Detection of enteric bacteria in two groundwater sources and associated microbial health risks

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ABSTRACT

The microbial quality of two groundwater sources (well and borehole) and associated risks were quantitatively assessed. Water samples from the selected borehole and well were collected over a period of 12 weeks ($n = 48$). The concentrations of *Escherichia coli*, faecal coliforms, *Salmonella*, *Shigella*, *Clostridium*, *Bifidobacterium* and *Campylobacter* were determined using standard microbiological methods, which involve the use of a membrane filter technique. The water samples were filtered through a 0.45 μm membrane filter using vacuum pump pressure and plated on selective agar for the bacteria under test. The number of colonies of the bacterial growth observed after the incubation period was counted and recorded. The physicochemical properties of the water were determined using standard methods. The risk of *Salmonella*, *Shigella*, *Clostridium* and *Campylobacter* infections resulting from the ingestion of water from the borehole and well was estimated. The results showed that the levels of enteric bacteria in the borehole were higher than those in the well. The mean levels of *E. coli* in water from the borehole and well were 3.3 and 1.7 $\log_{10}$ cfu/100 ml, respectively, and exhibited a negative relationship with salinity ($r = -0.53$). The estimated risks of infection associated with the pathogens in water from the borehole and well were greater than the acceptable risk limit of 10^{-4} and followed this order *Clostridium* < *Salmonella* < *Campylobacter* < *Shigella.* The findings of this study suggest recent and continuous faecal contamination of the two groundwater sources, thus exposing the residents relying on the water for drinking to potential risks of gastrointestinal infections.

Key words | dose–response, faecal indicator bacteria, human health, infection, microbial risk assessment

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HIGHLIGHTS

- Levels of *E. coli*, faecal coliforms, *Salmonella*, *Shigella*, *Clostridium*, *Bifidobacterium* and *Campylobacter* in the borehole and well water exceeded 1.5 log.
- Temperature and *Salmonella* had direct relationship while electrical conductivity and *Clostridium* had inverse relationship in borehole and well water.
- The risks of gastrointestinal illness from consumption of water from the borehole were greater than the well.
- Estimated risks of infection associated with *Salmonella*, *Shigella*, *Clostridium* and *Campylobacter* in water samples were greater than acceptable risk limit of 10^{-4}.

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- The risks of infection for *Shigella* and *Campylobacter* were more than 10 times greater than for *Salmonella.*

INTRODUCTION

Groundwater is present in the underground layer of the Earth's crust and constitutes about 37% of the Earth's non-ocean water (Trenberth *et al.* 2007). Water from groundwater sources represents approximately 50% of drinking water in the world, because it is usually less exposed to contamination, pathogen penetration and evaporation, thus making it more stable and suitable for supply when compared with surface waters (Zektser & Everett 2004). Contamination of groundwater sources may occur through anthropogenic activities such as improper sewage disposal, animal rearing and cross contamination from farmland, from runoff through soil infiltration and from natural land surface contamination (Zhang *et al.* 2014; Beer *et al.* 2015; Osvalda *et al.* 2016).

Human consumption of contaminated water from groundwater sources may result in an outbreak of infectious diseases that may lead to morbidity and mortality (Hunter *et al.* 2010; Ukpong & Okon 2013). It is estimated that 1.8 million people die every year from enteric diseases and that about 1000 children under 5 years in low- and middle-income countries die every month as a result of gastroenteritis or diarrhoea. A vast majority of these cases are due to poor drinking water (WHO 2014). For instance, Onyekachi *et al.* (2019) reported a regular increase of waterborne diseases in Enugu, Nigeria, from 2013 to 2015, with a slight decrease in 2016; typhoid fever was observed to have the highest frequency (48.9%), followed by diarrhoea (40%) and then dysentery (11.1%); these waterborne diseases are detected through clinical diagnosis. In addition, Olusa *et al.* (2017) reported that water sources in Ibulesoro community in Akure were heavily polluted and unsafe for human use.

Worldwide, *Escherichia coli* and faecal coliforms are used as classical faecal indicator bacteria in water quality monitoring according to the standards of the World Health Organization. Coliform bacteria are always found in the gastrointestinal tracts of humans and animals, thus making them a candidate for assessing the quality of waters used for drinking and other domestic purposes (Kolarević *et al.* 2011). The detection of *E. coli*, *Salmonella*, *Shigella* or *Clostridium perfringens* in water from groundwater sources is sufficient evidence that the water is not safe for drinking, except when treated. The presence of these bacteria in water often reflects faecal pollution that may originate from different sources (Olajubu & Ogunika 2014; Olalemi & Dauda 2018). Furthermore, the physicochemical properties of water such as temperature, pH, turbidity, electrical conductivity, salinity, total dissolved solids and dissolved oxygen (DO) are essential parameters to consider in the evaluation of water quality, because the intrinsic properties of water may be used to assess the nature and state of water in terms of quality (Kolarević *et al.* 2011).

Quantitative microbial risk assessment (QMRA) is a useful tool for determining the risk posed by pathogens in water and preventing waterborne diseases. It is composed of hazard identification, dose–response model, exposure assessment and risk characterization. Microbial risk assessment involves the estimation of the probability of experiencing an infection from exposure to pathogens (Haas *et al.* 1999; Choi *et al.* 2020). It takes into account the nature of the pathogen and the immunological status of the host such as low gastric acidity in old people, which decreases the infective dose of pathogens, and vaccination of the host, which increases the infective dose. Hosts with high levels of immunity are less susceptible to the risks of infections caused by pathogens. An evaluation of the dose–response relationship and exposure assessment are critical aspects in quantifying the risk of acquiring an illness from exposure to a pathogen (Medema *et al.* 1996; Teunis *et al.* 1999). This risk estimate approach is important for the management of water safety for human health protection.

This study set out to determine the microbial quality of two groundwater sources (borehole and well) used for drinking and other domestic purposes in Akure, Nigeria. The objectives of the study were to determine the level of faecal contamination in the borehole and well; evaluate the relationship between the physicochemical properties of water from the borehole and well and the amount of enteric bacteria; and quantitatively assess the microbial risks of the pathogens in water from the borehole and well.

