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ASSESSMENT OF BACTERIOLOGICAL QUALITY AND IDENTIFICATION OF CHLORINE-TOLERANT BACTERIA FROM DIFFERENT POTABLE WATER SOURCES IN GIREI, NIGERIA

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Domestic water sources such as wells, taps, and rivers are often used for drinking, cooking, and other household purposes, particularly in rural and peri-urban areas however in most places, their microbiological quality is not always ensured. In this study, 45 water samples were collected from key domestic water sources; 20 each from wells and taps, and 5 from River Benue in Girei, Nigeria following standard sampling techniques. Total aerobic bacterial count (in CFU/mL) for each sample was determined and river water had the highest average microbial count (285), followed by well water (248), and tap water had the lowest count (181). Identification of isolates involved cultural characteristics, Gram staining, motility, and biochemical tests. Bacterial species associated with the water sources were *Acinetobacter* sp., *Aeromonas* sp., *Bacillus subtilis, Citrobacter, Escherichia coli, Enterococcus faecalis, Enterobacter* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus* sp., *Providencia* sp., *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella* sp. and *Serratia* sp.. Chlorine tolerant isolates were determined after treating each sample with 9.9 mg/L (9.9 ppm) of Sodium hypochlorite (NaOCl) at a contact time of 30 minutes. They include 13 isolates from 9 genera, viz: *Acinetobacter* sp., *B. subtilis, Citrobacter* sp., *E. coli, Enterobacter* sp., *K. pneumoniae, P. aeruginosa, Proteus* sp. and *Serratia* sp. By subjecting the treatment tolerant isolates to higher concentrations of NaOCl, results indicated that high concentrations are required to neutralize the isolates with the highest bactericidal concentration observed in *B. subtilis* (70 ppm), and the lowest was 40 ppm observed in *Serratia* sp. and *E. coli*. The findings highlight significant bacterial contamination in water sources, some of which are pathogenic and chlorine-tolerant, emphasizing the need for proper water treatment methods, such as boiling, before consumption by residents.

Keywords: Bacterial count (CFU/mL), Chlorine tolerance, Contact time, Sodium hypochlorite, Water samples

INTRODUCTION

The use of water in human's daily life is quite indispensable. However, water can serve as a home for a diverse number of bacteria along with other microorganisms some of which may be pathogenic. These pathogens can be directly transmitted to the consumers if the water is not properly treated (1). In many regions of the world, chlorine is the most extensively used portable water disinfection technique. This technique has demonstrated exceptional efficacy against various microorganisms, such as molds, yeasts, protozoans, spore-forming bacteria, and viruses (2). The widespread use of chlorination has been credited to its ease of use and excellent results in significantly reducing the overall number of microorganisms in water, which helps to prevent the spread of illnesses like cholera, salmonellosis, shigellosis, and typhoid and paratyphoid fevers (3). Although chlorination has been praised for its efficacious water treatment, new research has demonstrated that it is not successful in eliminating certain types of bacteria from water (1, 4, 5). According to multiple research (6, 7), there is a growing concern for public health from the growth of bacteria that can withstand chlorine in drinking water sources.

Chlorine-resistant bacterial isolates encompass both Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Serratia* spp, *Micrococcus varians*, *Aeromonas hydrophilla*, *Rickettsia* and *Legionella* spp among others (1,4,7). Consequent to this, several health problems have been encountered with regard to waterborne diseases such as cholera, typhoid fever and dysentery among others (8-11).

Because chlorine has antibacterial properties by nature, some mechanisms may allow these bacteria to survive in water with comparatively high chlorine concentrations (12-14). Concern has been raised globally about certain bacteria's resistance to the effects of chlorination (4). The main objective of this study was to assess the bacteriological quality and identify chlorine-tolerant bacteria from different sources of potable water utilized in Girei, Nigeria.

Chlorination of major portable water supplies utilized for drinking and other domestic purposes in Girei Local Government Area, Adamawa State, has poorly been employed. Studies on the bacteriological quality of well water which has been utilized around the L.G.A. have shown a substantial level of contamination with bacteria including the enteric types (15).

