	10	0
Nkwelle (NK)	8	2	10	20
Umuoramma (UR)	8	2	10	20
Total	91	9	100	9.0

The agarose-gel image showed distinct amplification products in lanes UN4, UN5, UN6 and UN9 being the code for the four selected isolates for molecular identification. Visible PCR products of the four selected isolate are shown in figure 1. The products migrated close to the 1,500-bp marker position, indicating amplification of approximately 1.5-kb 16S rRNA gene products.

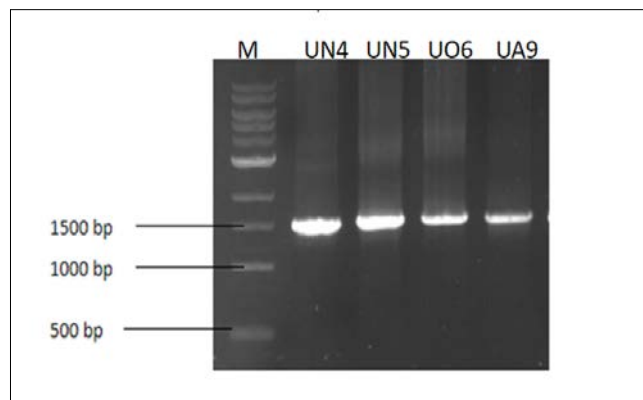


Fig 1: Agarose gel electrophoresis of PCR-amplified 16S rRNA gene products of selected *Chromobacterium* isolates; M, molecular DNA marker, UN4 – *Chromobacterium phragmitis*, UN5 – *Chromobacterium violaceum*, UO6 – *Chromobacterium paludis*, UA9 – *Chromobacterium violaceum*.

Molecular characterization revealed three *Chromobacterium* species among the selected isolates. although four selected isolates were characterized: *C. phragmitis*, *C. violaceum* and *C. paludis*, with *C. violaceum* represented twice.

Table 4: Molecular identification of selected *Chromobacterium* isolates

Sample	Species identified	Reference strain	GenBank accession
UN4	<i>Chromobacterium phragmitis</i>	IIBBL 274-1	CP029554.1
UN5	<i>Chromobacterium violaceum</i>	726249W	MG938493.1
UO6	<i>Chromobacterium paludis</i>	IIBBL 257-1	CP043473.1
UA9	<i>Chromobacterium violaceum</i>	CV1192	CP024028.1

Discussion

The present study investigated the prevalence of *Chromobacterium* species in selected borehole waters in Awka metropolis. A total of 100 borehole-water samples were examined and the isolates identified using biochemical analysis. The biochemical profile obtained in this study (Table 1) is consistent with the characteristic laboratory profile of *Chromobacterium* that was subsequently strengthened by molecular characterization. Biochemical identification is important because isolation of an environmental organism from drinking-water sources should ideally be supported by multiple identification methods. In the present study, phenotypic identification was complemented by molecular analysis, thereby increasing confidence in the identification of the test organism. The public-health relevance of this finding arises principally from the pathogenic potential of *C. violaceum*. Although human infections of it are rare, they can be severe. Alisjahbana *et al.* (2021) [1] reported rapid progression and potentially fatal outcomes in invasive infections and identified environmental water and soil exposure as important routes of exposure.

The detection of *Chromobacterium spp.* in nine of the 100 samples, giving an overall prevalence of 9.0% (Table 2), demonstrates that the organism was present in a proportion of the borehole-water sources investigated. The detection of *Chromobacterium* in borehole water is environmentally believable because members of the genus are naturally associated with soil and freshwater. The detection of *Chromobacterium* in borehole water is consistent with previous Nigerian evidence. Uba and Eze (2004) [18] reported persistent recovery of *C. violaceum* from borehole water in Port Harcourt. Their study demonstrated that borehole water could serve as an environmental reservoir for the organism despite its underground origin. Batista and da Silva Neto (2017) [3] described *C. violaceum* as an abundant component of soil and water microbiota in tropical and subtropical environments. The 9.0% prevalence observed in this study is also important in relation to previous studies of borehole-water quality in Nigeria. Studies in other Nigerian locations have reported bacterial contamination of borehole water, although the organisms detected and prevalence values vary according to location,

