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Bacteriological analysis of potable water in areas with reported cholera outbreaks in Ogun, Oyo and Lagos States, Nigeria

Deji-Agboola MA*[1], Museliu O[1], Olajubu FA[2], Osinupebi OA[1], Idowu AO[3]

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, OlabisiOnabanjo University, Sagamu, Ogun State, Nigeria
[2]Department of Microbiology, AdekunleAjasin University, Akungba-Akoko, Ondo State, Nigeria
[3]Department of Haematology and Blood Transfusion, OlabisiOnabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria

*Correspondence: Dr. M. A. Deji-Agboola, Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, P.M.B 2022, Sagamu, Ogun State, Nigeria.
Tel: +234-8023722600; Email: mopelola.agboola@oouagoiwoye.edu.ng; mopeagboola@yahoo.co.uk;
ORCHID: http://orcid.org/0000-0002-8341-4545

Abstract

Background: Cholera outbreaks, reported yearly in Nigeria, are a public health concern and transmission is attributed to drinking of contaminated water and poor sanitation.
Objective: To conduct a bacteriological analysis of potable water in areas with reported cholera outbreaks in Ogun, Lagos and Oyo States.
Methods: Water samples collected from the study areas were examined physically, cultured in MacConkey broth and Alkaline peptone water to determine the most probable number (MPN) of Coliforms and isolate *Vibro* species respectively. Bacteria were cultured from the broths and were identified using standard methods.
Results: All the other water samples examined were colourless, odourless, tasteless and lack particles except the well water which had yellowish brown colouration (4; 8%) and contained visible particles (5; 30%). The presumptive total coliform count ranged between < 3 and 1,100 MPN /100ml. *Enterobacter gergoviae* (34; 47.2%), *Escherichia coli* (16; 22.2%), *Klebsiella pneumonia* (7; 9.7%), *Sarratia liquefaciens* (6; 8.3%), *Vibrio parahaemolyticus* (5; 6.9%), *Citrobacter diversus* (2; 2.8), *Klebsiella oxytoca* (1; 1.4%) *and Enterobacter aerogenes* (1; 1.4%) were isolated from 33.5% of the water samples. The level of contamination of water sources within the different study areas was significant ($p<0.001$). *Vibrio cholerae* was not isolated, but *Vibrio parahaemolyticus* was isolated from 5 (10%) of the well water samples. Thermo-tolerant Coliforms were also identified from some samples.
Conclusion: The well water samples were grossly contaminated with coliform bacteria and *Vibrio parahaemolyticus* and should be treated before drinking.

Key words: Coliform, Cholera, MPN, Water, Vibrio parahaemolyticus

Introduction

Enteric infections (such as Typhoid fever and bacterial diarrhoea) are the most frequent diseases associated with the consumption of contaminated water and are often food-borne. In many instances, enteric diseases are relatively mild, self-limiting and are often not reported. People living in poor sanitary conditions are at risk of diarrhoeal diseases including cholera.[1]

Cholera is an acute enteric infection acquired through the ingestion of water or food contaminated with

faeces containing the bacterium *Vibrio cholerae*. Infections have been attributed to poor sanitation and inadequate access to safe potable water. The effects of cholera disease are more severe in areas with lack of access to basic environmental infrastructures such as potable water supply. [2]

In West Africa, only Nigeria and Togo reported more cases of cholera in 2012 than the previous year. [3] The Federal Ministry of Health in Nigeria reported sporadic cases of cholera in September 2013, which continued throughout December 2013. [3, 4] In that Nigerian report of 2013, a total of 6,600 cases, including 229 deaths [case fatality ratio (CFR) of 3.47%] were recorded from 94 local government areas in 20 out of the 36 States. [3] In 2015, the trend increased globally; 1304 deaths were reported from 42 countries, resulting in an overall CFR of 0.8%. Out of this, West Africa recorded the highest burden and mortality rate with 84% cholera cases and 91% deaths. In addition, 5290 cases, including 186 deaths (with CFR of 3.5%), were recorded in Nigeria in the same year. [5]

Safe drinking water is described as surface water, treated or untreated, that is uncontaminated; this includes water from protected boreholes, springs and sanitary wells. [6] Potable water must be odourless, colourless, tasteless and free from pathogenic microbes as well as faecal and other chemical pollutants in harmful amounts. [6, 7] The annual reports of cholera outbreaks from different parts of the country in recent times, [3 - 5] have been attributed to the drinking of contaminated water and poor sanitation. [8]

This study was carried out to evaluate the bacteriological quality of sources of drinking water in some areas with reported cholera outbreaks in Ogun, Lagos and Oyo States of Nigeria. The most probable number of Coliforms present in each sample was enumerated and the contaminating bacteria were isolated and identified.

