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Occurrence of coliforms and biofilm-forming bacteria in raw, treated, and distributed water from two waterwork systems in Osun State, Southwestern Nigeria

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ABSTRACT

This study assessed the bacteriological quality of raw, treated, and distributed water from Ede-Erinle and Opa reservoirs in Osun State, Nigeria. This was to determine the potability of water from these waterwork stations. Eighteen sampling points were established across the two reservoir networks for this study. Samples were collected bi-monthly for two annual cycles. Serial dilution and pour plate methods were employed for the enumeration of bacterial load. Total heterotrophic bacteria count (THBC) and total coliform bacteria count (TCBC) were enumerated on nutrient and MacConkey agar at 37 °C, respectively. Bacterial isolates were characterized using biochemical identification methods with reference to *Bergey's Manual of Determinative Bacteriology*. Bacterial isolates and biofilm formation were further identified molecularly through the PCR method using specific universal primers. Mean values of THBC and TCBC in distributed water from Ede-Erinle ($9.61 \times 10^4 \pm 1.50 \times 10^4$ CFU/mL; 69.56 ± 26.81 CFU/mL) and Opa waterworks ($9.58 \times 10^4 \pm 2.55 \times 10^4$ CFU/mL; 142.94 ± 44.41 CFU/mL) exceeded permissible limits for drinking water. *Paenibacillus lautus*, *Bacillus pseudomycoides*, *Pseudomonas aeruginosa*, and *Pseudomonas stutzeri* showed biofilm-forming capacity. The study concluded that the presence of coliforms and biofilm-forming bacteria in distributed water implies that the water is unfit for consumption without further treatment.

Key words: biofilm-forming bacteria, coliforms, reservoir, waterworks systems

HIGHLIGHTS

- Molecular identification of coliforms from the two reservoirs and their channels.
- Molecular detection of biofilm bacteria in water from the two waterwork systems.
- Comparison of molecular loads of water from the two waterwork systems.
- Health implications of the identified bacteria from the two waterwork systems.
- Potablilty of water from the two reservoirs and their stations.

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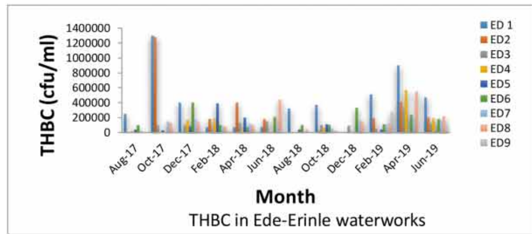
GRAPHICAL ABSTRACT

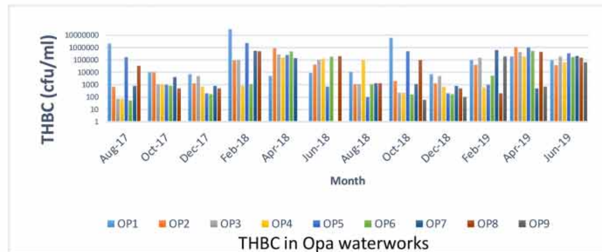
Occurrence of Coliforms and Biofilm – forming Bacteria in raw, treated and distributed water from two waterworks systems in Osun State, Southwestern Nigeria.

Ede-Erinle waterworks

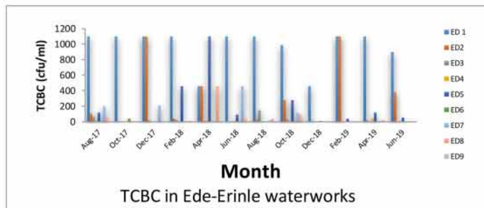
Opa waterworks



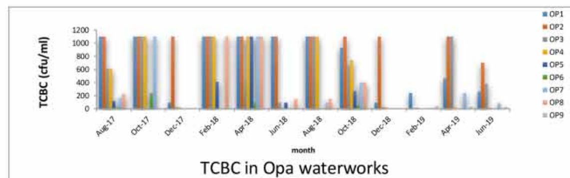
THBC in Ede-Erinle waterworks



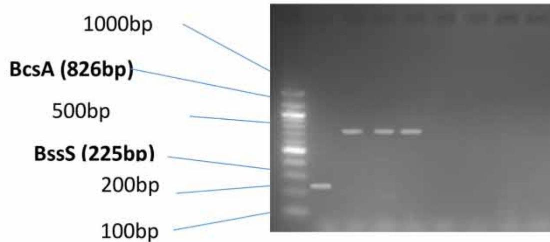
THBC in Opa waterworks



TCBC in Ede-Erinle waterworks



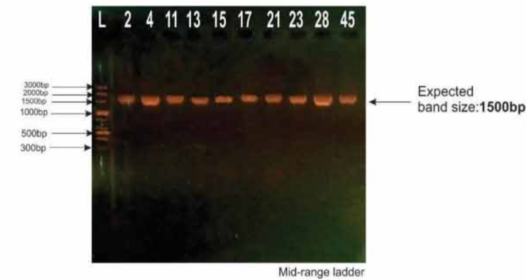
TCBC in Opa waterworks



PCR Amplification of *bcsA* and *BssS* genes

Gel picture of Biofilm-forming bacterial isolates detected by PCR from Ede-Erinle and Opa Waterworks systems

L = 100 bp DNA ladder; 1 = *Paenibacillus lautus*; 2 = *Bacillus paramycoides;* 3 = *Pseudomonas aeruginosa*; 4 = *Pseudomonas stutzeri*



Gel picture of amplified 16S rRNA genes of selected bacterial isolates from Ede-Erinle and Opa Waterworks systems.

L = 300 bp DNA ladder; 2 = *Bacillus pseudomycoides*; 4 = *Pseudomonas stutzeri*; 11 = *Bacillus cerus*; 13 = *Aeromonas jandaei*; 15 = *Bacillus paramycoides*; 17 = *Morganella morganii*; 21 = *Paenibacillus lautus*; 23 = *Klebsiella pneumoniae*; 28 = *Pseudomonas aeruginosa*; 45 = *Providencia rettgeri*

Conclusion

Presence of coliform bacteria in the treated and distributed water sources of Ede-Erinle and Opa waterworks systems is an indication of faecal pollution and could pose a high health risk to the consumer of these water sources without further treatment before use. Occurence of biofilm-forming bacteria in distribution water from the two waterworks may compromise the efficacy of water treatment operations. It could also lead to the evolution of antibiotic-resistant genes in aquatic bacteria.

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INTRODUCTION

Water supplies in underdeveloped nations are still insufficiently treated, forcing communities to rely on the most available sources (Sobsey 2002; Moyo *et al.* 2004). Many of these water sources are unprotected and vulnerable to contamination from runoff, windblown debris, human and animal faeces, and filthy collecting methods (Chidavaenzi *et al.* 1998; WHO 2000; Moyo *et al.* 2004). The quantity of microorganisms present in drinkable water influences its microbiological quality. However, because detecting each pathogenic organism in water is technically difficult, time-consuming, and expensive, it is not employed for routine water testing processes (Grabow 1996). As a result, indicator organisms are commonly used to assess the microbiological quality of water and provide a simple, quick, and reliable indication of water supply quality (Grabow 1996). Coliforms are members of the family *Enterobacteriaceae* and are defined as facultative anaerobic, Gram-negative, non-sporing, rod-shaped bacteria that ferment lactose with gas formation within 48 h at 35 °C (Prescott *et al.* 1999). Heterotrophic plate counts, total coliform bacteria (TCB), faecal coliform bacteria, and faecal *Enterococci* are utilized as a system indicator in water quality investigations, providing information on the efficacy of water treatment (APHA 1995; WHO 2000). The presence of coliforms in water samples indicates the existence of opportunistic pathogenic bacteria like *Klebsiella* and *Enterobacter* that can multiply in water environments, as well as pathogenic bacteria like *Salmonella* spp., *Shigella* spp., and *Escherichia coli*. These groups of microorganisms have the potential to cause illnesses such as gastroenteritis, dysentery, cholera, typhoid fever, and salmonellosis in consumers of these water sources (DWAF 1996; Grabow 1996). Biofilms can be found in industrial settings, hotels, wastewater channels, toilets, laboratories, and medical settings, and they typically form on hard surfaces that are submerged in or exposed to aqueous solutions. They can form on living and non-living surfaces (Rao *et al.* 2005).