MATERIALS AND METHODS

Sampling site and collection of samples

Akure is a city in south-western Nigeria and is the capital of Ondo State (Figure 1). Akure is situated at 7°18′29.8N and 7°12′46.5N latitude, 5°07′34.6E and 5°15′38.5E longitude and 396 m elevation above the sea level. The city has a human population of over 800,000. The borehole is located at Red-roof Akad junction, and the well is situated in Apata-piti; both groundwater sources are located around the Federal University of Technology, Akure (FUTA) south gate, Akure Ondo State. The two groundwater sources have separate sources with a distance of about 1 km between them. The borehole and well were selected based on their usage for drinking and other domestic activities by over 1,500 people in the area. The groundwater sources were sited in areas in close proximity to septic tanks (about 10 m from the borehole and well), sludge systems (approximately 15 m from the borehole and well) and waste dumpsites (about 20 m from the borehole and well). The well was shallow and uncovered, which exposed it to various kinds of chemical contamination that make the physicochemical properties of water unstable, such as the pH and electrical conductivity. Also, the temperature of the well environment influences the temperature of the

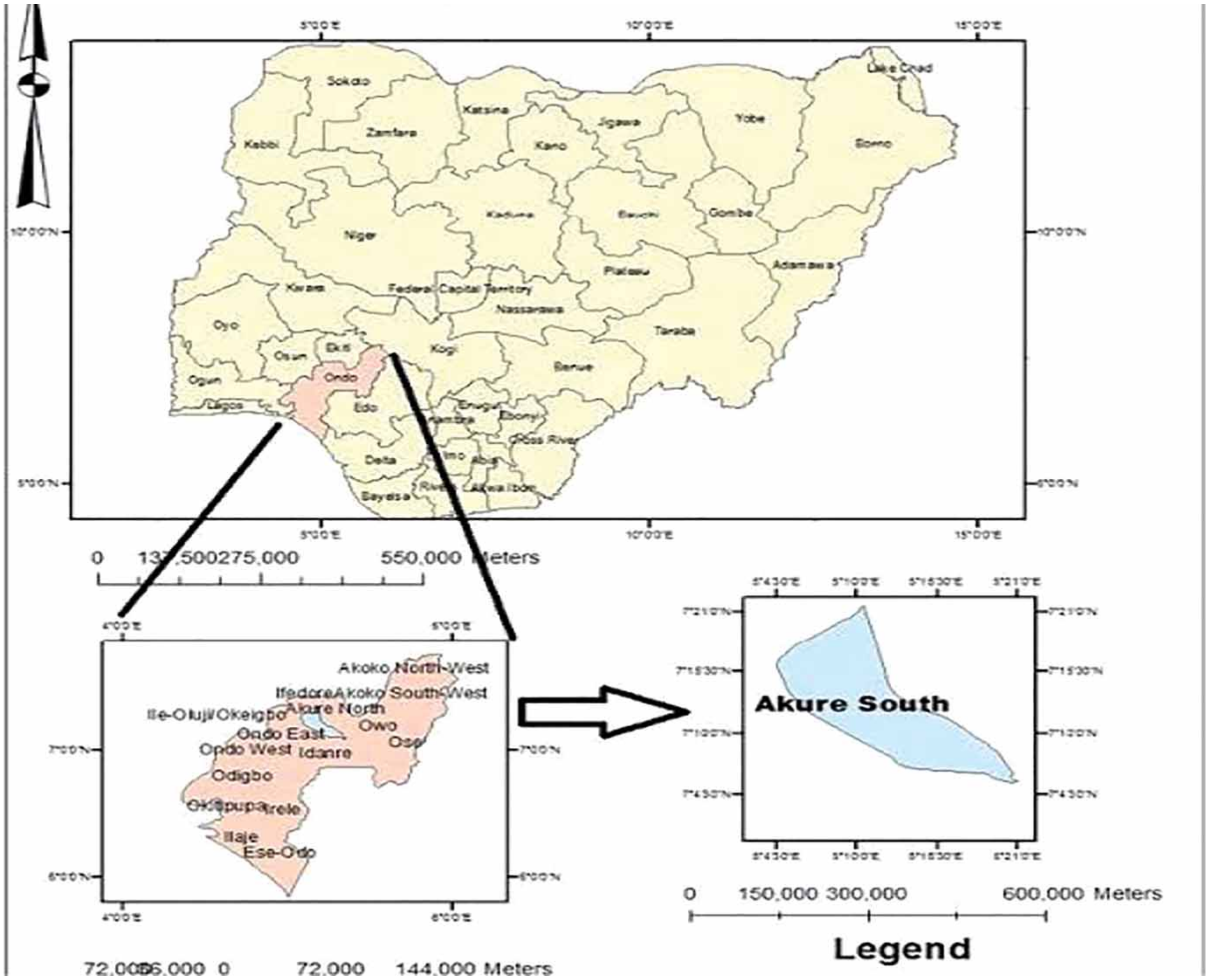

Figure 1 | Locality map showing the sampling area of the Red-roof borehole and Apatapiti well in Akure, Nigeria.

well water. Since the well was uncovered, rain water dropping into the well reduced its salinity and temperature and also added other chemical ions from the atmosphere into the water. The borehole was deeper, which caused the temperature to be low, and its storage tanks were not adequately maintained; the water pipes and storage tanks were not constantly washed to get rid of chemical deposits and biological contaminants, which influence the taste, acidic pH and other physicochemical properties of the borehole water.

Water samples from the borehole and well were collected weekly over a period of 12 weeks between May and September 2019, which was the rainy season in Nigeria in that year and which contributed to the runoff contamination of the water sources. A total of 48 non-sediment samples were collected over the study period; two samples (both microbiological and physicochemical analyses) for each groundwater source were collected every week. On each sampling occasion, about 500 ml were aseptically collected with sterile screw-capped bottles labelled appropriately and transported in a cool box with ice packs to the laboratory for analyses within 1 h.