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MATERIALS AND METHODS

Study area: The study was conducted in Girei, Adamawa State, North-East Nigeria. Girei is located between Latitude 9° 22' 11.83" N and Longitude 12° 33' 0.74" E (18,19). It has an estimated population of 200,200 and covers a land area of about 1040 km^2 with a population density of 192.5/km^2 as of 2022 (20, 21). Main water sources include public and private wells and hand pump boreholes (22).

Sample collection: A total number of 45 water samples (20 each from taps and wells and 5 from along River Benue) were collected around Girei Local Government Area, Adamawa State between December 2022 and April 2023. Samples were collected in sterile 1 liter screw-caped sampling plastic bottles. All samples were placed in an ice-packed cooler as suggested by Mabvouna et al. (23) and transported to the laboratory for further analysis.

Sample processing, culture and enumeration of bacteria: Upon arrival at the laboratory, the samples were inoculated using the spread plate method by aseptically dispensing 0.1 mL of each on plate count agar (PCA) and MacConkey agar (MCA). All plates were incubated at 37ºC for 24 hours.
Total aerobic plate count (TAPC) and total coliform count (TCC) were determined on nutrient agar and MacConkey agar respectively as suggested by Chikodili et al. (21).
The bacterial count on each plate was determined using the relation below:
Bacterial count (Colony Forming Units per Milliliter: CFU/mL) = $\frac{n}{v}$
Where n: is the number of microbial colonies counted on the plate.
v: is the volume of the diluted sample dispensed on petri dish (0.1 mL).

Isolation, characterization and identification of bacteria: After 24 hours of incubation, the cultural characteristics of the bacterial colonies on each plate were observed. Morphologically distinct colonies from each sample were subcultured on fresh nutrient agar plates to obtain pure cultures. All the isolates were further identified based on Gram reaction and biochemical characterizations viz; methyl-red, Vorges-Proskauer, Indole, Citrate utilization, catalase, oxidase, urease, hydrogen sulphide production and coagulase as suggested by Standard Operating Procedures (SOP) Bacteriology by Indian Council for Medical Research (25).

Biochemical Tests:
Citrate Utilization Test: Each isolate was inoculated onto Simmons' citrate agar and incubated at 37°C for 24 hours. A positive result was indicated by a color change in the medium from green to blue.
Catalase Test: A small amount of a well-isolated 24-hour colony was transferred onto a clean slide, and 2-3 drops of 3% hydrogen peroxide (H_2O_2) were added. The immediate formation of bubbles signified a positive test.
Sulfide, Indole, and Motility (SIM) Tests: A sterile inoculation needle was used to stab a portion of a well-isolated 24-hour colony into SIM medium, containing ferric ammonium citrate, sodium thiosulfate, tryptone, and yeast extract. The tubes were incubated at 37°C for 24 hours. Motility was indicated by diffused growth or a hazy appearance around the stab region, sulfide production by the presence of black precipitate, and indole production by the appearance of a red color after the addition of a few drops of Kovac's reagent (5% p-dimethylaminobenzaldehyde in 95% hydrochloric acid).
Urease Test: Each isolate was inoculated into urease test medium, which contained urea, phenol red, and yeast extract, using a sterile inoculation needle, and incubated at 37°C for 24 hours. A positive result was indicated by a pink to red coloration in the medium.
Methyl-Red and Voges-Proskauer (MR-VP) Tests: Each isolate was inoculated into MR-VP broth, which contained glucose, peptone, and potassium phosphate, and incubated at 37°C for 24 hours. The broth was then divided into two sterile tubes. For the methyl red test, 2-3 drops of methyl red indicator were added to one tube, with a positive result indicated by the production of a red or pink color. For the Voges-Proskauer test, 2-3 drops of alpha-naphthol and KOH were added to the second tube, with a positive result also indicated by a red or pink color in the medium.
Coagulase Test: A drop of human plasma was added to a clean slide, followed by a loopful of a 24-hour bacterial broth culture. The mixture was emulsified, and a positive result was indicated by visible clumping within 10-15 seconds.
Oxidase Test: A portion of a well-isolated colony was emulsified on a clean filter paper, and a few drops of oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) were added. A color change to purple indicated a positive result.