Drinking water networks may be considered as biological reactors hosting a wide variety of microorganisms, such as bacteria, protozoa, and fungi, both in bulk water and on the surfaces of pipes. The Gram-negative bacteria predominate over the Gram-positive bacteria, and *Pseudomonas* is the most prevalent bacterial organism in water supply systems, regardless of the source. In microbial ecology, the rRNA-targeted oligonucleotide samples are identified and increasingly used to classify the bacterial species found in biofilms produced in water delivery systems (Wagner *et al.* 1993; Kalmbach *et al.* 1997; Ludmány *et al.* 2006). There have been several investigations on the existence and significance of biofilms in drinking water distribution systems (Momba *et al.* 1999; Gouider *et al.* 2009; Paris *et al.* 2009). Flemming *et al.* (2002) proposed that 95% of the bacteria would adhere to the pipeline surface and that only 5% would be present in the bulk water. Biofilm can protect microorganisms from various stress conditions and disinfectants. Biofilm also provides the injured microorganisms with the micro- and macro-nutrients needed for recovery and growth (Davey & O'Toole 2000). Chlorine, a potent oxidizing agent, is the disinfectant most widely used in water distribution systems because of its effectiveness, stability, simple to use, and affordability. In iron pipes, however, doses of chlorine as high as 4 mg/L have little impact on biofilm reduction as iron corrosion products interfere with free chlorine disinfection. The demand for chlorine in iron pipes was found to be as much as ten times higher than in pipes of a different composition.

Ede-Erinle and Opa reservoirs are land-treated surface water sources, which supply potable water to about 10 respective Local Government Areas in Osun State and the Obafemi Awolowo University Campus, Ile-Ife, Nigeria. This work was carried out to identify coliforms as well as the presence of biofilm-forming bacteria in water samples from the waterworks stations with a view to determining the potability of distributed water from these reservoirs, thereby enhancing the actualization of sustainable development (SDG) goal 6 (provision of clean water and sanitation) in this region of the Sub-Sahara Africa.

MATERIALS AND METHODS

Study area and sampling stations

The study investigated the quality of water from the Ede-Erinle and Opa reservoirs. Ede-Erinle reservoir is located in Ede, Olorunda Local Government Area of Osun State, Nigeria. The reservoir, which is owned and operated by the Osun State Water Corporation, took its source from the ancient Erinle River. It has a catchment area of about 340 km^2 and a surface area of around 19 km^2. The reservoir network stations lay between Latitude 07°32′32″ and 07°44′28″N and Longitude 004°24′15″–004°32′41″E, with an elevation range of 278–304 m (amsl) (Table 1 and Figure 1). It supplies water to about 10 Local Government Areas in Osun State. Opa Dam and the associated reservoir were built within the confines of the University of Ife estate (now Obafemi Awolowo University, Ile-Ife) in 1978 by the impoundment of the Opa River, which took its

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Table 1 | Geographical location and description of sampling stations of Ede-Erinle and Opa waterwork systems

Sampling station	Description	Latitude (N)	Longitude (E)	Elevation (amsl) (m)	Distance from reservoir (m)
ED 1	Ede-Erinle raw water	07°45′32″	004°27′15″	278	At the reservoir
ED 2	Ede-Erinle aerator	07°45′35″	004°27′7″	304	150
ED 3	Ede-Erinle clarifier	07°45′35″	004°27′5″	303	150
ED 8	Ede-Erinle filter bed	07°45′37″	004°27′9″	283	200
ED 4	Ede-Erinle clear water tank	07°45′36″	004°27′11″	300	250
ED 5	Sekona distribution	07°38′3″	004°26′36″	278	20,000
ED 6	Moro distribution	07°32′22″	004°27′39″	270	28,000
ED 7	Ile-Ife distribution	07°29′28″	004°31′41″	284	35,000
ED 9	Ede distribution	07°45′36″	004°27′12″	292	500
OP 1	Raw water	07°30′8″	004°31′47″	248	At the reservoir
OP 2	Opa aerator	07°30′8″	004°31′41″	244	100
OP 3	Opa clarifier	07°30′8″	004°31′42″	246	200
OP 4	Opa filter bed	07°30′8″	004°31′43″	245	250
OP 5	Opa clear water tank	07°30′9″	004°31′40″	241	350
OP 6	Biological sciences distribution	07°31′9″	004°31′33″	287	7,000
OP 7	Staff quarters distribution	07°31′21″	004°30′59″	289	5,000
OP 8	Fajuyi Hall distribution	07°31′6″	004°30′49″	263	5,000
OP 9	Staff club distribution	07°31′33″	004°31′53″	271	6,000

ED: Ede-Erinle station; OP: Opa Station; amsl: above mean sea level.

source from the Oke-Opa Hills. Approximately 2.5 km long and 0.5 km at its widest point, the reservoir was primarily created to supply potable water to the university community, for which reason, fishing activities were permitted only for recreational and research purposes. The waterworks channels lay between Latitude 07°30′8″ and 07°31′33″N and Longitude 004°29′47″–004°32′53″E, with an elevation range of 248–289 m (amsl) (Table 1 and Figure 1).

The operating procedures in both water treatment plants are the same. A low lift machine draws raw water from the reservoir to the aerator, where dosing pumps deliver aluminium sulphate, lime, and hypochlorite into the water. The water then enters the clarifier chamber, where it undergoes flocculation and/or coagulation (alum reacts with and traps dirt in the water). Clear water will float to the top, while flocs will sink to the bottom. The water is then directed to the filter bed, where filter media is used to catch the suspended particles that escape from the clarifier, allowing clean water to percolate to a conserved region for further processing. Following that, some underground pipes will transport the water from the point of percolation to the final destination, which is the clear well tank or the main reservoir, where water is stored underground for later distribution to the general public. Before the final water is routed to the clear water tank, a process known as post-chlorination is performed to maintain a specified level of chlorine in the water.

Eighteen sampling stations were established for this study. Nine sampling stations were selected for each of the two waterworks investigated. Water samples were taken from the reservoirs (raw water; ED1, OP1), the treatment stages (treated water; ED2, ED3, ED4, ED8, OP2, OP3, OP4, OP5), and at the final consumer taps (distribution water; ED5, ED6, ED7, ED9, OP6, OP7, OP8, OP9). Water samplings were carried out in the morning (between 7.00 am and 10.00 am) bi-monthly and covered both the dry and rainy seasons for two annual cycles between August 2017 and June 2019.

