Journal of Biotechnology and Food Science
ISSN: 3078-7378
DOI: https://doi.org/10.61784/jbfs3004

THE PREVALENCE AND INCIDENCE OF *VIBRIO CHOLERAE* IN THE WELL WATER OF BILLE KINGDOM, RIVERS STATE

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Abstract: With the recent cases of cholera been reported in some parts of northern Nigeria. The spread of its causative agent, *Vibrio cholerae* towards the south have become a thing of concern. River tributaries through underground water wells are believed to carry pathogens and following this circumstance, the study seeked to determine the prevalence of *Vibrio cholerae* in well water of the Kingdom. A total of 60 water samples comprised of 20 each of Opu Imo, Angular Polo and Osia Polo wells were collected and coded; Well water A, B and C respectively for the purpose of the study. On transfer to the laboratory for microbiological analysis, result showed a mean *Vibrio* spp. count of 0×10^5, 0×10^5 and 9.2×10^7 CFU/ml for well A, B and C respectively. Morphological characteristics of isolates recovered showed the *Vibrio* spp. colonies appeared with a yellow color and a distinctive comma shaped, curved, rod-like structure. Biochemical classification of the isolates showed a positive catalase, oxidase, indole, methyl red, citrate, glucose, and sucrose tests while Voges Proskaurer and Lactose showed a negative reaction. Frequency of *Vibrio cholerae* prevalence across the water wells showed a high in Well C with a percentage prevalence of 100%. Well A and B had no *Vibrio cholerae* prevalence. Thus, the study noted the prevalence and incidence of *Vibrio cholerae* in well water C only which indicated a likelihood for cholera outbreak. Hence, inhabitants of Bille Kingdom may be predisposed to infectious doses of *Vibrio cholerae* when well water C is consumed. Therefore, well water C should be discontinued for drinking purposes.
Keyword: Bille Kingdom; Prevalence; *Vibrio cholerae*; Well water

1 INTRODUCTION

Following the importance of well water in Bille kingdom, it remains an age-old alternative source of water used in most households in Bille Kingdom for domestic purposes. As such, it is a common feature in most residences [1]. Consequently, cholera, a waterborne disease has been reported. with recent cases of Cholera been reported in some parts of Nigeria specifically, Lagos and Edo State [2]. The spread of the disease (cholera) towards the south of Nigeria has become a thing of concern, following the polluted state of Bille well water [3]. According to Amanda Well water in Bille Kingdom is in constant and continuous pollution following oil exploration and oil spill occasionally, experienced in the area [3]. Tourist outside the state who visit Bille sometimes introduce pathogenic microorganism via open defecation due to lack of toilet facilities in the Kingdom [4]. According to Benebo [4], the lack of toilet facility hence projects open defecation, which is mostly practiced by inhabitants and visitors to the Kingdom. Basically, well water have previously been reported contaminated with pathogenic microbes in the past [5]. Despite the reports, residents have no choice than to manage the water. However, steps taken to contain the challenge, continuously remains unsuccessful. Cholera is attributed with the consumption of Vibro cholerae infestation, the causative agent [6]. According to Dunkin [6], the infestation has been reported to cause death in some consumers of well water. Basically, several challenges associated with microbial contaminants in well water may be attributed to the proximity of the well to septic tanks, open drainages, leachates of dumpsites, flood, soil matter, and agricultural wastes [7]. Helard et al. [7] noted that inadequate disinfection of wells predisposes users to water borne diseases caused by a variety of pathogenic microbes. Thus, the presence Vibrio cholerae in water bodies in parts of Nigeria could be contaminated through water tributaries that recharge ground water(well) aquifers, hence bacteria and other microbes enter the tributaries through run-offs [8]. Pandey et al. [8] reported that several culture from well water have shown microbes could have contaminated water bodies through water tributaries. It has therefore become necessary to carry out an investigation in well water in Bille Kingdom, to evaluate there potability. Monitoring and managing bacterial contaminants are vital for preventing outbreaks and ensuring the safety of drinking water [9]. The study therefore, aimed at screening for Vibro cholerae in well water samples from Bille Kingdom of Degema Local Government Area of Rivers State, Nigeria, with a view of isolating, identifying and determining of the counts of Vibrio cholera in different water wells in Bille Kingdom.