Enumeration of enteric bacteria in water from the borehole and well

The concentrations of *E. coli*, faecal coliforms, *Salmonella*, *Shigella*, *Clostridium*, *Bifidobacterium* and *Campylobacter* in the water from the borehole and well were determined using a membrane filtration method; 10 ml of the water samples were filtered for each media used for each organism in order to have a moderate number of counts. The membrane filters (0.45 μm) were placed on freshly prepared selective media (media that contain components that allow the growth of the specific organism of interest): membrane lauryl sulphate agar (MLSA) for the isolation of Enterobacteriaceae; the colonies from MLSA were streaked on Eosin methylene Blue (EMB) agar for the specific count of *E. coli*, which appear as greenish metallic sheen on EMB, a feature that differentiates it from other Enterobacteriaceae, membrane faecal coliform agar (*m*-FC), *Salmonella Shigella* agar (SSA), membrane *Clostridium perfringens* agar (*m*-CP), Bifidobacterium selective agar (BSA) and charcoal cefoperazone deoxycholate agar (CCDA) for the isolation of *Campylobacter*. Each medium used was to selectively allow the growth of the bacteria of interest and inhibit the growth of other bacteria present in the water samples. Agar plates were incubated at 37 °C for 24 h (MLSA and EMB), 44 °C for 24 h (*m*-FC) and 37 °C for 24 h (SSA, *m*-CP, BSA and CCDA). BSA and *m*-CP plates were incubated anaerobically, and colonies were counted, calculated and expressed as colony-forming units (CFUs) in 100 ml^{-1} of water. The mean CFUs for each organism were calculated for the 12-week period of sampling. *Salmonella* and *Shigella* were distinguished by the colour of their colonies on SSA; the *Salmonella* colonies appear black, while *Shigella* colonies appear colourless/brown on SSA.

Determination of the physicochemical properties of water from the borehole and well

The temperature of the water was determined on-site during sample collection using a mercury-in-glass thermometer. The pH, electrical conductivity, salinity, total dissolved solids, turbidity and DO of the water samples were determined using a multi-parameter analyzer (HI98194, PH/ORP/EC/DO).

Microbial risk assessment

Salmonella, *Shigella*, *Campylobacter* and *Clostridium* are considered as pathogens that may be responsible for gastrointestinal illness in humans. *Salmonella* is the aetiological agent of typhoid fever and non-typhoidal salmonellosis. Humans may be infected through the ingestion of 10^3 and 10^5 cells (Mahendra & Uma 2007). *Shigella* causes shigellosis in humans, with symptoms ranging from mild to severe and acute diarrhoea. The ingestion of 10 to 100 viable organisms may result in infection (Crockett *et al.* 1996). *Campylobacter* is the aetiological agent of Campylobacteriosis, also known as *Campylobacter* enteritis, which presents symptoms such as fever, headache, abdominal pain, muscle ache, nausea, vomiting and diarrhoea. *C. jejuni* has been reported to be responsible for more than 80% of *Campylobacter* infections. The ingestion of about 500 viable *Campylobacter* cells may cause an infection (Medema *et al.* 1996). *Clostridium* is capable of producing enterotoxin (*cpe* gene) in the small intestine, which may cause gastroenteritis. Symptoms often include water diarrhoea and abdominal cramps. The ingestion of more than 10^6 vegetative cells or spores may result in infection. These pathogens are transmitted through the ingestion of food or water contaminated with the faeces of infected persons (FDA 2012; Choi *et al.* 2020). In order to determine the minimum human health risks from using the water from the borehole and well for drinking and other domestic purposes, microbial risk assessment methods were applied to evaluate the human dose–response data for the pathogens (Table 1). The species and strains of the pathogens are important factors to be considered in determining the health risk assessment of human pathogens, because the species and strains of a single pathogen can have a variable dose–response. For example, some species of *Shigella* have an infectious dose of less than 10 organisms, while those of *Salmonella* can be as high as $>10^5$ organisms (Mahendra & Uma 2007). However, considering all variables equal, the exponential model is a single-hit approach that describes the probability of an infection resulting from the consumption of an average number of a pathogen from a single exposure, whereas the beta-Poisson model assumes that the value defined by the dose–response curve is not a constant one and takes into account the variation in the level of infectivity of the pathogen and sensitivity of the host in their interaction (Haas *et al.* 1999).

The beta-Poisson model (Equation (1)) was utilized to determine the probability of infection associated with exposure to *Salmonella*, *Campylobacter* and *Shigella* in water from the borehole and well, while the exponential model (Equation (2)) was utilized to determine the probability of infection associated with exposure to *Clostridium* in water from the borehole and well. The annual probability of infection (Equation (3)) as a result of consuming water from the borehole and well was also evaluated.

Table 1 | Dose–response models adopted to calculate microbial health risks from exposures to pathogens in water from the borehole and well

Pathogens	Model	Parameters	References
Salmonella	Beta-Poisson	$\alpha = 0.3126; \beta = 23{,}600$	Haas *et al.* (1999)
Shigella	Beta-Poisson	$\alpha = 0.2099; \beta = 1120$	Crockett *et al.* (1996)
Campylobacter	Beta-Poisson	$\alpha = 0.145; \beta = 896$	Medema *et al.* (1996)
Clostridium	Exponential	$r = 1.82 \times 10^{-11}$	Choi *et al.* (2020)

$$P_{\mathrm{i}} = 1 - \left(1 + \frac{N}{\beta}\right)^{-\alpha} \tag{1}$$

$$P_{\mathrm{i}} = 1 - \exp\ (-rN) \tag{2}$$

$$P_{\mathrm{A}} = 1 - (1 - P_{\mathrm{i}})^{365} \tag{3}$$

where P_{i} is the probability of infection; α is a parameter defining the dose–response curve, β is an alternative parameter defining the dose–response curve, r is the rate of natural increase in the population of organism and N is the exposure (colony-forming unit); and P_{A} is the annual probability of infection.

For exposure assessment, the minimum human health risks from using the water from the borehole and well for drinking and other domestic purposes were estimated. An intake of 100 ml was assumed for intentional consumption and intakes of 1 and 10 ml were assumed for accidental consumption as the goal for estimating the minimum human health risks from the ingestion of water from the borehole and well.