Methods

Study Design and Area

Water samples were collected from Ogun, Oyo and Lagos States in areas with reported cholera outbreaks between 2013 and 2015. Simple random sampling method was used to collect water samples between July and October, 2015 across five communities which included the following: Ijaye (in Abeokuta South Local Government, Ogun State); Ago-Ika (in Abeokuta North Local Government, Ogun State); Ajegunle (in Ajeromi-Ifelodun Local Government, Lagos State), Ijora-Badia, (in Apapa Local Government, Lagos State) and Egbeda (in Egbeda Local Government, Oyo State).

Collection of water samples

A total of 200 water samples, were collected from each of the five different study sites. These samples included 10 bottled water samples, 10 tap water samples, 10 borehole water samples and 10 well water samples.

Different brands of bottled water were purchased from which 10 were randomly picked for analysis. The Borehole and Tap water were collected aseptically in labelled 250ml sterile bottles. In the case of tap water, the sample was collected into pre-sterilized 250ml bottles containing five drops of 1.8% (W/V) of Sodium Thiosulphate solution (to neutralize any residual chlorine in the sample). [9] The well water samples were collected in weighted 250ml sample sterile bottles lowered with a string into the well to a depth of about one meter to draw the water sample. [9] All the samples were transported to the laboratory in a cooling flask containing ice packs and the samples were examined within six hours of collection.

Physical examination and bacteriological assessment of water samples

The water samples were examined physically for colour, odour, taste and particles. The bacteriological quality of the water samples was determined using the multiple tubes fermentation test. [7, 10] The three tubes Most Probable Number (MPN) method was used to estimate the total coliform count. Three sets of bottles (each set containing three bottles) were used. The first set of bottles contained double strength sterile MacConkey broth (Oxoid) (10ml each). Other sets of bottles contained single strength sterile MacConkey broth (10ml each). The water samples were thoroughly mixed and 10ml, 1ml, and 0.1ml were aseptically dispensed into the sterile MacConkey broth. All the tubes were incubated after inoculation at 35°C for 24 hours; all the negative tubes were further incubated over additional 24 hours before final observations were made.

Sterile distilled water was used as negative control and standard *E. coli* culture of density corresponding to 0.5 McFarland was used as positive control. The number of bottles showing the production of acid and gas were counted and recorded as positive. The MPN of coliform bacteria in 100ml of sample was

determined by referring to McCrady's probability table. [9] Five millilitres of the water samples were added to 5.0ml double strength sterile alkaline peptone water and this was incubated at 37°C for 6 hours. Thereafter, the bottles were sub-cultured onto Thiosulphate Citrate Bile Salt-sucrose (TCBS) agar and were incubated at 37°C for 24-48 hours. The plates were examined for yellowish and greenish coloured colonies after incubation.

Confirmation of thermotolerant coliform bacteria

All the positive presumptive tubes were inoculated into sterile MacConkey broth containing Durham tubes for incubation at 44°C for 48 hours. Acid and gas production confirms the presence of thermo-tolerant coliform bacteria. [10]

Identification of Isolates

All MacConkey broth tubes were sub-cultured onto MacConkey agar plates. Pure isolates were identified using cultural characteristics, Gram's reaction, Microbact 12E test kit, oxidase reaction and indole test.

Data analysis: The data were analysed using the SPSS 17.0 software in the form of proportions and percentages. The proportions were compared using the Chi-Square test and p-values less than 0.01 were considered significant.

Results

Physical examination

The results of the physical examination of water samples revealed that all the water samples examined were odourless and tasteless. All the bottled, borehole and tap water samples were colourless, tasteless, odourless and were free of particles. However, out of the 50 well water samples examined physically, 4 (8%) of the samples had yellowish brown colour. None of the well water sample from Ajegunle and Ago-Ika had abnormal colour. In addition, there were visible particles in 15 (30%) of the well water samples (Table I).