2 MATERIALS AND METHODS

2.1 Study Area

The study area, Bille Kingdom is located on a latitude 4.5°N and longitude of 6.8°E in Degema Local Government Area of Rivers State, with a human population of 22, 204 as projected in 1996 [4]. It is an Ijaw speaking tribe and one of the Ancient Ijaw Kingdoms in the Eastern Niger Delta region. Water wells have been one of the major sources of water in this area outside bore-holes. The primary occupation of the Bille people is fishing. The fishing event is held like a festival and the ceremony done yearly to fosters unity in the community [4].

2.2 Water Sample Collection

Water sample were collected from Three (3) different water wells aseptically in a sterile 200 ml plastic bottles. The bottle caps were removed and immersed gently into the wells with the aid of a thread that was tied around the bottle. Following immersion, the bottle was drawn above the water gently without touching the sides of the wells and immediately covered [10]. The three commonly used wells were adopted for the study and coded as well A, B and C. Opu Imo Well was coded well A, Angular Polo and Osia Polo Wells were coded B and C respectively for the purpose of the study. After the water sample collections, the bottles were placed in cold boxes and transported to the Biology Laboratory, Ignatius Ajuru University of Education, Rumuolimeni Port-Harcourt, Rivers State within 4 hours for analysis.

2.3 Isolation of *Vibrio cholera* from Water Samples

2.3.1 Preparation of thiosulfate-citrate-bile salts-sucrose (TCBS) agar media

The preparation of the media as carried out by Dunkin [6], involved the preparation of thiosulfate-citrate-bile salts-sucrose (TCBS) agar culture media. Thus, 120ml of distilled water was measured and poured into conical flask containing 12g of thiosulfate-citrate-bile salts-sucrose (TCBS) agar. The flask containing the agar were thoroughly stirred to ensure the agars mixed properly with water. The opening of the conical flasks was sealed with cotton wool, foil and masking tape. The conical flask was then boiled and dispensed into petri dishes for further solidification of media and inoculation of the water sample.

2.3.2 Preparation of the water sample

The water samples were prepared for inoculation into the media by serial dilution. Thus, the sample was serially diluted to a 10-fold dilution. Approximately 9 ml of distilled water was dispensed into test tubes using a sterile pipette. Following this, 1 ml of the water sample was transferred into one of the tubes, to make the content 10 ml. thereafter, 1 ml was taken from the 10 ml tube into the another 9ml tube to get a 10-2 dilutions. The process continued accordingly till a 10-5 dilution is obtained for the investigation.

2.3.3 Enumeration of Vibrio spp. from the sample

Enumeration of the *Vibrio* spp. as documented by Obikpo et al. [10] using the spread plate technique involved the aseptically inoculation of the 10^{-5} sample dilution obtained via serial dilution into the freshly prepared TCBS media. The media after inoculation was incubated at 37°C for 24 hours. The colonies formed were counted and expressed as colony forming unit per mill (CFU/ml).

2.3.4 Morphological and biochemical characterization of Vibrio isolates

The isolates were characterized and identified based on their colonial morphological features such as; texture, color, shape, size and elevation. The biochemical test carried were as follows; catalase, indole, methyl red, Voges -Proskauer, citrate utilization, urease and sugar fermentation (glucose, lactose and sucrose) tests [11].

2.4 Experimentation and Testing

2.4.1 Sugar fermentation test

The sugar tested employed the use of glucose, sucrose and lactose in the right proportion accordingly. One gram (1g) either of the above stated sugar was added into eighty mill (80ml) of peptone water and stirred thoroughly to solubilize the sugar, thereafter twenty mill (20ml) of 0.2% (w/v) phenol red indicator was added to the sugar-peptone water solution. Following the addition, ten mill (10ml) of the sugar-peptone water solution was dispensed into test tubes contained in it inverted Durham's tubes and autoclaved at one hundred and twenty-one degrees centigrade (121°C) for fifteen (15) minutes. The test tubes were allowed to cool, and inoculated with the test organism. The tubes were then incubated at thirty-seven degrees centigrade (37°C) for twenty (24) hours. An orange color change of the medium signified a positive result for both fermentation and oxidation while the presence of bubbles in the Durham's tubes signified gas production [11].