Sample collection

A total of 108 water samples were collected from each sampling station (raw water – 12 samples; treated water – 48 samples; distribution water – 48 samples) of the two waterwork systems in 500-mL sterile corked bottles. Each sample after the collection was placed in an ice box prior to its immediate transfer to the laboratory within the holding period of 6 h for bacteriological analyses, which include heterotrophic plate count, total coliform count, faecal coliform count using the pour plate method, and most probable number (MPN) of the coliform presumptive test.

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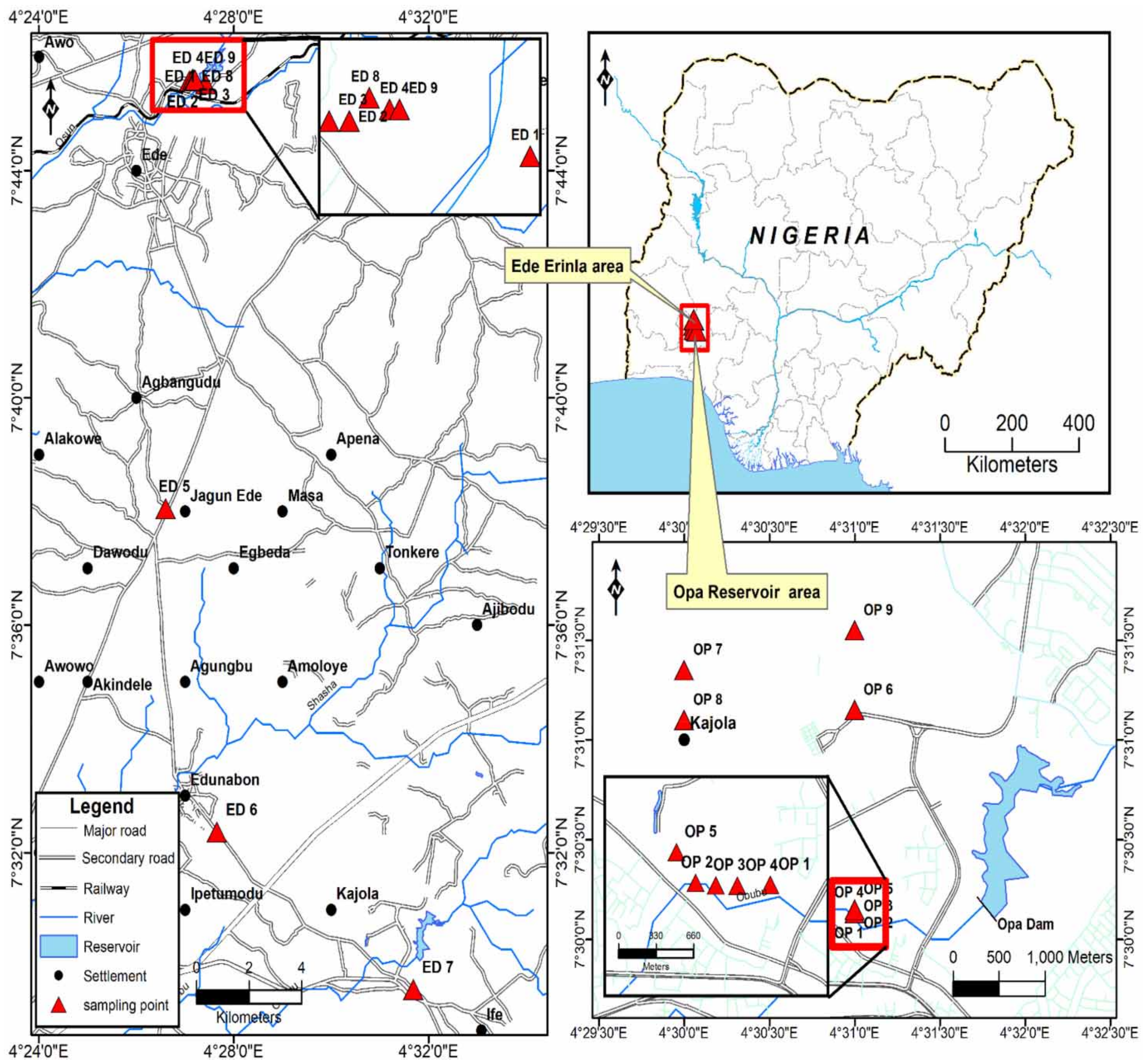

Figure 1 | Map of Ede-Erinle and Opa Reservoirs showing the sampling stations.

Determination of total heterotrophic bacteria, total coliform bacteria, faecal coliform count, and preliminary identification of bacteria

Enumeration of total heterotrophic bacteria (THB) and TCB was carried out using serial dilution technique by introducing 1 mL of water samples into 10^{-2} to 10^{-5} dilution folds and plated on sterile Petri dishes containing appropriately sterilized nutrient and MacConkey agar, respectively. The plates were then incubated at 37°C for 24 h, after which the colonies were counted using a manual colony counter.

The MPN of the coliform presumptive test was carried out on the water samples by placing three portions in each of three dilutions in geometric series employing the use of sterile single- and double-strength Lactose broth. For the dilution, a set of three test tubes, each containing 10 mL double-strength broth, and two sets of three test tubes, each containing 5 mL of sterile single-strength broth, were utilized. Ten millilitres of the sample was inoculated into each tube of the double-strength broth; 1.0 mL of the sample was inoculated into each of the first set of the three single-strength tubes, while 0.1 mL of the sample was inoculated into each of the other three single-strength broth. The culture tubes were incubated at 35 °C for 48 h, and each

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tube was observed for growth with acid and gas production. A tube in which acid and gas were produced was referred to as 'a positive tube'. The combined number of positive tubes in each set arranged in order of least diluted to the most diluted tubes was read out from the appropriate standard MPN table to obtain the estimated number of coliform cells present in 100 mL of the original sample.

Bacterial isolates obtained were preliminarily identified using cultural, morphological, and biochemical tests. Probable identification of bacteria in the water samples was carried out using *Bergey's Manual of Determinative Bacteriology* (Holt *et al.* 1994).

Phenotypic identification of biofilm producers

Phenotypic investigation for biofilm production by the isolates was performed using Congo Red Agar Medium, which was prepared using the combination of brain heart infusion agar 52 g/L, sucrose 50 g/L, and Congo red indicator 0.8 g/L, as described by Mathur *et al.* (2006). The Congo red was prepared as a concentrated aqueous solution separately from other medium constituents and then sterilized in different containers before adding them together when the agar had cooled to 55 °C before being distributed to sterile plates to solidify. The plates were then inoculated with the test organisms using point inoculation method and incubated at 37 °C for 24 h. The presence of black colonies on the positive plates, characterized by a dry crystalline quality, signifies the formation of biofilm.