Treatment of Water Samples with NaOCl: Using the USEPA (26) standard of 0.25 teaspoon of 6% NaOCl for 2 gallons of water, and considering 1 teaspoon equals 5mL (27), we arrived at 0.625 mL of 6% NaOCl per gallon of water sample. Similarly, considering 1 gallon as 3.785 liters, 165 µL of 6% NaOCl was added to 1 liter of each water sample and mixed by swirling. This yielded final NaOCl concentration of ≈ 9.9 mg/L (9.9 ppm) in each bottle. All the bottles were allowed to stand for a contact time of 30 minutes as recommended by WHO (28), Lantagne (27) and USEPA (23).

Identification of Tolerant Bacteria to NaOCl Treatment: After 30 minutes of contact with NaOCl, each of the water samples was swirled and 0.5 mL from each was spread on freshly prepared nutrient agar and MacConkey agar plates and the excess water was decanted. All plates were incubated at 37ºC for 24 hours and observed for growth. Bacterial colonies were subcultured and identified using Gram staining and the same biochemical tests as described earlier.

Determination of the Minimum Bactericidal Concentration of NaOCl: The chlorine-tolerant isolates were further subjected to a higher concentration of NaOCl in sterile phosphate-buffered saline as suggested by Owoseni et al. (29).
The turbidity of each isolate was adjusted to 0.5 MacFarland standard (1.5×10^8 cells/mL) using sterile phosphate buffered saline as a diluent.
Different concentrations of NaOCl ranging from 10 to 50 mg/mL were prepared in 10 mL in sterile test tubes with each volume deficient of 100 µL such that the addition of 100 µL microbial suspension will make a volume of 10 mL and final cell density of 10^6 cells/mL The tubes were incubated at room temperature for 30 minutes and were sub cultured on NA plates. The plates were incubated at 37ºC for 24 hours and observed for growth.
The lowest concentration of the disinfectants that produced no growth on the agar surface after 24 hours of incubation was regarded as its MBC against the test organism (30).

RESULTS

Average Aerobic Plate Count from the Water Sources

The results revealed varying aerobic plate counts across different water sources. River water samples exhibited the highest aerobic plate count, with a mean count of 285 CFU/mL, followed by well water samples, showing a mean aerobic plate count of 248 CFU/mL. Tap water samples exhibited the lowest mean count at 181 CFU/mL (Figure 1).

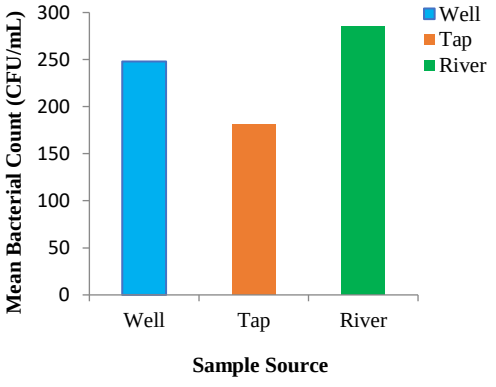

Figure 1. Average Aerobic Plate Count from the Water Sources.

Table 1: Cultural, microscopic and biochemical properties of the Bacterial Isolates.