Statistical analysis

Data obtained were transformed to $\log_{10}$ and examined using Statistical Package for Social Sciences (SPSS) Version 23.0. The Kolmogorov–Smirnov test for normality was used to determine the distribution pattern of enteric bacteria in the water from the borehole and well. Spearman's rank correlation analysis was used to determine the relationship between the concentration of enteric bacteria and the physicochemical properties of the water from the borehole and well. The dose–response models stated above were used to estimate the risk of infection.

RESULTS

Detection of enteric bacteria in water from the borehole and well

The mean concentrations of *E. coli* in water from the borehole and well were 3.7 and 1.7 $\log_{10}$ cfu/100 ml, respectively, while those of faecal coliforms in water from the borehole and well were 4.0 and 2.4 $\log_{10}$ cfu/100 ml, respectively. Similarly, the mean levels of *Salmonella* in water from the borehole and well were 3.2 and 2.3 $\log_{10}$ cfu/100 ml, respectively, while those of *Shigella* in water from the borehole and well were 3.3 and 2.3 $\log_{10}$ cfu/100 ml, respectively. In addition, the mean concentrations of *Bifidobacterium* in water from the borehole and well were 4.3 and 2.4 $\log_{10}$ cfu/100 ml, respectively, while those of *Campylobacter* in water from the borehole and well were 3.7 and 2.8 $\log_{10}$ cfu/100 ml, respectively. The mean concentrations of *Clostridium* in water from the borehole and well were 3.2 and 3.1 $\log_{10}$ cfu/100 ml, respectively.

Of all the bacteria, *Bifidobacterium* had the highest concentration in water from the borehole, whereas *Clostridium* had the highest concentration in water from the well. On the other hand, *Salmonella* and *Clostridium* had the least concentrations in water from the borehole, whereas *E. coli* had the least concentration in water from the well (Figure 2).

Physicochemical characteristics of water from the borehole and well

The mean values of temperature of water from the borehole and well were 24.3 and 28.1 °C, respectively, while those of pH of water from the borehole and well were 7.2 and 6.6, respectively. Similarly, the mean values of electrical conductivity in water from the borehole and well were 137.6 and 163.5 μS/cm, respectively, whereas those of total dissolved solids in water from the borehole and well were 70.1 and 76.7 mg/l, respectively. In addition, the mean values of salinity in water from the borehole and well were 0.16 and 0.07 ppt, respectively, whereas those of DO in water from the borehole and well were 2.3 and 3.6 mg/l, respectively. The mean values of turbidity of water from the borehole and well were 1.9 and 2.5 NTU, respectively (Table 2).

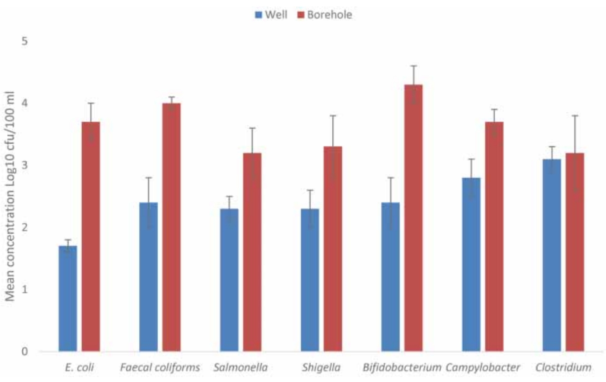

Figure 2 | Mean concentration of enteric bacteria in water samples from the borehole and well.

Distribution pattern of enteric bacteria in water from the borehole and well

Using Kolmogorov–Smirnov test for normality $H_0 = 0.05$, the distribution pattern of enteric bacteria in water from the borehole and well varies significantly. In water from the borehole, *E. coli*, *Salmonella*, *Shigella*, *Bifidobacterium*, *Campylobacter* and *Clostridium* with a significance of 0.200, 0.189, 0.093, 0.063, 0.200 and 0.200, respectively, were normally distributed; while faecal coliforms with a significance of 0.013 were not normally distributed. On the other hand, in water from the well, *E. coli*, faecal coliforms, *Salmonella* and *Clostridium* with a significance of 0.199, 0.147, 0.200 and 0.078, respectively, were normally distributed; while *Shigella*, *Bifidobacterium* and *Campylobacter* with a

Table 2 | Physicochemical characteristics of the water samples from the borehole and well

Parameters	Borehole	Well
Temperature (°C)	24.3 ± 0.8 (23.0–25.5)	28.1 ± 2.5 (23.7–32.0)
pH	7.2 ± 0.4 (6.6–7.9)	6.6 ± 0.5 (6.0–7.3)
Electrical conductivity (μS/cm)	137.6 ± 13.1 (123–173)	163.5 ± 32.7 (93–209)
Total dissolved solids (mg/l)	70.1 ± 8.2 (54–87)	76.7 ± 19.3 (47–104)
Dissolved oxygen (mg/l)	2.3 ± 1.8 (0.5–6.6)	3.6 ± 2.9 (0.5–8.5)
Salinity (ppt)	0.16 ± 0.01 (0.05–0.08)	0.07 ± 0.02 (0.04–0.10)
Turbidity (NTU)	1.9 ± 1.6 (0.1–5.8)	2.5 ± 1.3 (0.6–3.4)

Notes: Values are expressed as mean ± standard deviation (range: minimum – maximum); $n = 24$.

significance of 0.011, 0.008 and 0.030, respectively, were not normally distributed (Table 3).