Molecular identification of bacterial isolates

Molecular DNA extraction from the bacterial isolates was carried out using the boiling technique, as described by Gueimonde *et al.* (2004) and Naas *et al.* (2007). In further determining the identities of bacterial species isolated, polymerase chain reaction (PCR) was used by employing bacterial-specific universal primers. The primer developed by Horakova *et al.* (2008) amplified at 1465-bp sequence of *16srRNA* gene was used as follows: 27F 5′-AGAGTTTGATCCTGGCTCAG–3′ and 1429R 5′-GGTTACCTTGTTACGACTT-3′. The reaction mixture contained 12.5 μL of PCR Master Mix (New England Biolabs, SA), 0.5 μL each of Forward and Reverse Primers (Inqaba Biotech, SA), 3 μL of DNA template, and 8.5 μL of nuclease-free water to constitute a total reaction volume of 25 μL. The PCR conditions for the detection of 16*S rRNA* genes of the bacterial species were as described by Horakova *et al.* (2008) but with slight modification. Gene amplification was carried out in an Applied Biosystems 2720 Thermal Cycler. The mixture was subjected to an initial denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 1 min, annealing at 48 °C for 30 s, extension at 72 °C for 1 min for 37 times, and final extension step at 72 °C for 10 min. For the identification of biofilm-producing bacteria, the following two sets of primers were utilized: (i) *bcsA-F* GCT TCT CGG CGC TAA TGT TG; *bcsA-R* GAG GTA TAG CCA CGA CGGTG (826 bp), as described by Olowe *et al.* (2019), and (ii) BssS-F GATTCAATTTTGGCGATTCCTGC; BssS-R TAATGAAGTCATTCAGACTCATCC (225 bp), as described by Hassan & Abdalhamid (2014). The conditions for the forward and reverse biofilm primers were initial denaturation at 94 °C for 2 min followed by 40 s of denaturation also at 94 °C, annealing at 48 °C for 1 min, extension at 72 °C for 1 min for 35 times, and final extension step at 72 °C for 5 min.

Electrophoresis was done by loading 10 μL aliquot of the amplicons on 1.5% agarose gel CSL-AG 100 (Cleaver Scientific Ltd, SA) stained with 5 μL ethidium bromide (Sigma-Aldrich, USA) and ran in 1X TAE buffer (tri-acetate EDTA buffer) (BioConcept, SA) for 1 h at 80 V. The PCR fragments were visualized with a UV transilluminator (Uvitec 07 10322). The 16S rRNA PCR products were sequenced using the Nimagen, Brilliant-Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to the manufacturer's instructions. Sequence chromatogram analysis was performed using Finch TV analysis software version 1.4.0. All nucleotide sequences obtained were analysed by a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST).

Data analysis

The acquired data were analysed using descriptive, inferential (ANOVA), and multivariate (cluster analysis) statistics with applicable statistical software such as PAST version 2.17 and SPSS version 20. The student sample *t*-test ($p < 0.05$) was used to compare the bacteriological quality of the two reservoir systems to the World Health Organization (WHO) and Nigerian Standard for Drinking Water Quality (NSDWQ) permitted standards.

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RESULTS

Total heterotrophic bacteria and total coliform counts

In Ede-Erinle reservoir, THB over the period of study ranged from 0 to 1.30×10^6 CFU/mL, with overall mean and median values of 1.54×10^5 and 1×10^5 CFU/mL, respectively (Table 2), whereas total coliform bacteria counts (TCBC) in water samples collected from Ede-Erinle reservoir over the study period ranged from 0 to 1.1×10^3 CFU/mL, with overall mean and median values of 182.23 ± 33.91 CFU/mL and 10 CFU/mL, respectively (Table 2). Across the stations, the highest THB mean value of $3.94 \times 10^5 \pm 1.1 \times 10^5$ CFU/mL was recorded in the raw water station, while the lowest mean value of $9.61 \times 10^4 \pm 1.50 \times 10^4$ CFU/mL was recorded at the distributed water station. However, there was a significant spatial difference ($p < 0.001$) across the stations. The highest TCBC mean value was recorded at the raw water station ($9.67 \times 10^2 \pm 7.0 \times 10^1$ CFU/mL), while the lowest mean value occurred at the distributed water stations (69.56 ± 26.81 CFU/mL). In general, the mean values of TCBC across the stations were significantly different ($p < 0.001$) from each other (Table 2). The THB count was higher in the rainy season than in the dry season, but the seasonal mean values were not significantly different from each other ($p > 0.05$) (Table 3). Meanwhile, the TCBC was generally higher in the dry season than in the rainy season, but there was no significant difference ($p > 0.05$) between the two seasonal mean values (Table 3). The total heterotrophic bacteria count (THBC) in the Opa waterworks system ranged from 0 to 3.10×10^7 CFU/mL, with an overall mean of $4.91 \times 10^5 \pm 2.9 \times 10^5$ CFU/mL and median of 5.0×10^3 (Table 2). TCBC ranged from 0 to 1.1×10^2 CFU/mL, with overall mean and median values of $3.9 \times 10^2 \pm 4.5 \times 10^1$ CFU/mL and 93.00 CFU/mL, respectively (Table 2). The highest THB mean value of $3.31 \times 10^6 \pm 2.57 \times 10^6$ CFU/mL was recorded in the raw water, while the lowest mean value of $9.58 \times 10^4 \pm 2.55 \times 10^4$ CFU /mL occurred at the distributed water station. The highest mean TCBC value (723.00 ± 129.28 CFU/mL) was recorded at the raw water station, while the lowest mean value (142.94 ± 44.41 CFU/mL) occurred at the distributed water station. The difference in mean values across stations was significant at $p < 0.001$ (Table 2). The THB abundance was higher in the dry season than in the rainy season, but the seasonal mean values were not significantly different from each other ($p > 0.05$) (Table 3). Whereas TCBC was higher during the rainy season (458.40 ± 55.75 CFU/mL) than in the dry season (242.58 ± 95.66 CFU/mL), and there was a significant difference ($p < 0.05$) between the two seasonal mean values (Table 3).

The biochemical characteristics and probable bacterial isolates identified during this study from Ede-Erinle and Opa water samples are presented in Table 5.

Comparison of microbial loads in raw, treated, and distributed water between Ede-Erinle and Opa waterwork systems

From the bacteriological analysis carried out during the study, THBC was significantly higher ($p < 0.05$) in the Opa reservoir ($3.60 \times 10^6 \pm 2.57 \times 10^5$ CFU/mL) than in the Ede-Erinle reservoir station ($3.94 \times 10^5 \pm 1.1 \times 10^5$ CFU/mL), while TCBC were significantly higher ($p < 0.01$) in the Ede-Erinle reservoir (967.50 ± 70.74 CFU/mL) than in the Opa reservoir station (723.00 ± 129.28 CFU/mL) (Table 7). However, THBC and TCBC were higher in the Opa-treated water stations (1.43×10^5 CFU/mL and 545.83 ± 71.48 CFU/mL) than in the Ede-Erinle stations (1.02×10^5 CFU/mL and 98.58 ± 34.33 CFU/mL) during the study, and there was a significant difference ($p < 0.001$) in the mean values of TCBC during the study (Table 4). Similarly, analysis of the distributed water from the Ede-Erinle and Opa waterworks stations revealed that THBC and TCBC were both higher in the Opa distribution stations ($6.35 \times 10^4 \pm 2.20 \times 10^4$ CFU/mL and 143.02 ± 44.41 CFU/mL) than in the Ede-Erinle stations ($5.18 \times 10^4 \pm 1.42 \times 10^4$ CFU/mL and 69.56 ± 26.81 CFU/mL). There was a significant difference ($p < 0.05$) in the mean values of TCBC across the stations during the study (Table 4).