S/N	Culture		G. Stain/ Microscopy	Biochemical Tests										Isolates
	NA	MCA		Ci	Ca	H_2S	In	Ur	MR	VP	Co	Ox	Mo	
1	Circular gray Colonies	NLF	- Rods	+	+	+	+	+	-	+	-	+	+	*Acinetobacter* sp.
2	Smooth, round, convex, small colonies with entire margin.	NLF	- Rods	+	+	+	+	-	+	-	-	+	+	*Aeromonas* sp.
3	Large, rough, white irregular colonies	NG	+ Rods	+	+	-	-	-	+	+	-	+	+	*B. subtilis*
4	Smooth, convex, pale-yellow colonies	LF	- Rods	+	+	+	+	±	+	-	-	-	+	*Citrobacter* sp.
5	Circular, convex, smooth, milky colonies	LF	- Rods	-	+	-	+	-	+	-	-	+	+	*E. coli*
6	Small, round, convex, pale-yellow colonies	NG	+ Cocci in Pairs	-	-	-	-	-	-	+	-	-	-	*E. faecalis*
7	Large, white, mucoid, convex, colonies	LF	- Rods	+	+	-	-	-	-	+	-	-	+	*Enterobacter* sp.
8	Large, white, mucoid, colonies	LF	- Rods	+	+	-	-	+	-	+	-	-	-	*K. pneumoniae*
9	Large, greenish-yellow, flat, irregular margin colonies	NLF	- Rods	+	+	-	-	+	-	-	-	+	+	*P. aeruginosa*
10	Large, flat, white and spreading colonies	LF	- Rods	+	+	+	-	+	+	-	-	-	+	*Proteus* sp.
11	Small, convex, cream-coloured, smooth colonies	LF	- Rods	+	+	-	+	+	+	-	-	-	+	*Providencia* sp.
12	Large, round convex, golden yellow colonies	NG	+ Cocci	-	+	-	-	+	+	+	+	-	-	*S. aureus*
13	Small, white, round, convex colonies	NG	+ Cocci	-	+	-	-	-	+	±	-	-	-	*S. epidermidis*
14	Small, round, white, convex colonies	NLF	- Rods	+	+	+	-	-	+	-	-	-	+	*Salmonella* sp.
15	Small, round, convex, white colonies	LF	- Rods	+	+	+	-	+	-	+	-	+	+	*Serratia* sp.

Note: Ci, citrate utilization; Ca, catalase; H_2S, sulfide; In, indole; Ur, urease; MR, methyl-red; VP, Vorges-Proskauer; Co, Coagulase; Ox, oxidase; Mo, motility; NA, nutrient agar; MCA, MacConkey agar; LF, lactose fermenter; NLF, non-lactose fermenter; NG, no growth; +, positive; -, negative; ±, variable.

Table 2: Determination of Bactericidal Concentration of NaOCl against Tolerant Isolates.

S/N	Chlorine Isolates	Source	MBC of NaOCl (ppm)
1	*Acinetobacter* sp	River	50
2	*B. subtilis*	River	60
3	*B. subtilis*	Tap	70
4	*Citrobacter* sp	Well	50
5	*E. coli*	River	40
6	*Enterobacter* sp	Well	50
7	*Enterobacter* sp	River	40
8	*K. pneumoniae*	Tap	50
9	*K. pneumoniae*	Well	40
10	*P. aeruginosa*	River	60
11	*P. aeruginosa*	Tap	60
12	*Proteus* sp	Tap	60
13	*Serratia* sp	Well	40

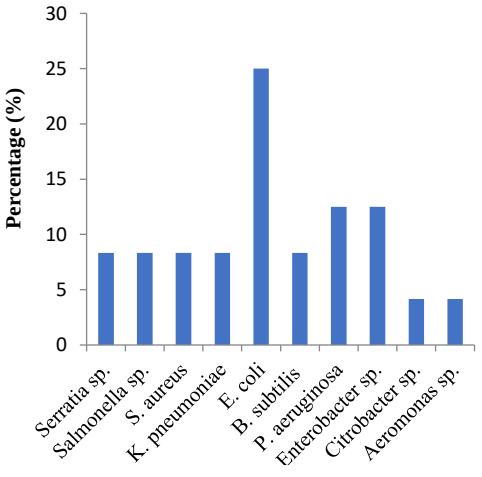

Figure 2: Occurrence of bacteria in well water samples.

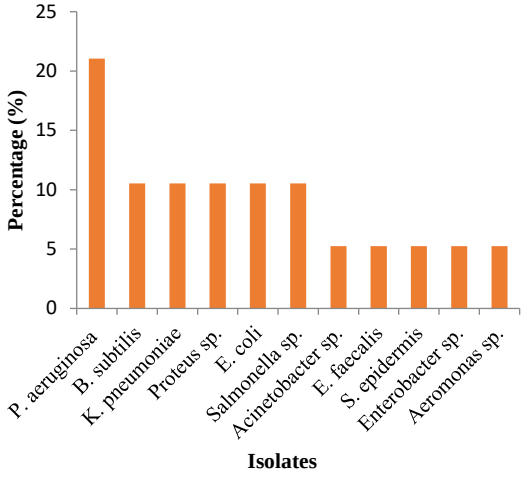

Figure 3: Occurrence of bacteria in tap water samples.

