Operational Paper

Incidence of *Chromobacterium violaceum* in borehole water in Port Harcourt metropolis, Rivers State, Nigeria

Beatrice N. Uba and Augustine Eze

ABSTRACT

A borehole located in Port Harcourt, Rivers State, Nigeria, drilled to a depth of 55 m, was analysed for its water quality between May and September 2001. All the physicochemical parameters analysed were within the limits stipulated by the World Health Organization (WHO) with the exception of pH, which fell within the range 4.98–5.15. Bacteriological analysis indicated a low count of total coliforms (3 cfu 100 ml^{-1}), faecal coliforms (0 cfu ml^{-1}) and heterotrophic bacteria (159 cfu ml^{-1}). The presence of *Chromobacterium violaceum*, a pathogen known to cause chromobacteriosis in humans, was detected in the heterotrophic plate count (3 cfu ml^{-1}). Other pathogenic organisms, as indicated in the WHO guidelines for water quality, were not detected. Repeated analysis in July indicated a low heterotrophic count (180 cfu ml^{-1}) with 5 cfu ml^{-1} *Chromobacterium violaceum*. Total coliforms and faecal coliforms were higher (45 cfu 100 ml^{-1} and 12 cfu 100 ml^{-1}, respectively). Other pathogenic organisms were not detected. Analysis of samples collected in September indicated a high level of heterotrophic bacteria (700 cfu ml^{-1}) while total coliforms and faecal coliforms were too numerous to count. The persistent occurrence of *Chromobacterium violaceum* in this borehole is a cause for concern. There is need for more research on the occurrence of *Chromobacterium violaceum* in drinking water sources and the epidemiology of its associated disease so that it can be included on the list of waterborne pathogens by WHO and the US Environmental Protection Agency (EPA).

Key words | borehole, *Chromobacterium violaceum*, EPA, research, quality, WHO

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INTRODUCTION

One of the major constraints to economic and social development in developing countries such as Nigeria is the difficulty in obtaining reliable water supplies for local populations. Where surface water is not perennial, or where it is often contaminated, groundwater is commonly the safest source of potable water (Bannerman 1998). To ensure the hygienic quality of the water, analysis of the physical, chemical and bacteriological qualities of the water is carried out. Routine bacteriological quality parameters analysed usually include total heterotrophic plate count, total coliforms and faecal coliforms, pathogenic organisms such as Vibrios, *Salmonella* and *Shigella* are also assayed in some instances. Standards for bacteriological quality of potable water rarely include the assay of pathogenic organisms such as *Chromobacterium* (WHO 1998). This organism is described as a mesophilic organism that is more likely to be met in a clinical laboratory. It is known to cause chromobacteriosis, a rare disease of humans and cows (Sneath 1960). The organism was reportedly isolated from an estuarine environment (Uba 1991) and from a wastewater equalization basin in a fertilizer factory (Uba 1995). Alexander (1977) listed this bacterium as a soil inhabitant able to hydrolyse starch, utilize chitin and carry out denitrification. The organism therefore can be found in aquatic environments as a transient organism from the soil.

This report is based on sporadic detection and persistence of this pathogenic organism in underground water located in a particular area of the Port Harcourt metropolis which serves as a source of potable water supply.

MATERIALS AND METHODS

Characteristics of the study site

The study site is a borehole drilled to a depth of 55 m, located in Rumuepirikom town, which is about 10 km from Port Harcourt city centre. The area is a medium density one, with an approximate population of 100,000 inhabitants.

Sample collection

Water samples were collected from a tap connected to the borehole. The mouth of the tap was thoroughly cleaned with absolute ethanol and flamed. The tap was then opened and allowed to run for 2 min. Thereafter the water sample was collected aseptically in a sterile 500-ml glass bottle and stored in an ice packed container. The sample was then taken to the laboratory for analysis.

Frequency of sampling

Replicate samples were collected from the borehole on three occasions in May, July and September 2001.

Analysis of samples

The samples were analysed for various physicochemical parameters which included pH, colour, odour, taste, conductivity, turbidity, salinity, acidity, alkalinity, total hardness, total solids, total dissolved solids, chloride, ammonia, nitrate, nitrite, phosphate, sulphate, calcium, iron, magnesium, zinc, lead and arsenic. These parameters were analysed using Standard Methods (1992).