2.4.2 Catalase test

In carrying out this test, a drop of hydrogen peroxide was placed on a clean microscope slide and with the aid of a sterile wire loop the test colony was introduced into the glass slide and the component smeared. Few seconds after the procedure, the slide was observed for bubbles. Catalase-positive bacteria are signified by the production of bubbles of oxygen [11].

2.4.3 Indole test

The indole medium, a composition of nutrient broth, peptone water and tryptone broth was freshly, prepared with the use of

autoclave at one hundred degrees Celsius (121°C) for fifteen (15) minutes and used for the test. Following the sterilization and subsequent dispense of the medium into test tubes. The test bacteria were then inoculated into the medium with the aid of a sterile wire loop and the medium incubate at thirty-seven degrees Celsius (37°C) for forty-eight (48) hours. After incubation, a red/pink layer formed on top of the broth indicated indole positive while a reverse of yellow indicated indole negative [11].

2.4.4 Methyl red

The test involved the use of MR/VP broth. The MR/VP broth was sterilized, allowed to cool before the isolate was inoculated. Following the inoculation, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Thereafter 5 to 6 drops of methyl red reagent were added to the broth, stirred thoroughly, and allowed to stand for 5 minutes. The broth culture was then examined for color change [11].

2.4.5 Voges proskauer

The test involved the use of glucose phosphate broth. The glucose phosphate broth was sterilized, allowed to cool before the isolate was inoculated. Following this, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Then, 1.5 ml of 5% alcoholic alpha napthtol and 0.5 ml of forty percent (40%) aqueous Potassium Hydroxide (KOH) was added and the test tubes, stirred thoroughly, and allowed to stand for 5 minutes. The test tubes were then examined for the appearance of pink color which denoted Positive Voges Proskauer test, while the formation of a yellow colored appearance indicated a negative result [11].

2.4.6 Citrate utilization test

This test was carried out to ascertain the ability of the isolate in the presence of bromothymol blue as an indicator to utilize citrate as its source of carbon and ammonium as its source of nitrogen. With the use of a sterile wire-loop the isolate was inoculated on the citrate medium and the medium incubated at thirty-seven degrees Celsius (37°C) for twenty-four (24) hours. A citrate positive result was indicated by a change in color of the indicator from green to blue [11].

2.4.7 Oxidase test

This test was done to detect the presence of the enzyme cytochrome oxidase. In carrying out the test, two to three drops of oxidase reagent were placed on a clean white filter paper, and with the aid of a sterile wooden applicator stick, the test bacteria were picked and inoculated on the saturated filter paper. After some seconds, an appearance of dark purple colour indicated a positive reaction whereas no discoloration indicated a negative oxidase reaction.

3 RESULTS

3.1 Enumeration of Bacteria Load in Well Water

Table 1, showed a mean *Vibrio cholerae* count in Well A, B and C. *Vibrio cholerae* had 0×10^5, 0×10^5 and 9.2×10^7 CFU/ml for Well A, B and C respectively.

Table 1 Mean Bacteria Count in Well A, B and C

Bacteria	Well A (CFU /ml)	Well B (CFU /ml)	Well C (CFU /ml)
Vibrio cholerae	0×10^5	0×10^5	9.2×10^7

Key: CFU/ml = Colony forming unit per mill, **Well A**= Opu Imo Well, **Well B**= Angular Polo Well, **Well C**=Osia Polo Well

3.2 Morphological Classification of the Isolates Recovered from the Well Water

Table 2 Showed the morphological characteristics of isolates recovered from well A, B and C. The colonies of *Vibrio cholerae* appeared with a yellow color and a distinctive comma shaped, curved, rod-like structure.

Table 2 Morphological Characteristics of the Isolates Recovered from the Water Samples

Bacteria	Color	Size	Shape	Texture	Elevation
V. cholera	Yellow	Small	Comma	Smooth	Low

3.3 Biochemical Classification of the Isolates Recovered from the Well Water

Table 3, showed biochemical reactions to identify *Vibrio cholerae*. catalase, oxidase, indole, methyl red, citrate, glucose, and sucrose tests all showed a positive reaction on inoculation of the test bacteria while Voges Proskaurer and Lactose showed a negative reaction.