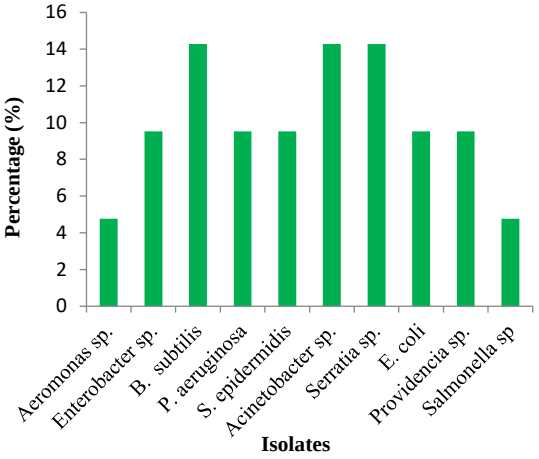

Figure 4: Occurrence of Bacteria in River.

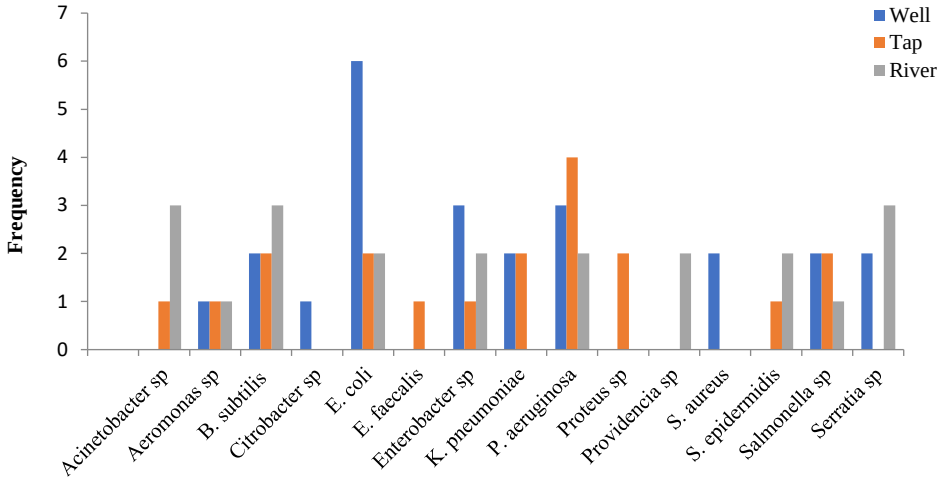

Figure 5: Distribution of bacterial species across the water sources.

Occurrence of Bacterial Species in Well Water Sample

A total of 24 bacterial isolates were identified from tapwater samples. Among these, *E. coli* demonstrated the highest occurrence at 25%, followed by *P. aeruginosa* and *Enterobacter* sp., both at 3% each. Subsequently, *Serratia* sp., *Salmonella* sp., *S. aureus*, *K. pneumoniae*, and *B. subtilis* each accounted for 8.33%. Finally, *Citrobacter* sp. and *Aeromonas* sp. showed the least occurrence, each at 4.17% (Figure 2 and 5).

Occurrence of Bacterial Species in Tap Water Samples

Nineteen isolates were identified from tap water samples. *P. aeruginosa* exhibited the highest occurrence at 21.05%, followed by *B. subtilis*, *K. pneumoniae*, *Proteus* sp., *E. coli*, and *Salmonella* sp., each at 10.53%. The lowest occurrences were observed in *Acinetobacter* sp., *E. faecalis*, *S. epidermidis*, *Enterobacter* sp., and *Aeromonas* sp., each at 5.26% (Figure 3 and 5).

Occurrence of Bacteria in River Water Samples

Twenty-one isolates were identified from river water samples. *B. subtilis*, *Serratia* sp., and *Acinetobacter* sp. demonstrated the highest frequency, each at 14.29%. Following them were *Enterobacter* sp., *P. aeruginosa*, *S. epidermidis*, *E. coli*, *Providencia* sp., each at 9.52%. Lastly, *Aeromonas* and *Salmonella* sp. exhibited the lowest occurrence, each at 4.76% (Figure 4 and 5).