Microbiological analysis of the samples included the enumeration of total heterotrophic bacteria on plate count agar (PCA), total coliforms, faecal coliforms, *Salmonella*, *Shigella*, Vibrios, *Pseudomonas* sp., filamentous fungi and yeasts. These were enumerated using appropriate media and standard procedure as indicated in *Standard Methods* (1992), Csuros & Csuros (1999) and as reported in Uba & Aghogho (2000). The analysis was replicated for each parameter and the mean results recorded.

Identification of isolate

While enumerating bacterial colonies on the plate count agar a violet-pigmented colony was repeatedly observed. The isolate was subcultured on fresh nutrient agar plates to obtain pure cultures. Characterization of the isolate was undertaken using various morphological, biochemical and physiological tests. These tests included gram stain reaction, motility, growth at 37°C, acid production from glucose, lactose and sucrose, starch hydrolysis, gelatin liquefaction, casein hydrolysis, arginine hydrolysis and chitin hydrolysis. These tests were carried out using the procedures outlined in Cowan (1981).

RESULTS

Table 1 shows the physicochemical quality of water samples from the borehole collected in May, July and September 2001. pH was in the range 4.98–5.15, colour was 4.0 Pt-Co, odour and taste were unobjectionable. Conductivity was in the range 43.5–46 μmhos cm^{-1}, turbidity ranged from 0.28 to 0.32 NTU, salinity was 0%, and acidity and alkalinity ranged from 16.06 to 17.1 and 12.5 to 13.25 mg $CaCO_3$ l^{-1}, respectively. Total solids ranged from 30.8 to 35.8 mg l^{-1}, while total dissolved solids ranged from 17.5 to 20.0 mg l^{-1}. Chloride was in the range 5.68–6.2 mg l^{-1}. Ammonia, nitrate and nitrite were not detected in the samples all through the study period.

Table 1 | Physicochemical quality of borehole samples in May, July and September 2001

Parameter	Unit	May	July	September
pH		5.15	4.98	5.0
Colour	Pt-Co	4.0	4.0	4.0
Odour		Unobjectionable	Unobjectionable	Unobjectionable
Taste		Unobjectionable	Unobjectionable	Unobjectionable
Conductivity	μmhos cm^{-1}	43.5	46	44.5
Turbidity	NTU	0.28	0.3	0.32
Salinity	%	0.0	0.0	0.0
Acidity as $CaCO_3$	mg l^{-1}	16.06	17.1	16.8
Alkalinity as $CaCO_3$	mg l^{-1}	13.25	12.25	12.8
Total solids	mg l^{-1}	30.8	35.1	35.8
Total dissolved solids	mg l^{-1}	20.0	19.8	17.5
Chloride (Cl^-)	mg l^{-1}	5.68	6.2	5.9
Ammonia (NH_3)	mg l^{-1}	0.0	0.0	0.0
Nitrate (NO_3)	mg l^{-1}	0.0	0.0	0.01
Nitrite (NO_2)	mg l^{-1}	0.0	0.0	0.0
Phosphate (PO_4^{3-})	mg l^{-1}	0.121	0.2	0.18
Sulphate (SO_4^{2-})	mg l^{-1}	0.0	0.05	0.1
Calcium	mg l^{-1}	0.02	0.08	0.15
Magnesium	mg l^{-1}	0.0	0.08	0.05
Iron	mg l^{-1}	0.23	0.15	0.21
Zinc	mg l^{-1}	0.04	0.05	0.02
Lead	mg l^{-1}	0.0	0.0	0.0
Arsenic	mg l^{-1}	0.0	0.0	0.0

Phosphate was in the range 0.18–0.2 mg l^{-1}. Sulphate ranged from 0 to 0.1 mg l^{-1}, and calcium and magnesium ranged from 0.02 to 0.15 and 0 to 0.08 mg l^{-1}, respectively. Iron ranged from 0.15 to 0.23 mg l^{-1} while zinc ranged from 0.02 to 0.05 mg l^{-1}. Heavy metals such as lead and arsenic were not detected in the samples.