Correlation between enteric bacteria and the physicochemical characteristics of water from the borehole and well

In water from the borehole, the Spearman's correlation showed that *E. coli* exhibited a negative relationship with salinity ($r = -0.53$), whereas *Salmonella* had a positive relationship with temperature ($r = 0.51$), pH ($r = 0.50$) and total dissolved solids ($r = 0.50$) but showed a negative correlation with turbidity ($r = -0.50$). Similarly, *Shigella* demonstrated a positive relationship with electrical conductivity ($r = 0.56$) and total dissolved solids ($r = 0.50$). *Bifidobacterium* had a positive correlation with pH ($r = 0.50$) and turbidity ($r = 0.80$) and a negative relationship with salinity ($r = -0.60$). *Campylobacter* showed a positive correlation with turbidity ($r = 0.68$), while *Clostridium* demonstrated a negative relationship with electrical conductivity ($r = -0.67$), total dissolved solids ($r = -0.50$) and DO ($r = -0.54$) (Table 4). In water from the well, the Spearman's correlation showed that faecal coliforms had a positive relationship with pH ($r = 0.50$), whereas *Salmonella* demonstrated a positive relationship with temperature ($r = 0.50$) and DO ($r = 0.50$) and a negative relationship with turbidity ($r = -0.56$). In addition, *Shigella* exhibited a negative relationship with turbidity ($r = -0.61$), while *Campylobacter* showed a positive relationship with salinity ($r = 0.50$), and *Clostridium* showed a positive and negative relationship with temperature ($r = 0.50$) and electrical conductivity ($r = -0.53$), respectively (Table 5).

Probability of infection from the consumption of water from the borehole and well

The probability of *Salmonella* infection from the consumption of 1 ml of water from the well and borehole ranged

Table 3 | Kolmogorov–Smirnov test for normality in water from the borehole and well

	Borehole			Well		
Enteric bacteria	**Statistic**	**df**	**Significance**	**Statistic**	**df**	**Significance**
E. coli	0.140	24	0.200	0.200	24	0.199
Faecal coliforms	0.274	24	0.013	0.211	24	0.147
Salmonella	0.202	24	0.189	0.192	24	0.200
Shigella	0.225	24	0.093	0.278	24	0.011
Bifidobacterium	0.236	24	0.063	0.284	24	0.008
Campylobacter	0.194	24	0.200	0.255	24	0.030
Clostridium	0.174	24	0.200	0.231	24	0.078

Table 4 | Significant Spearman's correlation coefficient (r) between enteric bacteria and the physicochemical characteristics of water from the borehole ($p = 0.5$)

	Temp. (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Sal. (ppt)	Turb. (NTU)
E. coli	−0.21	0.18	0.18	0.15	−0.14	**−0.53**	0.10
Faecal coliforms	−0.26	0.05	0.34	0.12	0.34	0.21	0.01
Salmonella	**0.51**	**0.50**	0.40	**0.50**	−0.15	−0.09	**−0.50**
Shigella	0.05	−0.03	**0.56**	**0.50**	0.13	0.41	0.01
Bifidobacterium	0.22	**0.50**	−0.12	0.06	−0.16	**−0.60**	**0.80**
Campylobacter	0.18	0.33	0.05	0.12	−0.08	−0.37	**0.68**
Clostridium	−0.25	0.41	**−0.67**	**−0.50**	**−0.54**	−0.38	0.30

Notes: Values in bold figures indicate significant correlation.
Temp., temperature; EC, electrical conductivity; TDS, total dissolved solids; DO, dissolved oxygen; Sal., salinity; Turb., turbidity; $n = 24$.

Table 5 | Significant Spearman's correlation coefficient (*r*) between enteric bacteria and the physicochemical characteristics of water from the well ($p = 0.5$)

	Temp. (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Sal. (ppt)	Turb. (NTU)
E. coli	−0.14	0.35	0.22	−0.15	−0.05	0.36	0.13
Faecal coliforms	−0.13	**0.50**	−0.17	−0.43	0.31	−0.34	−0.01
Salmonella	**0.50**	−0.03	0.28	0.16	**0.50**	0.26	**−0.56**
Shigella	0.13	−0.07	−0.07	0.10	0.32	0.16	**−0.61**
Bifidobacterium	−0.20	0.19	0.40	0.31	0.38	0.22	−0.06
Campylobacter	−0.18	−0.10	0.13	0.34	0.23	**0.50**	−0.28
Clostridium	**0.50**	−0.02	**−0.53**	0.21	0.32	0.14	0.03

Notes: Values in bold figures indicate significant correlation.
Temp., temperature; EC, electrical conductivity; TDS, total dissolved solids; DO, dissolved oxygen; Sal., salinity; Turb., turbidity; $n = 24$.

from 0.000011 to 0.00006 and from 0 to 0.00037, respectively. The consumption of 10 ml of water from the well and borehole revealed the probability of infection that ranged from 0.00011 to 0.0006 and from 0 to 0.0037, respectively. The probability of *Salmonella* infection from the consumption of 100 ml of water from the well and borehole ranged from 0.0011 to 0.006 and from 0 to 0.037, respectively. The mean probability of *Salmonella* infection from the consumption of 100 ml of water from well was 0.0024 and 0.019 from the borehole (Figure 3). The model predicts the probability of infection with exposure to a single CFU of *Salmonella* in water from the groundwater sources at 1.3×10^{-5}. Similarly, the probability of *Shigella* infection from the consumption of 1 ml of water from the well and borehole ranged from 0.00014 to 0.00077 and from 0.0013 to 0.003, respectively. The consumption of 10 ml of water from the well and borehole revealed the probability of infection that ranged from 0.0014 to 0.0077 and from 0.013 to 0.03, respectively. The probability of *Shigella* infection from the consumption of 100 ml of water from the well and borehole ranged from 0.014 to 0.077 and from 0.13 to 0.3, respectively. The mean probability of *Shigella* infection from the consumption of 100 ml of water from the well was 0.032 and 0.19 from the borehole (Figure 3). The model predicts the probability of infection with exposure to a single CFU of *Shigella* in water from the groundwater sources at 1.9×10^{-4}.