Treatment Tolerant Isolates and their Minimum Bactericidal Concentrations (MBC)

Thirteen bacterial isolates arising from 9 different genera survived conventional treatment with NaOCl at 9.9 mg/L (9.9 ppm). These included *Acinetobacter* sp. (from river), two *B. subtilis* (from river and tap samples), *Citrobacter* sp. (from the well), *E. coli* (from river), two *Enterobacter* sp. (from well and river), two *K. pneumoniae* (from tap and well samples), two *P. aeruginosa* (from river and tap), *Proteus* sp. (from tap), and *Serratia* sp. (from well).

To determine the MBC, the isolates were exposed to higher concentrations of NaOCl. The results indicated that *B. subtilis* from river samples was the most resistant isolate with an MBC of 70 ppm, followed by *B. subtilis* (well), two *P. aeruginosa* (river and tap), and *Proteus* sp. (from tap), all with an MBC of 60 ppm. *Acinetobacter* sp. (river), *Citrobacter* sp. (well), *Enterobacter* (well), and *K. pneumoniae* (tap) each exhibited an MBC of 50 ppm. Isolates with the lowest MBC were *E. coli* (river), *Enterobacter* sp. (river), *K. pneumoniae* (well), and *Serratia* sp. (well), all with an MBC of 40 ppm.

DISCUSSION

The ability of some bacteria to withstand the effect of disinfectants and water treatment chemicals particularly, chlorine and chlorine-containing compounds at concentrations used in water treatment has posed great concern. In this study, bacterial isolates from water samples collected from three major sources of domestic water viz: wells, River Benue and taps around Girei Local government area of Adamawa state were tested for susceptibility and tolerance to sodium hypochlorite (NaClO) treatment.

Bacterial counts across all the water sources have been determined to be in relatively substantial numbers (Figure 1). Aerobic plate count of bacteria in river water was the highest in number (285 CFU/mL), followed by well (248 CFU/mL) and lastly, tap water samples (181 CFU/mL). This is attributed to the fact that river water is more prone to contamination with different biological wastes emanating from different sources including animal and human excreta, flow of both untreated sewages and run-offs from the surrounding lands. Similarly, Rodriguez-Tapia and Morales-Novelo (31) also reported that river water is open and prone to contamination mainly due to environmentally unfriendly human activities such as discharge of waste water from industrial and agricultural wastes and untreated domestic sewages which add to their high microbial numbers.

Wells that are properly located and constructed are expected to be free or have very low bacterial counts. However, results from this study indicated that well water samples were observed to have high microbial counts. Most wells around the study area are open wells which exposes them to different sources of contamination from the surrounding environment. Similarly, the conventional practice of using a fetcher to draw water from the wells makes it easy to introduce bacteria to the well water especially, when the handlers fail to adhere to proper hygiene. A report from the Wisconsin Department of Natural Resources, Bureau of Drinking Water and Groundwater (32), indicated that well water particularly, those without adequate caps or seals usually become contaminated with bacteria from different sources such as rodents, reptiles and insects. They also added that several strains of bacteria can have their way into the underground water by moving through coarse soils, hallow fractured bedrocks, and can access water in adequately grounded wells or wells with cracks in the wall casing.

Tap water samples had fewer bacterial counts compared to river and well water samples. This is due to their less vulnerability to sources of contamination as they are usually drawn from deep ground and stored in protected water tanks. However, bacteria can access and contaminate the water if tanks are not well sealed or if there is a breach along the pipelines.