As shown in Figure 2, the hierarchical cluster analysis (HCA) plot indicated the relationship between THB and TCB distribution in the Ede-Erinle stations. ED 3 (Ede Clarifier), ED 4 (Ede clear water), Ed 7 (Ile-Ife distribution), Ed 6(Moro distribution), and ED 8 (Ede filter bed) were independently related to each other. There was also a close relationship between ED 2 (Ede aeration chamber) and ED 1 (Ede raw water) in one of the major clusters formed. Similarly, Figure 3 shows the similarities that exist in the distribution of THB and TCB in the stations of Opa waterworks. The HCA plot showed relatedness in OP 8 (Opa Fajuyi Hall distribution), OP 5 (Opa clear water), OP 9 (Opa staff club water), and OP 1 (Opa raw water). Also, there was a relationship between OP4 (Opa filter bed), OP 3 (Opa clarifier), OP 2 (Opa aeration chamber), and OP 6 (Opa biological sciences) stations, while OP7 demonstrated a distant relationship to the group.

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Table 2 | ANOVA statistics of bacterial load in raw, treated, and distributed water from Ede-Erinle and Opa waterwork systems (August 2017–June 2019)

Ede-Erinle reservoir	Raw water ($n = 12$)		Treated water ($n = 48$)		Distributed water ($n = 48$)		Overall ($n = 108$)		ANOVA	
	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	F	p
THBC (CFU/mL)	9.0×10^2–1.3×10^6	$3.94 \times 10^5 \pm 1.1 \times 10^5$	1.12×10^2–1.28×10^6	$1.53 \times 10^5 \pm 3.16 \times 10^5$	0–4.0×10^5	$9.61 \times 10^4 \pm 1.50 \times 10^4$	0–1.30×10^6	$1.54 \times 10^5 \pm 2.12 \times 10^2$	10.317	0.000***
TCBC (CFU/mL)	4.60×10^2–1.1×10^3	$9.67 \times 10^2 \pm 7.0 \times 10^1$	0–1.1×10^2	98.58 ± 34.35	0–1.1×10^2	69.56 ± 26.81	0–1.1×10^3	182.23 ± 33.91	88.608	0.000***
Opa reservoir										
THBC (CFU/mL)	5.0×10^3–3.10×10^7	$3.31 \times 10^6 \pm 2.57 \times 10^6$	73–2.33×10^6	$1.83 \times 10^5 \pm 5.88 \times 10^4$	0–6.30×10^6	$9.58 \times 10^4 \pm 2.55 \times 10^4$	0–3.10×10^7	$4.91 \times 10^5 \pm 2.92 \times 10^5$	6.394	0.002**
TCBC (CFU/mL)	93–1.1×10^3	723.00 ± 129.28	0–1.1×10^2	545.83 ± 71.48	0–1.1×10^2	142.94 ± 44.41	0–1.1×10^3	$2.92 \times 10^2 \pm 4.5 \times 10^1$	15.663	0.000***

THBC, total heterotrophic bacterial count; TCBC, total coliform bacterial count.
**Significant ($p < 0.01$).
***Significant ($p < 0.001$).
***Significant ($p < 0.001$).

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Table 3 | ANOVA statistics of the seasonal variation in bacterial load of water samples from Ede-Erinle and Opa waterworks system (August 2017–June 2019)

Ede-Erinle reservoir	Rainy season ($n = 72$)		Dry season ($n = 36$)		Overall ($n = 108$)		ANOVA	
	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	F	p
THBC (CFU/mL)	0–1.30 × 10^5	$1.65 \times 10^5 \pm 2.99 \times 10^4$	0–5.10 × 10^5	$1.32 \times 10^5 \pm 2.21 \times 10^4$	0–1.30 × 10^5	$1.54 \times 10^6 \pm 2.12 \times 10^5$	0.546	0.462
TCBC (CFU/mL)	0–1.10 × 10^2	178.49 ± 39.71	0–1.10 × 10^2	189.72 ± 64.41	0–1.10 × 10^2	182.23 ± 33.91	0.024	0.877
Opa reservoir								
THBC (CFU/mL)	0–6.21 × 10^6	$2.41 \times 10^5 \pm 9.23 \times 10^4$	0–3.10 × 10^7	$9.92 \times 10^5 \pm 8.60 \times 10^5$	0–3.10 × 10^7	$4.91 \times 10^5 \pm 2.93 \times 10^5$	1.472	0.228
TCBC (CFU/mL)	0–1.10 × 10^2	458.40 ± 55.75	0–1.10 × 10^2	242.58 ± 95.66	0–1.10 × 10^2	386.46 ± 45.20	5.269	0.024*

THBC, total heterotrophic bacterial count; TCBC, total coliform bacterial count.
*Significant difference ($p < 0.05$).

Table 4 | *t*-test statistics of the raw water samples from Ede-Erinle and Opa waterwork systems

Parameters (unit)	Reservoir		*t*-test		NSDWQ maximum permitted level	WHO maximum permitted level
	Ede-Erinle Mean ± S.E (N = 12)	Opa Mean ± S.E (N = 12)	t	p		
Raw water						
THBC (CFU/mL)	$3.94 \times 10^5 \pm 1.1 \times 10^5$	$3.60 \times 10^6 \pm 2.57 \times 10^5$	−1.1133	0.029*	10	0
TCBC (CFU/mL)	967.50 ± 70.74	723.00 ± 129.28	1.659	0.001**	10	0
Treated water						
THBC (CFU/mL)	$1.02 \times 10^5 \pm 3.20 \times 10^4$	$1.43 \times 10^5 \pm 5.74 \times 10^4$	−0.622	0.155	10	0
TCBC (CFU/mL)	98.58 ± 34.33	545.83 ± 71.48	−5.640	0.000***	10	0
Distributed water						
THBC (CFU/mL)	$5.18 \times 10^4 \pm 1.42 \times 10^4$	$6.35 \times 10^4 \pm 2.20 \times 10^4$	−0.450	0.141	10	0
TCBC (CFU/mL)	69.56 ± 26.81	143.02 ± 44.41	−1.416	0.028*	10	0

Source: NSDWQ (2015); WHO (2017).
*Significant ($p < 0.05$).
**Significant ($p < 0.01$).
***Significant ($p < 0.001$).

Identified bacterial isolates

The biochemical characteristics and probable bacterial isolates identified during this study from Ede-Erinle and Opa water samples are presented in Table 5.

Molecular identification of bacterial isolates

Table 6 shows the respective sequences, accession numbers, and the identified species of 10 randomly selected bacterial isolates subjected to 16S rRNA PCR amplification. This result revealed the isolates' identity to be *Bacillus pseudomycoides*, *Pseudomonas stutzeri*, *Bacillus cereus*, *Aeromonas jandaei*, *Bacillus paramycoides*, *Morganella morganii*, *Paenibacillus lautus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Providencia rettgeri*. Gel electrophoresis showed that the band size of all the selected isolates fell within the range of the expected band size of 1,500 base pairs (bp), as indicated in Figure 4.