Table I: Physical characteristics of the well water samples from different sites

Sites	*Number of samples*	*Abnormal Colour*	*Presence of an Odour*	*Particles Present*	*Abnormal Taste*
Ago-Ika	10	0	0	3	0
Ijaye	10	1	0	4	0
Ajegunle	10	0	0	2	0
Ijora-Badia	10	1	0	3	0
Egbeda	10	2	0	3	0
Total	***50***	***4 (8.0%)***	***0 (0.0%)***	***15 (30.0%)***	***0 (0.0%)***

Bacteriologic analysis

The presumptive total coliform count of the water samples using the Most Probable Number (MPN) ranged between < 3 and 1,100 MPN/100ml. Well water samples (1,100 MPN/100ml) had the highest presumptive total coliform count. Out of the 200 water samples examined, 67 (33.5%) showed bacterial growth (Table II).

Table III shows the percentage of bacterial contaminations within each study area. Although, there was no statistically significant difference in the level of bacterial contaminations (p = 0.341, p = 0.348, p = 0.554 and p = 1.000 for borehole water, tap water, bottled water and well water sample, respectively) of different water sources, the differences in the level of bacterial contamination between the different study sites were statistically significant (P<0.001) (Table IV). A total of 72 isolates were obtained from the water samples and the distribution of these isolates showed the following: *Enterobacter gergoviae* (34; 47.2%), *Escherichia coli* (16; 22.2%), *Klebsiella pneumoniae* (7; 9.7%), *Sarratia liquefaciens* (6; 8.3%), *Vibrio parahaemolyticus* (5; 6.9%), *Citrobacter diversus* (2; 2.8%), *Klebsiella oxytoca* (1; 1.4%), *Enterobacter aerogenes* (1;1.4%).

Vibrio parahaemolyticus was isolated from 5 (10%) of the well water samples (2 from Ago-Ika and the remaining 3 from Ijaye). The coliform bacteria isolated from the samples were confirmed to be thermo-tolerant except *Sarratia liquefaciens and Vibrio parahaemolyticus*. The well water samples were contaminated with thermo-tolerant (faecal) Coliforms (*K. pneumoniea, K. oxytoca, E. gergoviae, E. coli,* and *C. diversus*) while *E. coli* was the only thermo-tolerant isolate identified in a borehole water sample and 2 tap water samples.

Table II: The MPN index per 100ml of Water samples and the frequency of bacterial growth

Water samples	*Total number of water samples*	*Frequency of MPN/100ml bacterial growth [n (%)]*		*Samples with MPN/100ml < 3*
Bottled water	50	2 (4)	< 3-29	48
Tap water	50	6 (12)	< 3-27	44
Borehole water	50	9 (18)	< 3-35	41
Well water	50	50 (100)	210 - 1,100	0
Total	***200***	***67 (33.5)***	***<3-1,100***	***133***

Table III: Comparison of the level of contamination of water sources according to the sites of collection

Source	*Sites*	*Number of Samples*	*Number and Percentage contamination*	X^2	*P values*
Well water	Ago-Ika	10	10 (100%)	0.000	1.000
	Ijaye	10	10 (100%)		
	Ajegunle	10	10 (100%)		
	Ijora-Badia	10	10 (100%)		
	Egbeda	10	10 (100%)		
	Total	50	50 (100%)		
Borehole water	Ago-Ika	10	0 (0%)	4.515	0.341
	Ijaye	10	1 (10%)		
	Ajegunle	10	3 (30%)		
	Ijora-Badia	10	2 (20%)		
	Egbeda	10	3 (30%)		
	Total	50	9 (18%)		
Tap water	Ago-Ika	10	2 (20%)	4.455	0.348
	Ijaye	10	0 (0%)		
	Ajegunle	10	0 (0%)		
	Ijora-Badia	10	2 (20%)		
	Egbeda	10	2 (20%)		
	Total	50	6 (12%)		
Bottled water	Ago-Ika	10	0 (0%)	3.062	0.547
	Ijaye	10	1 (1%)		
	Ajegunle	10	0 (0%)		
	Ijora-Badia	10	0 (0%)		
	Egbeda	10	1 (10)		
	Total	50	2 (4%)		

Discussion

Water is described as polluted when it contains substances or impurities which distort its physical and microbiological qualities. [7] This study showed that, most samples of well water were odourless and tasteless, but 8% had yellowish brown discolouration while 30% contained visible particles. Colour has been described as an important physical quality of water which affects its acceptability by consumers. [11] The colour of potable water is mostly due to the presence of coloured organic matter, the presence of iron and other metals, as natural impurities. [12] Abnormal colour may also result from the contamination of the water source with industrial effluents and this may be the first indication of a hazardous situation. [12]