Bacterial species associated with the water samples include *Acinetobacter* sp., *Aeromonas* sp., *B. subtilis, Citrobacter, E. coli, E. faecalis, Enterobacter* sp., *K. pneumoniae, P. aeruginosa, Proteus* sp., *Providencia* sp., *S. epidermidis, Salmonella* sp. and *Serratia* sp. (Table 1 and Figure 5). Consistent to the results of this study, the report from Pindi et al. (33) also highlighted *Acinetobacter, Aeromonas, P. aeruginosa, Citrobacter,* and *Bacillus* spp. among the bacterial isolates commonly isolated in drinking water. Similarly, studies by Prevost et al. (34) and Mathias et al. (35) also identified *Aeromonas* sp., *Salmonella* spp. along with other bacterial isolates as aquatic pathogens commonly associated with domestic water supplies. Another study by Hayward et al. (36) highlighted the presence of some of the bacteria identified in this study in domestic water samples including, *Bacillus* spp., *Enterobacter, Enterococcus* spp., *E. coli* strains, *Salmonella* spp., *Staphylococcus* spp. and *Serratia* spp. The presence of the bacterial isolates in drinking water samples, especially the enteric types is an indication of fecal contamination and this poses great threat to public health. Additionally, a study conducted in Bareilly, India by Singh et al. (37) also reported occurrence of *Proteus* spp., *Citrobacter* spp., *Providencia* spp., *Klebsiella* spp., *Enterococcus* spp. and *S. aureus* among the most frequently isolated pathogens in drinking water. Similarly, Stevens *et al*. (38) also reported that domestic water is contaminated with so many bacteria which include the coliform family which is the prime indicator of faecal contamination. Contamination of water by bacteria is a major problem in rural areas (39, 40). The consumption of water with the presence of many pathogenic microbes of fecal origin is a major risk to the health of human beings (12).

The presence of indicator bacteria, such as *E. coli*, is used for assessing microbial water safety. According to the World Health Organization's (WHO) 2022 guidelines, drinking water is considered safe only if it has zero *E. coli* and total coliform bacteria counts per 100 mL (41). While heterotrophic plate count (HPC) is not a direct indicator of health risk, the guidelines also suggested that the level should be as low as possible to ensure overall water safety. Our analysis revealed that none of the water sources met the WHO's criteria for safe drinking water, given their average bacterial counts and presence of *E. coli* and other coliform bacteria, posing significant health concerns.

The presence of chlorine tolerant bacteria in potable

water has been a major concern globally. Sodium hypochlorite is usually used at concentrations ranging from 2 ppm to 10 ppm in most water treatment approach (4, 29, 42-46). The amount required is also dependent on the level of contamination of the water being treated. On accessing the water samples for chlorine tolerant bacteria by treatment with NaOCl (9.9 ppm), a total of 13 isolates comprising of 9 species across the different water sources were identified viz: *Acinetobacter* sp., *B. subtilis*, *Citrobacter* sp., *E. coli*, *Enterobacter sp., K. pneumoniae, P. aeruginosa, Proteus* sp. and *Serratia* sp. A consistent result was also reported from a study conducted by Gupta et al. (13) where they identified *Acinetobacter* spp., *Citrobacter, E. coli, Enterobacter* spp., *K. pneumoniae, P. aeruginosa* and *Serratia* spp. among chlorine tolerant isolates associated with treated water samples. The resistance by *Bacillus* spp. to chlorination was also reported by Luo et al. (11). This ability of bacteria to resist chlorine is a public health concern.

To determine the bactericidal concentration of NaOCl, the isolates chlorine-tolerant isolates were further subjected to higher concentrations of chlorine maintaining a contact time of 30 minutes as recommended by WHO (28), Lantagne (24) and USEPA (26). The isolates exhibited different killing concentrations with *B. subtilis* from tap water samples having the highest MBC (70 ppm). Members of the genus *Bacillus* are known as aerobic spore formers and this feature gives them the advantage to resist and survive adverse environmental conditions including treatments with chemicals and antibiotics. A consistent report from the work of Martins et al. (14) who accessed the chlorine resistance profile of different *Bacillus* strains indicated that the isolates were highly resistant to chlorine accounting for 8 out of 12 isolates survived up to 1000 ppm of chlorine at 30 minutes contact time. Upon subjecting them to higher concentrations of chlorine, they also found out that 7 of them survived 5000 ppm at 10 minutes. Same authors also stated that the resistance of *Bacillus* spp. endospores to toxic substances and adverse environmental conditions could be due to several factors including external layer of protection and the relative impermeability of the internal spore membrane. *Pseudomonas aeruginosa* and *Proteus* sp. were also inactivated at 60 ppm. Other isolates were inactivated at viz; *Citrobacter* sp., *Acinetobacter* sp. and *Enterobacter* sp. were inactivated at 50 ppm whereas *Serratia* sp, *E. coli* and one *K. pneumoniae* isolates were inactivated at 40 ppm. Compared to the standard for chlorination of domestic water sources using NaOCl (i.e. ranging from 2 -10 ppm), these killing concentrations observed are incredibly high. A similar finding was also reported by Al-Berfkani et al. (4) who reported some bacterial isolates including *Aeromonas* spp. survives chlorine even at 100 ppm at different contact times.

