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Comparative hazard evaluation of enteric bacteria in two surface water sources in Akure, Nigeria

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Abstract

This study was carried out to determine the level of enteric bacteria in two surface water sources (river and stream) commonly used for domestic, recreational and agricultural purposes in Akure, Nigeria. This is to gain a better understanding of the level of faecal pollution of the water sources and the potential health risks associated with usage of the waters for human activities. Water samples were collected from the river and the stream from May to September, 2019 ($n = 24$). The concentration of enteric bacteria in the water samples was determined using the membrane filtration technique, while the physicochemical characteristics of the water samples were determined using the standard method. Results revealed that the concentration of *Escherichia coli* ranged from 3.00 to 4.78 $\log_{10}$ cfu/100 ml and 3.48 to 5.75 $\log_{10}$ cfu/100 ml in water samples from the river and stream respectively; *Bifidobacterium* ranged from 4.18 to 5.00 $\log_{10}$ cfu/100 ml and 3.87 to 4.66 $\log_{10}$ cfu/100 ml in water samples from the river and stream respectively; *Salmonella* ranged from 3.30 to 4.30 $\log_{10}$ cfu/100 ml and 2.60 to 4.32 $\log_{10}$ cfu/100 ml in water samples from the river and stream respectively. Water temperature ranged from 22.1 to 28.5 °C and 23.64 to 25.56 °C in the river and stream respectively; turbidity ranged from 12.28 to 29.11 NTU and 17.07 to 61.80 NTU in the river and stream respectively. Spearman's rank correlation showed that *Salmonella* had positive relationship with temperature ($r = 0.556$) in water samples from the stream whereas *Bifidobacterium* exhibited a positive relationship with dissolved oxygen ($r = 0.557$) in water samples from the river. While the stream appeared to have a higher turbidity than the river, the level of enteric bacteria in the river was higher than that in the stream. Based on microbiological water quality categories, the findings from this study demonstrated that the level of enteric bacteria in the river and stream suggests strong faecal pollution that may pose potential risks of diarrheal diseases to humans. Water from these two surface water sources must be treated before use in order to protect human health.

Key words: faecal indicator bacteria, human health, pathogens, risk assessment, surface water

Highlights

- The level of faecal pollution in the river and stream were 'strong' and 'excessive' respectively.
- The concentration of enteric bacteria in the surface waters does not conform to WHO guidelines on drinking and bathing water.
- All the water samples showed *E. coli*, faecal coliforms, *Salmonella*, *Shigella*, *Clostridium*, *Bifidobacterium*, *Campylobacter* and enterococci exceeding 2 log.
- *E. coli*, faecal coliforms, *Salmonella* and *Campylobacter* were normally distributed, while enterococci were not normally distributed.
- Rainfall, salinity, dissolved oxygen influenced the levels of enteric bacteria in the surface waters.

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INTRODUCTION

In Nigeria, about 74% of all the water used is from surface water sources and approximately 47 million people, representing about 27% of the human population, rely exclusively on streams, ponds, rivers and rainwater for domestic, recreational and agricultural purposes (Longe *et al.* 2010; Raji & Ibrahim 2011; Aladejana & Talabi 2013). Studies have shown that incidence and prevalence of outbreak of waterborne diseases such as cholera, diarrhoea, dysentery and gastroenteritis are higher in areas that solely depend on water sources prone to faecal contamination (Edberg *et al.* 2000; Oguntoke *et al.* 2009; Olalemi & Dauda 2018). Josiah & Joseph (2018) reported that respondents that suffered from waterborne diseases as a result of consumption of water from different water sources in Akure, Ondo State showed that 5% had cholera while another 15% suffered from typhoid fever. Another related study in Nigeria demonstrated an increase from 14.61 to 50.56% in the number of patients that suffered from waterborne diseases as a result of drinking water from midstream in Amassoma community, Niger Delta from 2005 to 2007 (Nwidu *et al.* 2008). In addition, Oguntoke *et al.* (2009) studied residential areas with cases of waterborne diseases in Ibadan, Oyo State and reported high cases of waterborne diseases in Oja-Oba, Gbekuba and Ago-Taylor and low cases in Oluyole, Jericho, Ring-Road, Iyaganku and Popoyemoja. Typhoid fever (39.3%) and bacillary dysentery (26.7%) were mostly reported, with about 50% of the diseases occurring between July and September which are periods of heavy rainfall in Nigeria. Omole *et al.* (2015) also reported the occurrence of typhoid, cholera and skin diseases in Ota, Ogun State as a result of drinking and bathing in polluted surface water sources.