Most of the well water sampled were observed to be either poorly covered or left uncovered and the surroundings were dirty in some cases. All the well water samples were heavily contaminated with coliform bacteria. The presumptive coliform counts of well water samples in this study were higher than the permissible Nigerian standards for potable water. [13] Although, coliform bacteria are not generally considered risky to health, infections due to Coliforms may be fatal in infants, the elderly and the immune-compromised individuals. [13]

Table IV: Comparison of the level of contamination of different water sources within sites of collection

Sites	*Sample description*	*Number of samples*	*Percentage contamination*	X^2	*P values*
	Ago-Ika Well water	10	10 (100%)	31.571	<0.001
	Borehole water	10	0 (0%)		
	Tap water	10	2 (20%)		
	Bottled water	10	0 (0%)		
Ijaye	Well water	10	10 (100%)	30.643	<0.001
	Borehole water	10	1 (10%)		
	Tap water	10	0 (0%)		
	Bottled water	10	1 (10%)		
Ajegunle	Well water	10	10 (100%)	29.667	0.000
	Borehole water	10	3 (30%)		
	Tap water	10	0 (0%)		
	Bottled water	10	0 (0)		
Ijora-Badia	Well water	10	10 (100%)	25.286	0.000
	Borehole water	10	2 (20%)		
	Tap water	10	2 (20%)		
	Bottled water	10	0 (0%)		
Egbeda	Well water	10	10 (100%)	16.939	0.001
	Borehole water	10	3 (30%)		
	Tap water	10	2 (20%)		
	Bottled water	10	1 (10%)		

E. coli or thermo-tolerant coliform bacteria are not supposed to be present in any 100ml of sample of water intended for drinking. [13] The well water samples in the present study, contained thermo-tolerant (faecal) coliform bacteria (*K. pneumoniea, K. oxytoca, E. gergoviae, E. coli,* and *C. diversus*) indicating that these water samples were faecally contaminated and thus, unsafe for human consumption. This finding agreed with the report of Akinyemi *et al.*, which showed gross contamination of well water samples with *E. coli* and other bacterial pathogens in Lagos State. [14] Similarly, Ayantobo *et al.* reported that hand-dug wells were highly contaminated with *E. coli* in another study conducted in Ibadan, Oyo State. [15]

It is important to note that, some of the wells lacked protective coverings, and the containers used for drawing water from the wells were carelessly placed on the ground or where dirty stagnant water was observed in the immediate surroundings of the wells. These observations could have contributed to the high level of contamination of the wells. Furthermore, this study was conducted during the rainy season and this could also have been a contributing factor to the high level of contamination. Poor design and construction of wells may result in the draining of polluted surface water into the ground water, without filtering through the soil, thus contaminating the well water. [15, 16]

Vibrio cholerae was not isolated in this study. However, 10% of the well water sampled contained *V. parahaemolyticus.* The latter organism naturally inhabits marine and estuarine environments and is known to cause gastroenteritis, wound infections, and septicaemia. [17] *V. parahaemolyticus* has been recognized as a common cause of food-borne illness in the world. [17] It is attractive to attribute the presence of *V. parahaemolyticus* to the proximity between contaminated wells to major flowing water bodies such as streams and the Ogun River. Indeed, environmental waters are known to support the growth of *V. parahaemolyticus*. [18]

Although, some of the bottled water samples (4%) were contaminated with coliform bacteria, none contained thermo-tolerant *E. coli* and *Vibrio spp.* Thermo-tolerant *E. coli* was identified in few tap water and borehole water samples. Burst pipes laid in dirty drainages were observed in these areas and may probably be responsible for the observed level of contamination of tap water samples. The contamination of potable water has been linked with pipe leaks, damaged valves, laying of pipes in poorly drained gutters and insufficient chlorination. [15, 19, 20]

Conclusion

The sources of potable water in areas with reported cholera outbreaks (Oyo, Ogun and Lagos States) revealed that all well water samples were grossly contaminated and did not conform with the standards recommended by the WHO for safe drinking water. No *Vibrio cholerae* was isolated from the samples of water studied, but *Vibrio parahaemolyticus* were isolated from some of the well water. Though, few of the bottled, tap and borehole water analysed were contaminated, the finding in this study showed that they were more reliable for drinking as they were mostly free of coliform bacteria. While constant monitoring of the sources of drinking water in the study areas is important, there is also a need to scale up preventive measures including adequate protection and treatment of water sources and improved sanitation in order to avert cholera and other water borne diseases.

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