Furthermore, the probability of *Campylobacter* infection from the consumption of 1 ml of water from the well and borehole ranged from 0.00052 to 0.00088 and from 0.00052 to 0.0037, respectively. The consumption of 10 ml of water from the well and borehole revealed the probability of infection that ranged from 0.0052 to 0.0088 and from 0.0052 to 0.037, respectively. The probability of *Campylobacter* infection from the consumption of 100 ml of water from the well and borehole ranged from 0.052 to 0.088 and from 0.052 to 0.37, respectively. The mean probability of *Campylobacter* infection from the consumption of 100 ml of water from the well was 0.071 and 0.22 from borehole (Figure 3). The model predicts the probability of infection with exposure to a single CFU of *Campylobacter* in water from the groundwater sources at 1.6×10^{-4}. On the other hand, the probability of *Clostridium* infection from the consumption of 1 ml of water from the well and borehole ranged from 7.3×10^{-11} to 3.4×10^{-10} and from 3.6×10^{-11} to 5.8×10^{-10}, respectively. The consumption of 10 ml of water from the well and borehole revealed the probability of infection that ranged from 7.3×10^{-10} to 3.4×10^{-9} and from 3.6×10^{-10} to 5.8×10^{-9}, respectively. The probability of *Clostridium* infection from the consumption of 100 ml of water from the well and borehole ranged from 7.3×10^{-9} to 3.4×10^{-8} and from 3.6×10^{-9} to 5.8×10^{-8}, respectively. The mean probability of *Clostridium* infection from the consumption of 100 ml of water from the well was very low, at 2.4×10^{-8} and 3.0×10^{-8} from the borehole (Figure 3). The model predicts the probability of infection with exposure to a single CFU of *Clostridium* in water from the groundwater sources at 1.82×10^{-11}. Based on these predictions, the risks of infection associated with the pathogens in water from the borehole and well were in this order *Clostridium* < *Salmonella* < *Campylobacter* < *Shigella*. In addition, the risks of infection from the consumption of water from the borehole were greater than those from the well.

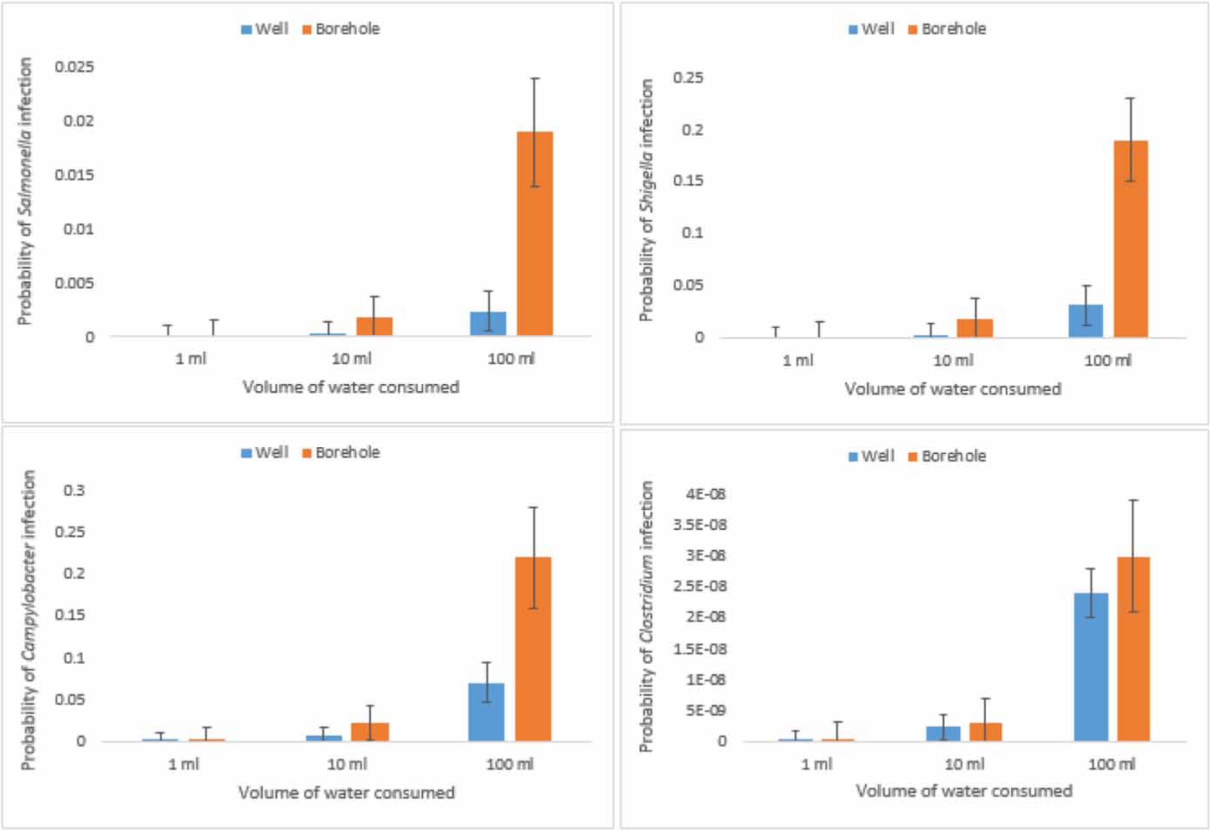

Figure 3 | Mean probability of infection from the consumption of water from well and borehole.

To compare the level of risk of infection associated with the bacteria, the mean annual probabilities of infection as a result of consuming water from the well and borehole contaminated with *Salmonella*, *Shigella*, *Campylobacter* and *Clostridium* were determined. The mean annual probability of *Salmonella* infection due to the ingestion of 1 ml of water from the well and borehole was 0.0087 and 0.067, respectively; 10 ml of water from the well and borehole was 0.084 and 0.5, respectively, whereas the mean annual probability of *Shigella* infection due to the ingestion of 1 ml of water from the well and borehole was 0.11 and 0.5, respectively; 10 ml of water from the well and borehole was 0.69 and 1.0, respectively. Similarly, the mean annual probability of *Campylobacter* infection due to the ingestion of 1 ml of water from the well and borehole was 0.23 and 0.55, respectively; 10 ml of water from the well and borehole was 0.93 and 1.0, respectively, whereas the mean annual probability of *Clostridium* infection due to the ingestion of 100 ml of water from the well and borehole was 8.8×10^{-6} and 1.1×10^{-5}, respectively (Figure 4).