Surface water is an important natural resource used for many purposes, especially for domestic, recreational and agricultural activities. The majority of water used for public supply, irrigation, mining and industrial purposes are from surface water sources. A river is a naturally formed water body that flows on a course. The water collected in a river usually comes from precipitation at a higher altitude and then flows towards another river, lake, sea or ocean (McCabe 2011). A stream is a surface water body that flows within the bed and banks of a channel. The difference between rivers and streams lie solely on their sizes, because the river is a bigger body of water while a stream is smaller (Alexander *et al.* 2015). The river is a collection of streams, whereas the stream is a single flowing body of water. On the basis of location and certain characteristics, streams are referred to as different names; long large streams are sometimes called river as streams form the upper arm of the river. The river's shape is defined by its waterbeds and its volume varies depending on the shape of its riverbeds and meanders. The streams usually have a fast current, often determined by their descent from higher grounds. The character of a stream depend on its gradient which is determined by its base level of erosion, and also responsible for formation of flood plain and meander by the stream (McCabe 2011). The increase in population, pollution and environmental degradation have a resultant effect on the contamination of surface water by chemical and biological substances (Malhotra *et al.* 2015). Contamination of water with faecal materials from human origin may be considered to be a human health risk upon consumption (Scott *et al.* 2003). Warm-blooded animals excrete enteric bacteria such as total coliforms, faecal coliforms (thermotolerant coliforms), *Escherichia coli* and intestinal enterococci (faecal streptococci), that may survive for a period of time in the aquatic environment.

Worldwide, the microbiological quality of water is determined on the basis of enumeration of indicator bacteria such as total coliforms, faecal coliforms, *E. coli* and intestinal enterococci (WHO 2001; Anyamene & Ojiagu 2014; Olalemi 2019). The presence of these indicator bacteria in water suggests faecal contamination and this may lead to evaluation of how the contamination occurred, the level of the contamination and the right steps to eliminating or preventing future occurrence of the contamination. In addition, the presence of these indicator bacteria serves as potential risk to public health. The higher the level of indicator bacteria, the higher the level of faecal contamination and the greater

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the risk of waterborne diseases (Pipes 2001; Nwachukwu & Otokunefor 2006; Baudart *et al.* 2009; Anyamene & Ojiagu 2014). A wide range of pathogenic microorganisms may be transmitted to humans through water contaminated with faecal material. These include enteropathogenic agents such as *Salmonella*, *Shigella*, enteroviruses and multicellular parasites as well as opportunistic pathogens like *Pseudomonas aeroginosa*, *Klebsiella*, *Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Hodegkiss 2002).

Physicochemical properties of water such as dissolved oxygen (DO), temperature, electrical conductivity (EC), salinity, total dissolved solids (TDS), pH and turbidity can be used to analyze the water quality. For instance, DO of water produces information about the biochemical oxygen demand (BOD) which explains the concentration of organic matter present in the water, and chemical oxygen demand (COD) reveals the rate of industrial waste contamination of the water (Najafzadeh *et al.* 2018). The water quality parameters are essential for the survival of bacterial pathogens in the water which makes them important factors to consider in microbiological water quality of water (Singh *et al.* 2010; Najafzadeh *et al.* 2018).

This study aims to determine the level of enteric bacteria in two surface water sources (river and stream) commonly used for domestic, recreational and agricultural purposes in Akure, Nigeria. This is to gain a better understanding of the level of faecal pollution of the water sources and the potential health risks associated with usage of the waters for human activities. The objectives of the study are to examine the distribution pattern of enteric bacteria in the water samples and to assess the effect of meteorological (rainfall) and physicochemical factors on the occurrence of enteric bacteria in the water samples from the river and stream.