Table 2 | Microbiological quality of borehole water samples in May, July, and September 2001

Parameter	Unit	May	July	September
Total heterotrophic count	cfu ml^{-1}	1.59×10^2	1.8×10^2	7.0×10^2
Total coliforms	cfu 100 ml^{-1}	3	45	TNTC
Faecal coliforms	cfu 100 ml^{-1}	0	12	TNTC
Salmonella sp.	cfu ml^{-1}	0	0	0
Shigella sp.	cfu ml^{-1}	0	0	0
Vibrios	cfu ml^{-1}	0	0	0
Chromobacterium violaceum	cfu ml^{-1}	3	5	6
Pseudomonas	cfu ml^{-1}	0	0	0
Filamentous fungi	cfu ml^{-1}	0	0	0
Yeasts	cfu ml^{-1}	0	0	0

TNTC=too numerous to count.

The results of the microbiological analysis of the samples are shown in Table 2. The samples collected in May 2001 indicated a total heterotrophic bacteria count of 1.59×10^2 cfu ml^{-1}. Three colonies of *Chromobacterium violaceum* were detected in the medium. Total coliforms were 3 cfu 100 ml^{-1}. Faecal coliforms and other pathogenic bacteria were not detected. The water sample collected in July 2001 had a total count of 1.8×10^2 cfu ml^{-1} of heterotrophic bacteria. Five colonies were *Chromobacterium violaceum*. Total coliforms and faecal coliforms were 45 and 12 cfu 100 ml^{-1}, respectively. Pathogenic organisms, namely *Salmonella*, *Shigella*, Vibrios and *Pseudomonas* were not detected.

In September 2001 the number of heterotrophic bacteria was 7.0×10^2 cfu ml^{-1}, six of these colonies were *Chromobacterium violaceum*. Total coliforms and faecal coliforms were too numerous to count.

Table 3 lists the characteristics used for the identification of the violet-pigmented bacteria found in the sample. The test result indicated that the organism was *Chromobacterium violaceum*, a human pathogen. All the physicochemical parameters analysed in the borehole water were within the World Health Organization (WHO) standards for potable water, except pH, which was low (5.15 as against 7.0–8.5).

DISCUSSION

The WHO guidelines for drinking water quality have helped to shape drinking water legislation in many countries of the world (Chorus 2000). The standard for drinking water as laid down by WHO emphasizes a total heterotrophic count of not more than 500 cfu ml^{-1}, less than 10 total coliforms per 100 ml, less than one faecal coliform per 100 ml and the absence of pathogenic bacteria such as *Salmonella*, *Shigella*, Vibrios and *Pseudomonas* (WHO 1998). Based on this guideline, the result of the analysis of the water sample from the borehole in May 2001 indicated that the water was bacteriologically fit for consumption, since the heterotrophic plate count was 159 cfu ml^{-1} which is obviously less than 500 cfu ml^{-1}. Total coliforms were 3 cfu 100 ml^{-1}, which was also less than the guideline, and faecal coliforms were less than 1 cfu 100 ml^{-1}.

Table 3 | Characteristics used for the identification of the violet-pigmented isolate

Test	Result
Pigmentation	Violet
Gram stain reaction	–
Motility	+
Growth at 37°C	+
Acid production from glucose	+
Acid production from lactose	–
Acid production from sucrose	–
Starch hydrolysis	+
Gelatin liquefaction	+
Casein hydrolysis	+
Arginine hydrolysis	+
Chitin hydrolysis	+
Denitrification	+

+ = positive; – = negative.

Most water quality analysts would be tempted to certify water from such a borehole fit for human consumption; however the presence of violet-pigmented colonies in the culture plate arose curiosity for the further investigation of the identity of the isolate. The result was quite revealing and indicated the presence of a pathogenic bacterium not previously included in the WHO standard. Subsequent sampling of the borehole water in July and September 2001 indicated the continued presence of the pathogenic organism, with an increase in the number of coliform organisms. Based on the number of coliform organisms in the water samples during these times, the water was unfit for human consumption. However, the other pathogens were still not detected. It is widely known that indicator bacteria, for instance, may give an imbalanced view of the risk posed by individual pathogens; their environmental resistance and transport pathways may be very different from individual pathogens and natural die-off and attenuation potentials may vary greatly (Foster 1985).