Table 3 Biochemical Characterization of the Isolate Recovered from the Well Water

Bacteria	Oxidase	Catalase	Indole	MR	Citrate	Glucose	VP	Lactose	Sucrose
V. cholorae	+	+	+	+	+	+	-	-	+

Key; +=Positive, -=Negative

3.4 Frequency of *Vibrio cholerae* Prevalence Across the Wells

Table 4 showed the percentage frequency of prevalence of the *Vibrio cholerae* isolates recovered from the water samples. The bacteria, *Vibrio cholerae* were noted high in well C with a percentage prevalence of 100. Well A and B had no *Vibrio cholerae* occurrence.

Table 4 Bacterial Prevalence Across the Beverages

Media	Frequency of Prevalence	% Frequency of Prevalence
Well A	0	0
Well B	0	0
Well C	56	100

Key: Well A= Opu Imo Well, **Well B**= Angular Polo Well, **Well C**=Osia Polo Well

4 DISCUSSION

The study reports the presence of Vibrio spp. load exclusively in well C, with a concentration of (9.2×102cfu/ml), however there were no records of the Vibrio spp. in Well A and B. Well A and B may have not had Vibrio spp. counts due to the believe of the water wells in good quality devoid of fecal route [12]. The absence of Vibrio spp. in well A and B suggests the water facilities are consistently locked or less abused by inhabitance [13]. Basically, Obikpo et al. [10] study on bacteriological quality of community well water, could not isolate Vibrio spp. as an indicators of water quality. The rise in the Vibrio spp. count in well C, could be attributed to runoff water that enters some of the wells during raining seasons and particles from the environment which gain access into the wells from time to time. Bulus et al. [12] also noted that factors such as unhygienic practices, improperly covered wells, poor drainage system were wells are located and the proximity of water wells to septic tanks could be attributed to bacterial contaminated well water. Consequently, the presence of Vibrio spp. may also result from the horrendous sea erosion and regular flooding that occurs. In this context, following biochemical characterization and subsequent identity of the Vibrio spp. as Vibrio cholerae which well C possess, their presence is a significant indicator of potential health risks. Thus, the emergence of Cholera, caused by Vibrio cholerae, leads to severe diarrhea and dehydration, which can be fatal if untreated [14]. This level of contamination as reported in well C is particularly alarming as it suggests that consumers of this well water are at substantial risk of contracting cholera, highlighting the need for immediate remedial actions. The presence of Vibrio cholerae in well C suggest that the presence of human encroachment (fecal contamination) or environmental factors (due to natural contamination) acting as a carrier or might have been introduced by tourist [15]. The results from well C is in contrast with those of Okojokwu et al. [16], who did not detect Vibrio cholerae. The isolation of Vibrio cholerae from well C suggest localized issues of contamination that could differ from the broader trends reported in wells A and B. The findings of this study, particularly the isolation of Vibrio cholerae from well C, offer important insights into water quality and safety, and present a contrasting view to recent literature on the subject. The presence of Vibrio cholerae highlights a critical issue as it indicates potential fecal contamination and a health risk for consumers [16]. The findings of this study diverge from those of Obikpo et al. [10], who did not isolate Vibrio cholerae among their indicators of water quality. Their results suggest that, in their study area, well water might be free from Vibrio cholerae contamination. However, they still observed other microbial indicators of pollution, which underscores that Vibrio cholerae is not the sole bacterium of concern in water quality assessments [10]. In contrast, Agwaranze et al. [17] reported the isolation of Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris and Staphylococcus aureus from wells in Wukari, Taraba State, Nigeria. Thus, the emergence of Cholera, caused by Vibrio cholerae, leads to severe diarrhea and dehydration, which can be fatal if untreated, hence timely treatment is recommended [18].

5 CONCLUSION

The study revealed the prevalence and incidence of *Vibrio cholerae* in some wells in Bille kingdom. Although the prevalence of *Vibrio cholerae* in the well water samples was minute while in a few others high. Inhabitants of Bille Kingdom were the well C is located may be predisposed to infectious doses of *Vibro cholerea* when Well C is consumed.

6 RECOMMENDATIONS

The study therefore recommends a total close down of well C (Osia Polo well) as this could prevent further spread of cholera.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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