MATERIALS AND METHODS

Sampling site and collection of samples

The study areas were the River Malaika located around Obanla, Federal University of Technology, Akure (FUTA) north gate and Glory of God stream located around Roadblock, FUTA junction, Akure (Figure 1). The river and stream were selected because of their exposure to various sources of pollution as a result of various human activities taking place in and around the water sources. Other pollution sources are runoff from agricultural soils during rainfall or storm events, direct defecation by animals such as cattle, goats, birds, and sheep, and close proximity to sewer channels. The river emerged from the meeting of two different rivers: River Atinikaro, which flows from the direction of Deeper Life camp ground and River Aule, which flows from direction of Igbokoda. River Malaika is located near Ipinsa Community, where some of the inhabitants (approximately 1,000 people) use the water from the river for drinking and other domestic purposes. The water is also used for construction of buildings and agricultural (irrigation of crops) activities. The water from Glory of God stream is also used for domestic activities such as washing, bathing and some agricultural activities such as farming and drinking water for animals. Sampling activities were carried out weekly over a period of 12 weeks ($n = 24$) between the months of May and September, 2019, which are the months of heavy rainfall and runoff. The river is approximately 1.5–2.5 metres deep and 2–3 metres wide, whereas the stream is approximately 1–1.5 metres deep and 1–2 metres wide. The representative monitoring points on the river and stream were about 0.1 metres from the bank. On each sampling occasion, a grab sample of approximately one litre of water from the river and stream was collected at a depth of about 20–30 cm in a pre-sterilised plastic bottle. The water samples were transported to the laboratory in a cool box with ice packs and processed immediately within less than one hour.

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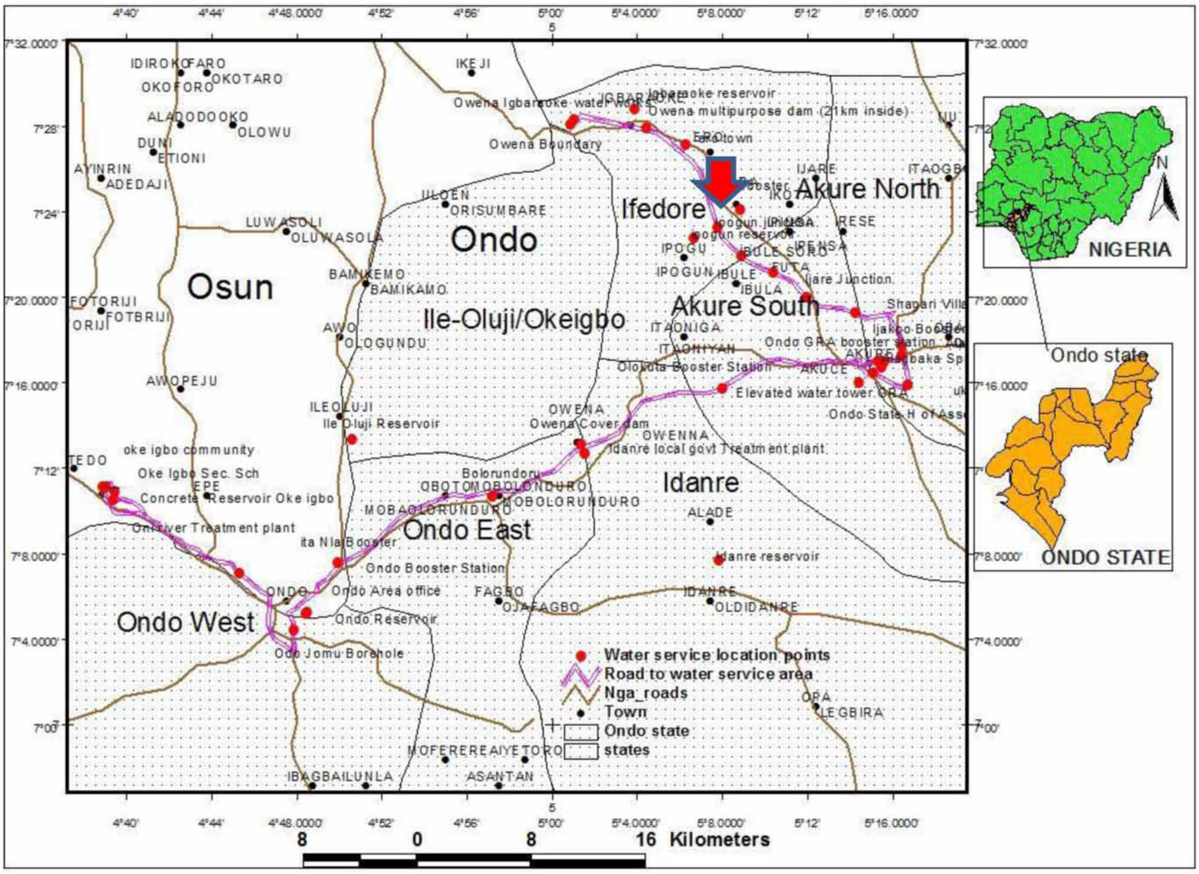

Figure 1 | Locality map showing sampling area of River Malaika and Glory of God Stream (red arrow) in Akure, Nigeria.