DISCUSSION

The microbial quality of the two groundwater sources (borehole and well) used for drinking and other domestic purposes in Akure, Nigeria, was determined. The influence of the physicochemical characteristics of water on enteric bacteria detected in the water from the borehole and well was evaluated; and the risks of infection associated with the usage of the water from the borehole and well for drinking were quantitatively assessed. The concentration of *E. coli* in water from the well was lower than those observed by Osvalda *et al.* (2016) in water from 21 wells in Gargano, southern Italy. It was also higher than those observed in six wells (W1–6), but lower than those observed in three wells (W7–9) in the

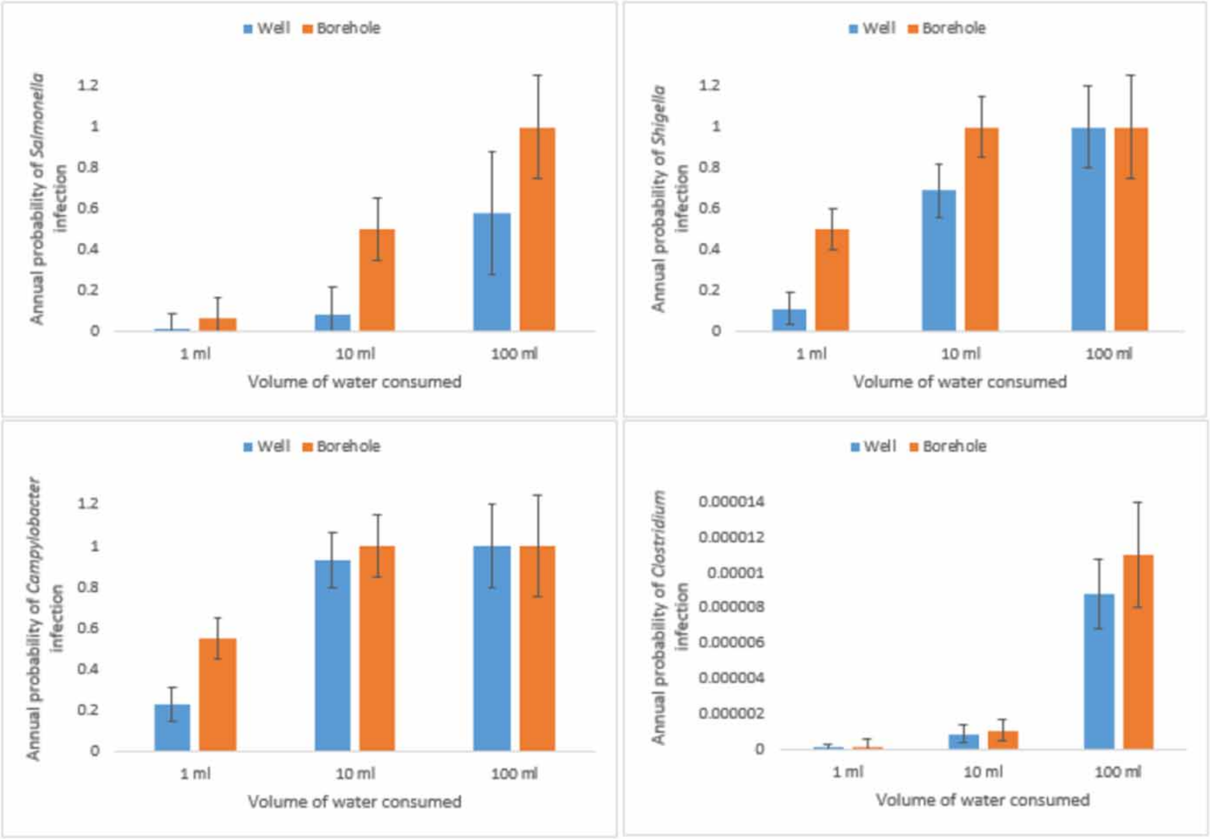

Figure 4 | Annual probability of infection from the consumption of water from well and borehole.

peri-urban tropical lowlands of Dar es Salaam, Tanzania (Mushi *et al.* 2012). Similarly, the concentration of faecal coliforms in water from the borehole was higher than those reported by Ohimain *et al.* (2013) in boreholes situated in Yenagoa metropolis, Bayelsa State, Nigeria. It is important to note that the levels of *E. coli* and faecal coliforms in water from the borehole were higher than those from the well. This observation is likely a result of inadequate maintenance of the borehole and the storage tanks. The presence of septic tanks, sludge systems and waste dumpsites around the borehole and well is probably responsible for the levels of *E. coli* and faecal coliforms detected. The levels of *E. coli* and faecal coliforms in the water from the borehole and well do not conform to the guideline on the microbial quality of drinking water (WHO 2011).

The concentrations of *Salmonella* in water from the borehole and well were higher than those observed by Osvalda *et al.* (2016) in 47 aquifers in Salento, Italy. Most waterborne diseases associated with *Salmonella* are mostly due to the ingestion of contaminated water and inadequate water management practices (Kozlica *et al.* 2010). In this study, *Shigella* was detected in water from the borehole and well on all sampling occasions, but the concentration was higher in the borehole than in the well. Faecal contamination of water may expose the inhabitants of an area to shigellosis as the percentage of *Shigella* can be very high in water samples. The results obtained by Oyedum *et al.* (2016) showed that *Shigella* was about 11.7% of 13 bacterial isolates detected in the water obtained from public boreholes in Bosso Town, North Central, Nigeria. Also, Adeiza *et al.* (2018) detected *Shigella* in five out of 10 borehole water sources in Kano State, Nigeria. *Campylobacter* is known to be responsible for most gastroenteritis transmitted through food and water (Moore *et al.* 2005). The concentration of *Campylobacter* observed in this study was higher than those reported by Savill *et al.* (2001) in New Zealand recreational and drinking waters. *Bifidobacterium* survive for a limited time in water outside the GIT of man and animals and their presence in waters indicates recent contamination (WHO 2010). The high concentration of *Bifidobacterium* in water from the borehole and well suggests a recent and continuous contamination of the groundwater sources. The concentration of *Clostridium* observed in this study was higher than those observed by Potgieter *et al.* (2006) in the groundwater sources used by rural communities in Limpopo Province, South Africa, but lower than that observed by Abok *et al.* (2018) in site 5, LMD borehole water, Isiolo County, Kenya.