The occurrence of *Chromobacterium violaceum* was also reported in borehole water in the northern part of Nigeria (IPAN 2001, unpublished information); based on WHO bacteriological quality, this water would have been fit for human consumption. *Chromobacterium violaceum* is described as a mesophilic organism which is more likely to be met in a clinical laboratory (Cowan 1981). Cases of human and animal infection have been described in Europe, the USA and the Far East (Collins & Lyne 1985). The presence of this pathogen in borehole water and its apparent omission from the WHO list of pathogens that impair drinking water quality should be a cause for concern. *Chromobacterium violaceum* is a mesophilic heterotrophic bacterium which is easily detected in a heterotrophic plate count on plate count agar because of its pigmentation. The presence of this organism in a water sample just like other pathogens renders it unfit for human consumption, irrespective of the number of heterotrophs. The high level of coliform organisms in the samples in July and September 2001 suggest possible sewage contamination of the borehole. The absence of ammonia, nitrite and nitrate cannot be interpreted as indicating an absence of sewage contamination. These nitrogenous compounds are subject to rate-limited input in groundwater and subsequent impact on their stability due to nitrification and denitrification processes. Barrett *et al.* (1998) stated that correlations between the presence of microbes and the level of NO_3 in groundwater should not be expected, given the entirely different controls on their transport in aquifers.

Although there have been no reported cases of chromobacteriosis from drinking water, this could probably be due to a lack of awareness of the existence of this disease and its possible causes. The Safe Drinking Water Act Amendment of 1996 requires the United States Environmental Protection Agency (EPA) to establish a list of contaminants of public health concern that are known or anticipated to occur in drinking water systems and may require future regulations under the Safe Drinking Water Act. The list, known as the Contaminant Candidate List (EPA 1998), is intended to generate scientific research that will assist the EPA in creating new regulations to protect the public from health risks associated with drinking water. For example, *Aeromonas* has been indicated on this list as an emerging pathogen associated with drinking water. Research is ongoing to confirm this assertion (Borchardt *et al*. 2003).

This paper serves to alert the public about the occurrence of this pathogen in a drinking water source and to generate interest for more research on the organism and the disease it causes for possible inclusion in the list of waterborne pathogens by WHO and EPA.

CONCLUSION

Chromobacterium violaceum, a pathogenic bacteria known to cause chromobacteriosis, an occasional disease of humans, was found repeatedly on a heterotrophic plate count agar of water samples from a borehole drilled to a depth of 55 m. The WHO bacteriological standard for water quality does not include this organism among the list of pathogens to be assayed in water quality analysis. This study shows that *Chromobacterium violaceum* could be detected in a heterotrophic plate count at a level below the WHO stipulated limit and with a limited number of total and faecal coliforms, i.e. routine faecal pollution indicator bacteria. Based on the WHO standard, drinking water contaminated with this organism could be certified fit for human consumption. This could be a threat to public health particularly in areas where this organism is endemic.

The disease chromobacteriosis, although a rare disease of humans, could assume an epidemic level where there is gross contamination of water by the causative organism. The bacteriological quality standard of water should include not just the number of colonies on a heterotrophic plate count, but also the types of colony. Detection of *Chromobacterium violaceum* on a heterotrophic plate count of a water sample should render such water unsafe for human consumption, irrespective of the number of colonies on such a plate. *Chromobacterium violaceum* was present at a level of 6 cfu ml^{-1} while other pathogens were not detected. The persistent occurrence of *Chromobacterium violaceum* in this borehole and other boreholes reported in northern Nigeria suggests that it is a potential contaminant in boreholes. Its presence in borehole water should be viewed seriously because of its pathogenic ability. More research is needed on the occurrence of this pathogen in drinking water sources and the epidemiology of its associated disease so that it can be included on the list of waterborne pathogens by the various regulating bodies such as WHO and EPA.

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EDITOR'S NOTE

This paper addresses a very little known hygienic problem: the presence of the pathogen *Chromobacterium violaceum* in a drinking water source. It is unknown whether this occurs on a wider scale and it is impossible–on the basis of this short research paper–to judge whether chromobacteriosis deserves more attention and research by regulating agencies and/or research institutes as called for in the paper. Therefore readers with knowledge of and/or experience with this subject are invited to contact Professor Gimbel at the Editorial Office, or alternatively, to submit a paper or comment to the journal.

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