Enumeration of enteric bacteria in water samples from the river and stream

The concentrations of *E. coli*, faecal coliforms, *Salmonella, Shigella, Clostridium, Bifidobacterium, Campylobacter* and intestinal enterococci in the water samples from the river and stream were determined using the membrane filtration method. The membrane filters (0.45 μm) were placed on freshly prepared selective media: Membrane lauryl sulphate agar (MLSA), Eosin methylene Blue (EMB) agar, Membrane faecal coliform agar (*m*-FC), *Salmonella Shigella* agar (SSA), Membrane *Clostridium perfringens* agar (*m*-CP), *Bifidobacterium* selective agar (BSA), Charcoal Cefoperazone Deoxycholate Agar (CCDA) and Membrane enterococcus agar (*m*-EA). Agar plates were incubated at 37 °C for 24 hours (MLSA, EMB), 44 °C for 24 hours (*m*-FC) and 37 °C for 24 hours (SSA, *m*-CP, BSA, CCDA), 37 °C for 48 hours (*m*-EA). BSA and *m*-CP plates were incubated anaerobically and colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml^{-1} of water.

Determination of meteorological and physicochemical properties of water samples from the river and stream

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 dissolved oxygen of the water samples were determined using a multi-parameter analyzer (HI98194, PH/ORP/EC/DO) in the laboratory in less than an hour of sampling. The water parameters were determined by dipping the probe of the instrument into the water samples in the sterile bottle and the readings observed were recorded. The rainfall data of the sampling area at 48

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hours, 24 hours before sample collection and on sampling day were obtained from the Department of Meteorology, FUTA.

Statistical analysis

Data obtained were transformed to $\log_{10}$ and examined using Statistical Package for Social Sciences (SPSS) Version 23.0. Kolmogorov-Smirnov test for normality was used to determine the distribution pattern of enteric bacteria in the water samples. Spearman's rank correlation analysis was used to determine the relationship between the concentration of enteric bacteria, meteorological and physico-chemical properties of the water samples.

RESULTS

Detection of enteric bacteria in water samples from the river and stream

The mean total viable count of enteric bacteria in the water samples collected over 12 weeks showed that *Bifidobacterium* species had the highest count in the river with a mean total count of 4.57 $\log_{10}$ cfu/100 ml; the mean total count of *Bifidobacterium* species in the stream was 4.51 $\log_{10}$ cfu/100 ml. Faecal coliforms had the highest count in the stream with a mean total count of 5.45 $\log_{10}$ cfu/100 ml, the mean total count of faecal coliform in the river was 4.53 $\log_{10}$ cfu/100 ml, enterococci had the lowest count at 2.52 $\log_{10}$ cfu/100 ml and 2.92 $\log_{10}$ cfu/100 ml in both river and stream respectively. *E. coli* had a mean total viable count of 4.35 $\log_{10}$ cfu/100 ml and 5.33 $\log_{10}$ cfu/100 ml in the river and stream respectively, *Clostridium* had a mean total viable count of 4.32 $\log_{10}$ cfu/100 ml and 4.41 $\log_{10}$ cfu/100 ml in the river and stream respectively, *Salmonella* had a total mean viable count of 4.10 $\log_{10}$ cfu/100 ml and 3.77 $\log_{10}$ cfu/100 ml in the river and stream respectively, *Shigella* had a total mean viable count of 3.76 $\log_{10}$ cfu/100 ml and 3.97 $\log_{10}$ cfu/100 ml in river and stream respectively, *Campylobacter* had a total mean viable count of 3.60 $\log_{10}$ cfu/100 ml and 3.59 $\log_{10}$ cfu/100 ml in river and stream respectively (Figure 2).