Out of the ten bacterial isolates that showed biofilm-forming ability phenotypically, as shown in Table 7, only four were genotypically confirmed. These confirmed biofilm-forming bacterial isolates were *P. lautus* (isolated from the Ile-Ife distribution station of Ede-Erinle waterworks), *B. pseudomycoides* (isolated from the raw water station of Ede-Erinle

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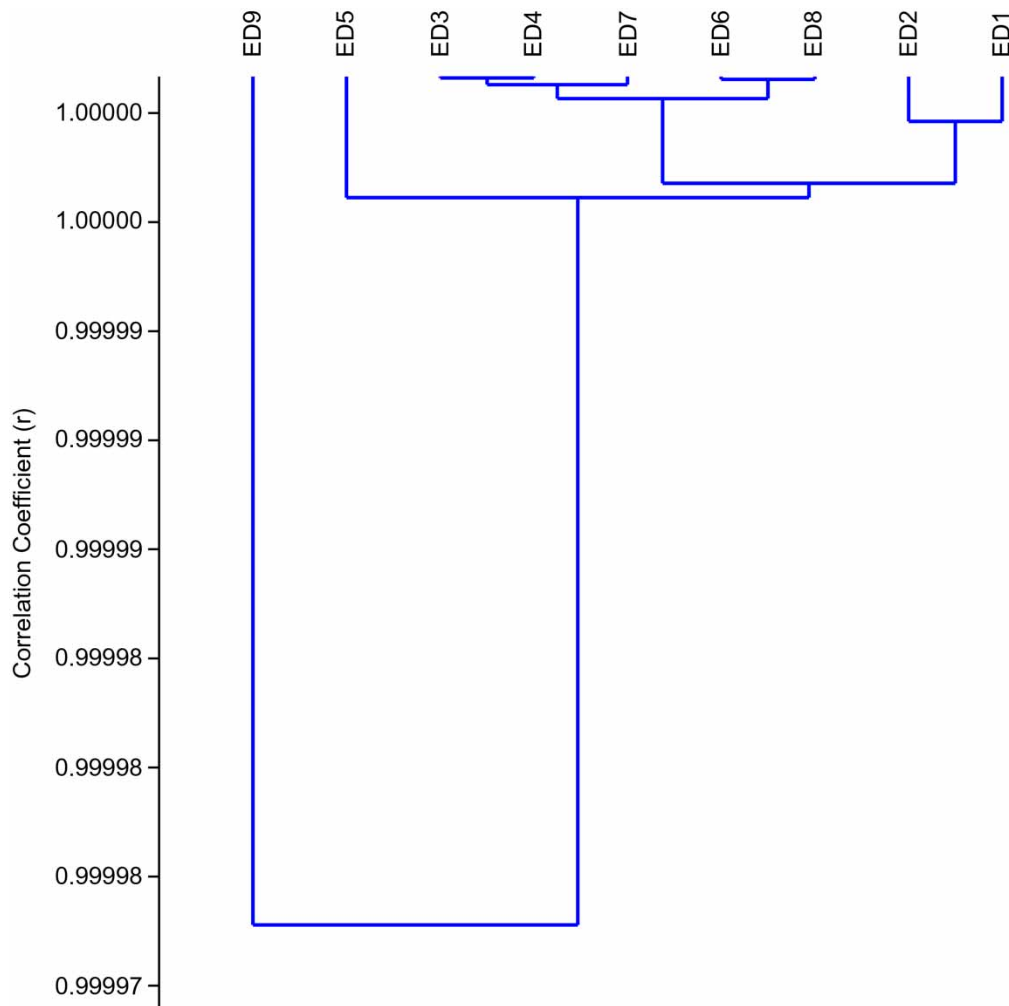

Figure 2 | Cluster diagram showing the relationship among sampling stations of Ede-Erinle reservoir based on bacterial population (THB and TCB).

waterworks), *P. aeruginosa* (isolated from the clear water tank of Ede-Erinle waterworks), and *P. stutzeri* (isolated from the raw water station of Ede-Erinle waterworks). The gel electrophoresis with the band size is as shown in Figure 5.

DISCUSSION

Water pollution happens when particles, chemicals, or bacteria invade a water supply. Some of the major causes of water contamination are agricultural runoff, industrial contamination, insufficiently treated water, natural catastrophes, and sewage leaks. Although it is reasonable to assume the presence of certain contaminants in drinking water because pure unpolluted water does not occur in nature, some contaminants in drinking water may be dangerous if ingested in certain quantities. Also, the presence of these contaminants in water may lead to biofilm formation. In this study, THBC and TCBC exceeded the NSDWQ (2015) and WHO (2017) permissible limit for drinking water standards in the stations of Ede-Erinle and Opa waterwork systems, notably at the distribution water stations. This poses a significant threat to the health of consumers of these water supplies in the region. Also, a higher population of bacterial species was isolated in the rainy season than in the dry season in the Ede-Erinle and Opa waterwork systems. This could be attributed to the discharge and washing of organic matter into the water bodies, which promotes the proliferation of these bacterial species. This was consistent with the findings of many researchers (Ogbonna 2010; Oluyege *et al.* 2011; Adesoji & Ogunjobi 2013; Balogun *et al.* 2013) who hypothesized that the presence of a greater number of heterotrophic bacterial species in rivers and other sources of drinking water during

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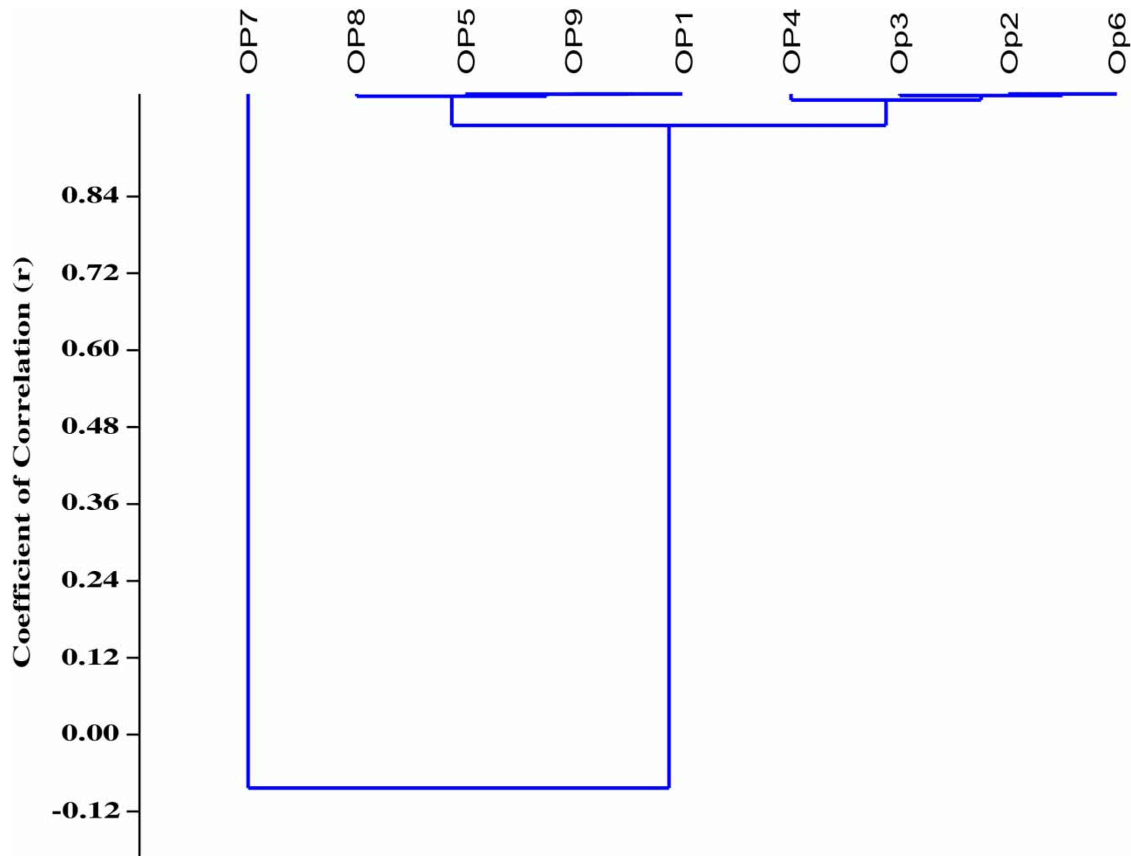

Figure 3 | Cluster diagram showing the relationship among sampling stations of Opa reservoir based on bacterial population (THB and TCB).

the wet season compared to the dry season could be due to favourable physicochemical conditions, which could be attributed to the presence of allochthonous materials from the catchment area of the river during the rainy season.