sampling period, environmental conditions and analytical methods. Bamigboye *et al.* (2020) ^[2], for example, reported multiple-antibiotic-resistant bacteria and poor water-quality indicators in groundwater sources in Ogbomoso. The finding is also consistent with evidence that groundwater in Nigeria may contain bacterial contaminants. Enemor *et al.* (2020) ^[9] reported several bacterial species in residential borehole waters in Awka, including *E. coli* and *P. aeruginosa*. Nwaiwu *et al.* (2020) ^[12] similarly reported bacterial contamination in borehole water in southeastern Nigeria, indicating that groundwater contamination is not restricted to a particular region. The present finding adds *Chromobacterium* to the range of environmental bacteria that may be encountered in groundwater sources in the area. The occurrence of positive samples in six of the ten sampled villages, as seen in Table 3, indicates that the organism was geographically distributed across multiple parts of the study area rather than being restricted to one sampling location. The variation in occurrence between villages may be associated with differences in the environmental conditions surrounding individual boreholes. Potential factors may include proximity to drainage channels, surface runoff, sanitation practices, borehole construction, contamination of the surrounding soil and possible infiltration of microorganisms into groundwater. The WHO recommends risk-based surveillance, sanitary inspections and water-safety planning as important approaches for small drinking-water supplies (World Health Organization [WHO], 2024) ^[20]. However, the absence of positive samples in a village should not be interpreted as proof that *Chromobacterium* is absent from all boreholes in that village. Only ten samples were investigated per village, and the study represents a cross-sectional assessment. Temporal changes in water quality, borehole depth, hydrogeology, sanitation and environmental conditions could influence detection.

One of the most important findings of this study was the identification of three *Chromobacterium* species among the molecularly characterized isolates (Table 4). Molecular approaches, particularly 16S rRNA gene sequencing, therefore, provide useful additional information for bacterial identification. All four selected isolates produced visible PCR products of approximately 1.5 kb (Figure 1), indicating successful amplification of the target bacterial 16S rRNA region. UN4 was identified as *C. phragmitis*, UN5 and UA9 as *C. violaceum*, and UO6 as *C. paludis*. This finding demonstrates that the environmental *Chromobacterium* population investigated was more diverse. The result agrees with the growing recognition of substantial diversity within the genus. *C. phragmitis* and *C. paludis* are both formally recognized species described from environmental water-associated habitats (Blackburn *et al.*, 2019, 2020). Batista and da Silva Neto (2017) ^[3, 4, 5] discussed the increasing recognition of additional *Chromobacterium* species recovered from environmental samples. The finding highlights the importance of molecular methods in environmental bacterial surveillance.

The present study therefore contributes location-specific information concerning an organism that has received comparatively less attention than conventional indicators such as *E. coli* and total coliforms. It should also be emphasized that detection of *Chromobacterium* is not equivalent to detection of fecal contamination. *C. violaceum* is principally an environmental organism, and its occurrence

may reflect ecological and groundwater conditions rather than direct fecal pollution. Consequently, the detection of *Chromobacterium* should complement conventional drinking-water quality indicators.

Conclusion

The present study established that *Chromobacterium* species, an environmental organism with recognized opportunistic pathogenic potential, occur in selected borehole waters in Awka metropolis, Anambra State, Nigeria. The detection of *Chromobacterium* in selected borehole waters should encourage greater attention to microbiological surveillance of groundwater sources. Although the prevalence observed was relatively low, its detection is relevant because invasive *C. violaceum* infection can be severe and potentially fatal. The study therefore concludes that routine microbiological surveillance of borehole-water sources is warranted, particularly where groundwater is used for drinking and other domestic purposes.

Recommendations

Based on the findings of the study, it is recommended that borehole-water sources in Awka metropolis should be subjected to regular microbiological assessment and improved protection and management of borehole-water sources adopted. Further studies should include a larger number of boreholes and repeated sampling across wet and dry seasons. Communities should be educated about the fact that groundwater is not automatically free from microorganisms.

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