The mechanism of action of chlorine on bacteria is mainly attributed its ability to interact with the cell membrane lipids, proteins and nucleic acids resulting in oxidative damage to these cellular components (46). Similarly, Gupta et al. (12) stated that the major targets of reactive chlorine species on bacterial cells are sulfur containing amino acids and subsequent protein denaturation. Reports also highlighted that reactive chlorine species (RCS) reacts with the DNA forming chlorinated nucleic acids and double-strand breakage which is lethal to the bacterial cells (12, 13). However, chlorine-tolerant bacteria are able to withstand the aforestated toxic effects of chlorine by different mechanisms.

The mechanism by which bacteria tolerate chlorination has been widely investigated by different researchers and the results highlighted different cellular structures that interfere with the activity of free chlorine rendering it ineffective. The extracellular capsule has been identified as one of the main structures that protects bacteria from chlorine as it can serve as permeability barrier by binding free chlorine extracellularly (12). This is consisted with another study conducted by Lecherillier et al. (14) who reported that the capsulated strains of *K. pneumoniae* were able to survive chlorination more than the non-capsulated ones. Further evidences revealed that capsulated bacterial strains produce more extracellular polymeric substance and can easily form biofilm which can interact with free chlorine thereby impeding its access into the cells (11). Some of the chlorine tolerant isolates identified in this study, namely, *Enterobacter* spp, *E. coli*, *P. aeruginosa, K. pneumoniae* were highlighted among water borne pathogens that are found to possess capsule and can form biofilms that serve as protection against chlorination (12).

Enzymatic resistance to chlorine was also reported, most notably, peroxidases and catalases which reacts with reactive chlorine species (RCS) as reported by Gray et al. (13). Gupta et al. (12) also reported that chlorine tolerant *E. coli* was found to possess hypochlorite response transcription factor (YjiE), reactive chlorine specific transcription factor like (RclP) protein and redox regulated chaperon (Hsp 33) all of which aids the bacterium to survive chlorine treatment. *Acinetobacter* has been reported to significantly produce superoxide dismutase and ascorbate peroxidase after exposure to chlorine (47).

Several studies reported clinical cases that are linked to infection with chlorine resistant bacteria in drinking water; *Acinetobacter* has been reported to cause upper respiratory tract infections, meningitis, urinary tract infections and sepsis (48). Water borne *P. aeruginosa* has also been shown to cause urinary tract infections, bacteremia and sepsis, wound infections and suppurative otitis media (9, 11). *Enterococcus* spp. have been found to involve in the cases of pelvic infections, infective endocarditis, and urinary tract infections. *Citrobacter* has also been reported to cause pneumonia and meningitis in neonates, urinary tract infections as well as abdominal problems (10). *Bacillus* species are among the remarkable spore formers and are found

widely distributed in different environments. The endospores give them the ability to survive NaOCl and other chemical treatments as the endospore layers serve as permeability barriers to these chemicals. Members of *Bacillus* spp. have been reported to cause food poisoning, diarrhoea, respiratory tract infections and bacteremia (11, 48). The presence of chlorine-tolerant bacteria such as *Bacillus* spp. and *Pseudomonas aeruginosa* presents serious public health concerns and therefore emphasizes the need for stricter water treatment protocols.

CONCLUSION

In conclusion, this study unveiled considerable bacterial counts, in the River, Tap, and well water samples from Girei Local Government Area. Some of these bacterial isolates are potential human pathogens particularly, those of the enteric types. Therefore, there is a critical need for effective water treatment measures in the study area. The identification of resistant bacterial isolates to conventional chlorination using sodium hypochlorite signifies the importance of this research to suggest alternative and more potent disinfection strategies against the bacterial isolates in these water sources. This study also contributes to the understanding of water quality in the region and emphasizes the urgency of implementing effective water treatment protocols to ensure the safety of water for domestic purposes in the study area.

CONFLICTS OF INTERESTS

The authors have indicated no conflict of interest.

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