High microbial loads (higher than the WHO permissible limits for heterotrophic and coliform bacteria in any water meant for drinking) documented at the distribution stations of the two waterwork systems despite treatment may be due to leakage as observed during the collection of samples in the pipelines at some distribution channels. Leakage in pipe water transmission pipelines might have enabled pollutants to reach the water transmission line. This is analogous to the observation of Van Zyl (2004) that there is a renewed international concern that water delivery systems are ageing and decaying worldwide, as the demands on these systems, and thus on our natural water supplies, are growing. In several towns and cities around the world, non-revenue water in water delivery systems is reaching alarming levels. In their findings, the United Nations (2002) and Hall (2006) stated that about half of the water in the drinking water supply systems in the developing world is lost due to leakage, illegal hook-ups, and vandalism that could eventually lead to poor microbial quality water production and eventually to a public health crisis. Another possible explanation for this could be that the disinfectants used or their dose might be bacteriostatic and not effective enough to completely eliminate the microbes. This can trigger the static microbes to reactivate a few days after disinfection. Another reason may also be the lack of adequate residual chlorine in some of the treated water being pumped out into the various distribution stations of Ede-Erinle and Opa waterwork systems.

The presence of *P. aeruginosa* and other genera of *Enterobacteriaceae* (coliforms) such as *E. coli* (faecal coliform), especially in the Opa waterworks system, is an indication that the water sources are of poor microbiological quality. The presence of these coliforms and non-coliform pathogenic bacteria in the Ede-Erinle and Opa waterwork systems over the two seasons considered in the course of this study could have consequential health effects on the final consumer of the water sources. *P. aeruginosa* and other genera of *Enterobacteriaceae* are associated with gastroenteritis (Adeniyi & Olabanji 2005). A few strains of *E. coli* have been implicated to cause diarrhoea and kidney failure (Karch *et al.* 2005). *K. pneumoniae*

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Table 5 | Biochemical characteristics of bacterial isolates obtained from Ede-Erinle and Opa reservoir water sampling stations

Isolate code	Cell shape	Gram's stain	Catalase	Oxidase	Triple Sugar Iron (TSI) reaction	Sulfur; Indole; Motility (SIM) I-M-H_2S	Citrate	Methyl Red (MR)	Voges Proskauer (VP)	Glucose	Maltose	Mannitol	Sucrose	Lactose	O-F	Nitrate reduction	Possible organism
COP1[b]	SR	–	+	–	YGY +	+ – +	+	+	–	YG	YG	YG	YG	YG	F	+	*Citrobacter freundii*
EED5[d]	LR	–	+	+	YNcNc	– + –	–	+	–	Y	Y	Nc	Nc	Nc	F	+	*Chromobacterium violaceum*
DED1[a]	SR	–	+	+	NcNcNc	–	+	–	–	Nc	Nc	Nc	Nc	Nc	F	+	*Chromobacterium sp.*
DED4[b]	LR	+	+	+	NA	– + –	–	+	+	Y	Y	Nc	Y	Nc	F	+	*Bacillus alvei*
EOP1[a]	SR	–	+	–	YGYNc	+ – +	–	+	–	YG	YG	YG	YG	YG	F	+	*Escherichia coli*
EED7[a]	SR	–	+	+	YGYNc	+ + –	+	+	–	YG	YG	YG	YG	YG	F	+	*Paenibacillus lautus*
AOP1[a]	MLR	–	+	–	YYNc	–	+	+	–	YG	YG	YG	YG	YG	F	+	*Klebsiella pneumoniae*
COP8[a]	LR	–	+	+	NcNcNc	– + –	–	–	–	Nc	Nc	Nc	Nc	Nc	–	–	*Alcaligenes faecalis*
DED5[b]	Cocci	+	+	–	–	–	–	+	–	Y	Y	Y	Y	Y	F	+	*Staphylococcus aureus*
BED7[a]	MLR	–	+	–	YGNc +	– + +	+	+	+	YG	Nc	Nc	YG	Nc	–	+	*Proteus mirabilis*
COP5[b]	LR	–	–	–	YGY +	+ + +	–	+	+	YG	YG	Nc	YG	Nc	–	–	*Proteus vulgaris*
CED1[c]	LR	–	+	+	YNcNc	–	–	+	+	Y	Y	Nc	Nc	Y	F	+	*Bacillus pseudomycoides*
BED1[c]	MLR	–	+	+	YGNcNc	+ – –	–	+	+	YG	YG	YG	YG	YG	F	+	*Pseudomonas stutzeri*
CED4[b]	LR	–	+	+	YNcNc	–	–	+	–	Y	Y	Y	Y	Y	F	+	*Pseudomonas aeruginosa*
COP8[c]	LR	–	+	+	YNcNc	–	–	+	–	Y	Y	Nc	Nc	Nc	F	+	*Aeromonas jandaei*
EED5[b]	MLR	–	+	+	YNcNc	–	–	+	–	Y	Y	Nc	Nc	Y	F	–	*Klebsiella*

SR: short rod; MLR: medium long rod; LR: long rod; YG: acid and gas production; Y: acid production; Nc: no change; I: indole production; M: motility; H_2S: hydrogen sulphide production; F: fermentative; Ox: oxidative; –: negative; +: positive.

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Table 6 | Molecular identification of selected bacterial isolates obtained from Ede-Erinle and Opa Waterworks system

Isolate code	Sequence	Accession number	Organism identified
CED1[c]	GTAAG…TTTCT	NR_114422.1	*Bacillus pseudomycoides*
BED1[c]	CTCGG…GACCC	NR_118798.1	*Pseudomonas stutzeri*
CED4[d]	ATCCG…GAGCG	NR_115526.1	*Bacillus cereus*
COP8[c]	CCCGG…TTACT	NR_119040.1	*Aeromonas jandaei*
COP5[c]	CCGGG…TTTTT	NR_157734.1	*Bacillus paramycoides*
FOP7[c]	GGTTA…TATCG	NR_113580.1	*Morganella morganii*
EED7[a]	TCCAG…GAACG	NR_040882.1	*Paenibacillus lautus*
AOP1[a]	ATTCG…ATTCA	NR_037084.1	*Klebsiella pneumoniae*
CED4[b]	ATCAA…GGGGG	NR_114471.1	*Pseudomonas aeruginosa*
JOP9[d]	CTGAT…AGACG	NR_115880.1	*Providencia rettgeri*

Note: Full sequences available upon request.