Temperature is part of the factors that determine the distribution of pathogens in groundwater (O'Dwyer *et al.* 2014; Engström *et al.* 2015). Temperature was observed to have a positive relationship with the concentration of *Salmonella* in water from the borehole and well, and *Clostridium* in water from the borehole. This is in agreement with the findings of Olalemi & Dauda (2018), where the authors observed a positive relationship between temperature and enteric bacteria in the water from selected groundwater sources in four geographical locations within Akure metropolis. The pH of water from the borehole and well was within the range of the 6.5–8.5 standard for drinking water (Istifanus *et al.* 2013; WHO 2017). The values of pH that correlated positively with *Salmonella* and *Bifidobacterium* in water from the borehole, and faecal coliforms in water from the well, agreed with the findings of Youssef *et al.* (2014), where the authors observed a positive correlation between pH and faecal coliforms in El Jadida city, Morocco. The EC of water from the borehole and well fall within the maximum limit of 1000 µS/cm for drinking water (Istifanus *et al.* 2013). The values of EC showed a positive correlation with *Shigella* in water from the borehole and a negative correlation with *Clostridium* in water from the borehole and well. The TDS in water from the borehole and well conform to the maximum limit of 500 mg/l for drinking water (Istifanus *et al.* 2013). TDS showed a negative correlation with *Clostridium* in water from the borehole, and the result was not expected, as TDS are meant to promote the growth of *Clostridium* in the water. This observation is contrary to the findings of Olalemi *et al.* (2020a), where the authors reported a positive relationship between TDS and *Clostridium* in water samples from a river in Akure, Nigeria. DO was observed to have a direct relationship with *Salmonella*; this is in agreement with Tirumalesh *et al.* (2015), where the authors observed a positive relationship between DO and faecal indicator bacteria. The DO in water from the borehole and well fall within the maximum limit of 5 mg/l for drinking water (WHO 2017). Although the level of salinity in the well was low, a positive relationship with *Campylobacter* was observed; this finding aligns with the report of Vahid *et al.* (2017), where the authors observed a positive correlation between salinity and enteric bacteria in the Persian Gulf in the Bushehr beach zone. Turbidity showed a positive correlation with *Bifidobacterium* and *Campylobacter* in water from the borehole; this is an expected result, because the higher the turbidity of water, the higher the availability of nutrients that support the growth of bacteria in water. This is in agreement with studies that have demonstrated that turbidity has a direct relationship with the load of bacteria in water (Azhdarpoor *et al.* 2019; Olalemi *et al.* 2020b). The turbidity of water from the borehole and well conforms to the maximum limit of 5 NTU (Istifanus *et al.* 2013).

The mean annual probabilities of *Salmonella* and *Shigella* infections due to the ingestion of 10 ml of water from the well were low compared with those from the borehole. The mean annual probabilities of *Salmonella* and *Shigella* infections observed in this study were lower than those observed by Teklehaimanot *et al.* (2015), where the authors reported high annual risks of *S. typhimurium* and *S. dysenteriae* infections due to the ingestion of water from upstream and downstream sampling points on rivers receiving effluents from some wastewater treatment facilities in South Africa. Conversely, the mean probability of *Campylobacter* infection from the consumption of water from the well and borehole was higher than the value observed by Lee *et al.* (2019), where the authors reported the risk of illness caused by *Campylobacter jejuni* from consuming ground meat to be 5.68×10^{-10}. In addition, the probability of *Clostridium* infection from the consumption of water from the well and borehole was considered to be low, but higher than the value observed by Choi *et al.* (2020), where the authors reported the mean risk of *C. perfringens*-associated illness from the consumption of Kimchi in South Korea to be 1.21×10^{-17}.

In the present study, an intake of 100 ml of water was assumed for intentional consumption, and intakes of 1 and 10 ml of water were assumed for accidental consumption as the goal for determining minimum human health risks. The World Health Organization estimated the highest acceptable risk limit of enteropathogen infection in drinking water to be 10^{-4} (WHO 2001). The mean probabilities of *Salmonella*, *Shigella* and *Campylobacter* infections from both accidental and intentional consumption of water from the borehole and well were higher than the acceptable risk limit, whereas the mean probability of *Clostridium* infection from accidental and intentional consumption of water from the borehole and well was lower than the acceptable risk limit. In the study area, a human population of approximately 1500 relies on the water from the borehole and well for drinking and other domestic activities. Assuming that there was uniform risk and if each person in the population was exposed to 1000 CFU (3 log) of *Salmonella* or *Shigella* or *Campylobacter* in the water from the borehole and well once in a year, the annual cases of infection associated with exposure to *Salmonella* would be predicted to be 20 cases; those associated with exposure to *Shigella* would be predicted to be 290 cases and those associated with exposure to *Campylobacter* would be predicted to be 240 cases. The risks of infection for *Shigella* and *Campylobacter* were 10 times greater than those for *Salmonella.* This is a result of the lower infective ability of *Salmonella* compared with those of *Shigella* and *Campylobacter*, since the population is predicted to be exposed to the same number of colonies of the bacteria.

In order to reduce or eliminate the risks of infection associated with the usage of water from the borehole and well for drinking, adequate treatment of the water must be a routine exercise. This will protect residents relying on the water sources from gastrointestinal illnesses. This will also reduce morbidity and ill health from diarrheal diseases in children under 5 years. Sinking boreholes and digging wells near septic tanks, sludge systems and waste dumpsites should be discouraged. In addition, boreholes and their storage tanks must be adequately maintained and wells must be covered properly.

CONCLUSION

The findings of this study demonstrated that the two groundwater sources (borehole and well) used for drinking and other domestic purposes do not have high microbial quality. The physicochemical characteristics of water from the borehole and well have a correlation with the level of enteric bacteria in the water sources. The risk of *Salmonella* or *Shigella* or *Campylobacter* infections from the ingestion of water from the borehole and well was greater than the acceptable risk limit, thus exposing the residents relying on the water for drinking to potential risks of gastrointestinal illness. The high rates of enteric diseases reported in the communities where the borehole and well are located are the ones associated with *Salmonella*, *Shigella* and *Campylobacter*. These pathogens have a low infective dose, which reflects in the high cases of enteric diseases in these communities.

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DATA AVAILABILITY STATEMENT

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

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