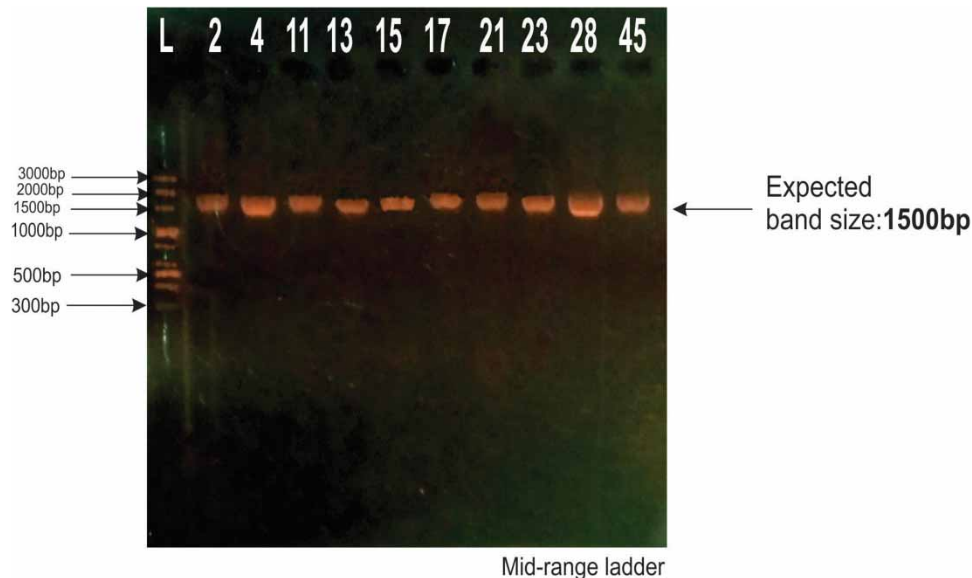

Figure 4 | A representative gel picture of amplified 16S rRNA genes of selected bacterial isolates from Ede-Erinle and Opa waterwork systems. L: 300 bp DNA ladder; 2: *Bacillus pseudomycoides*; 4: *Pseudomonas stutzeri*; 11: *Bacillus cereus*; 13: *Aeromonas jandaei*; 15: *Bacillus paramycoides*; 17: *Morganella morganii*; 21: *Paenibacillus lautus*; 23: *Klebsiella pneumoniae*; 28: *Pseudomonas aeruginosa*; 45: *Providencia rettgeri.*

are important causes of different bacterial infections such as bacteraemia, respiratory and urinary tract infections, neonatal meningitis, and pneumonia (Vasaikar *et al.* 2017).

The molecular identification of *B. pseudomycoides*, a bacterium generally known to be found in the soil worldwide in the reservoir station of the Ede-Erinle waterworks system, could be a result of erosion or runoff of sub-surface soil into the water body. The bacterial species may also have found their way into the water body from the bottom sediments of the reservoir. Similarly, *P. stutzeri*, a common clinical isolate that was identified in the Ede-Erinle reservoir water station, might be a result of runoff from hospital wastes or urines from humans and animals into water bodies, which could find their way into the reservoir. The isolation of *A. jandaei* from the distribution station in the Fajuyi Hall of Opa waterwork systems was in agreement with the work of Kühn *et al.* (1997), who reported the presence of *A. jandaei* from a drinking water distribution system in Sweden. This bacteria is capable of producing toxins, which could be harmful to the consumers of the distributed water.

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Table 7 | Phenotypic and molecular detection of biofilm-forming abilities of bacterial species isolated from Ede-Erinle and Opa waterwork systems

Bacterium	Phenotypic detection			Molecular detection
	16–18 h	18–24 h	48 h	
Paenibacillus lautus	−	+	+	+
Bacillus pseudomycoides	−	++	++	+
Pseudomonas aeruginosa	+	+++	+++	+
Pseudomonas stutzeri	+	+	+	+
Bacillus cereus	−	+	+	−
Klebsiella pneumoniae	++	++	++	−
Aeromonas jandaei	++	++	++	−
Citrobacter freundii	+	++	++	−
Staphylococcus aureus	+	+	+	−
Providencia rettgeri	−	+	++	−

−: non-biofilm forming; +: weak biofilm forming; ++: moderate biofilm forming; +++: strong biofilm forming. Molecular detection, +: positive; −: negative.

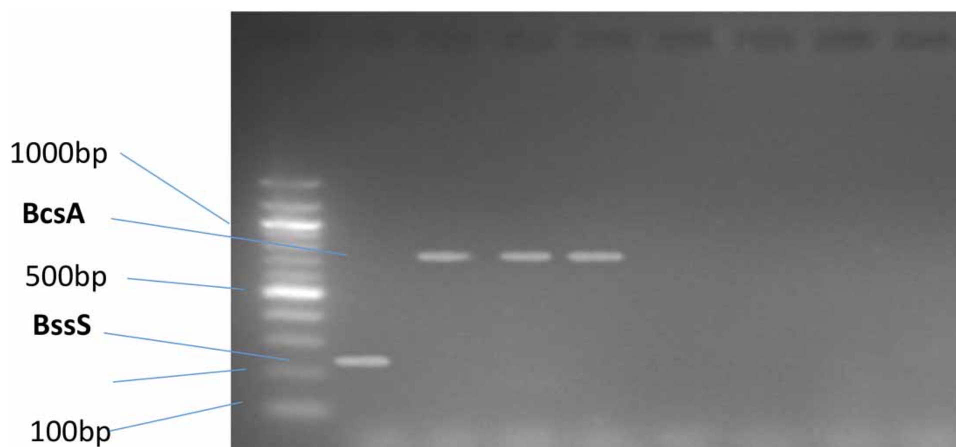

Figure 5 | A representative gel picture of biofilm-forming bacterial isolates detected by PCR from Ede-Erinle and Opa waterwork systems. L: 100 bp DNA ladder; 1: *Paenibacillus lautus*; 2: *Bacillus paramycoides*; 3: *Pseudomonas aeruginosa*; 4: *Pseudomonas stutzeri*.

The isolation of *P. rettgeri* and *M. morganii* from the Staff Club distribution station and Staff Quarters distribution station of the Opa waterworks system, respectively, could cause a wide variety of human infections in the consumer of water from this station without prior treatment. This may range from urinary tract infections to gastroenteritis and bacteraemia (O'Hara *et al.* 2000). *P. rettgeri* has previously been isolated from poultry, reptile and amphibian faeces, and surface waters. It is only sometimes isolated from human faeces and the urinary tract. The bacterium generates urease, which causes urine alkalinization and catheter encrustation. Wounds, burns, and blood infection have also been linked to *P. rettgeri* (Traub *et al.* 1971; Kaslow *et al.* 1976; Müller 1986; Yoh *et al.* 2005). Bacterial biofilm formation and subsequent resistance to antibiotics and disinfectants is a slow but substantial hazard to human health. Biofilm production has become a common occurrence not just in human and animal illnesses but also in non-biological settings. The biofilm-forming bacteria recorded at distribution stations of the Ede-Erinle and Opa waterworks could be attributed to treatment processes or distribution infrastructure deficiencies, as suggested by Lehtola *et al.* (2004).

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Molecular identification of bacteria complements the phenotypic methods, as conventional biochemical identification methods have been shown to be insufficient in the identification of bacterial isolates to species level; hence, to avoid misidentification of such isolates, molecular identification is more reliable.

CONCLUSION

The study concluded that the identification of coliform bacteria in the treated and distributed water sources of the two waterwork systems, most especially the isolation of *E. coli* and *K. pneumoniae* at the Opa distributed water station, is an indication of faecal pollution. This could pose a high health risk to the consumers of these water sources without further treatment before use. The presence of biofilm-forming bacteria in the distributed water from these waterwork systems may compromise the efficacy of water treatment operations. It could also lead to the evolution of antibiotic-resistance genes in aquatic bacteria, making treatment harder for affected consumers of these water sources.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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