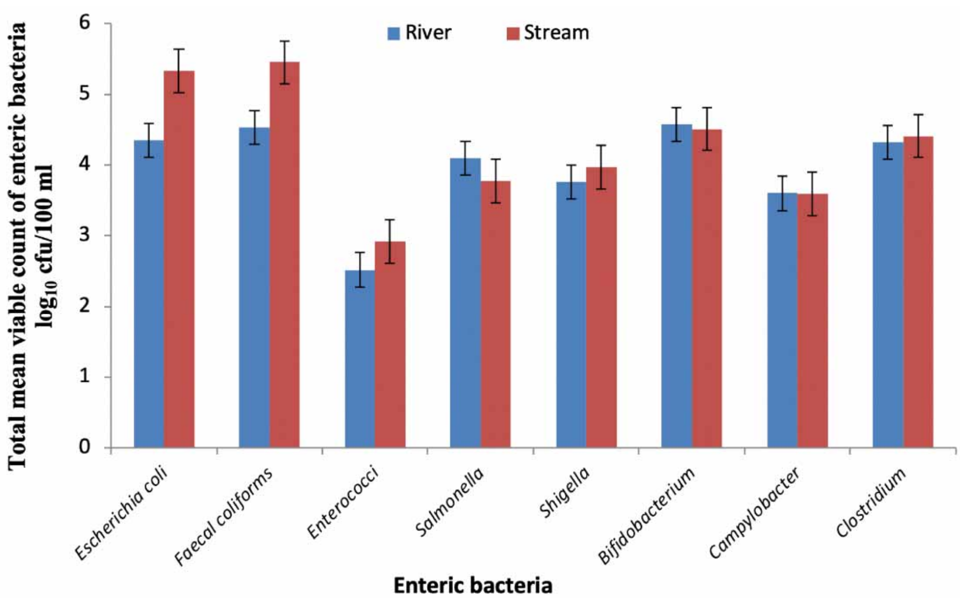

Figure 2 | Total viable count of enteric bacteria in water samples from the river and stream.

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Meteorological and physicochemical characteristics of the water samples from the river and stream

The temperature of the water samples ranged from 22.10 to 28.50 °C in the river and 23.64 to 25.56 °C in the stream; the temperature values in both river and stream tends to be stable, with little variation observed in the river, this stability is essential for enteric bacteria to grow and survive in the water as it supports their metabolic activities. The pH values ranged from 6.19 to 7.83 in the river and 7.07 to 7.43 in the stream; these values are within the range of neutral and therefore conducive for enteric bacteria and other pathogens. The electrical conductivity ranged from 91 to 165 μS/cm in the river and 154 to 314 in the stream; these values reflect a low amount of ions and salinity, which allows the bacteria to carry out metabolic activities needed for their growth and survival. Turbidity values ranged from 12.28 to 29.11 NTU in the river and 14.99 to 61.80 NTU in the stream, indicating that the stream was more turbid and may contain higher levels of contaminants than the river. Salinity ranged from 0.04 to 0.08 ppt in the river and 0.07 to 0.15 ppt in the stream. Total dissolved solids ranged from 40 to 89 mg/l in the river and 77 to 157 mg/l in the stream; showing large variation of organic and inorganic ions that are suitable for various kinds of bacteria in water. The amount of dissolved oxygen ranged from 0.48 to 7.81 mg/l in the river and 0.51 to 7.63 mg/l (Table 1).

Table 1 | Physicochemical characteristics of the water samples from the river and stream

Physicochemical parameters	Mean ± S.D (Min.–Max.) (river)	Mean ± S.D (Min.–Max.) (stream)
Temperature (°C)	25.01 ± 0.52 (22.1–28.5)	24.65 ± 0.17 (23.64–25.56)
pH	7.02 ± 0.14 (6.19–7.83)	7.09 ± 0.08 (7.07–7.43)
Electrical conductivity (μS/cm)	118.67 ± 8.56 (91–165)	215.33 ± 18.05 (154–314)
Total dissolved solids (mg/l)	62.06 ± 5.54 (40–89)	109.67 ± 9.09 (77–157)
Dissolved oxygen (mg/l)	3.34 ± 0.75 (0.48–7.81)	2.67 ± 0.64 (0.51–7.63)
Salinity (ppt)	0.05 ± 0.00 (0.04–0.08)	0.11 ± 0.01 (0.07–0.15)
Turbidity (NTU)	20.64 ± 2.00 (12.28–29.11)	33.36 ± 3.90 (14.99–61.8)

Key: Values are expressed as Mean ± Standard Deviation ($n = 12$) (Range: Min. 'Minimum' – Max. 'Maximum').

The values of rainfall for the river and stream ranged from zero to 15.00 mm at 48 hours before sampling and zero to 76.80 mm at 24 hours before sampling; there was no rainfall during sampling activities throughout the study period. The rainfall was observed to have negative impacts on temperature and salinity but positive impacts on turbidity in both river and stream; rainfall was also observed to have negative impacts on EC and TDS of the stream water (Table 5 and 6).

The residence time of the river and stream was observed to be 7 and 13 days respectively.

Distribution pattern of enteric bacteria in the water samples from river and stream

Kolmogorov-Smirnov[a] test for normality $H_0 = 0.05$ showed that the distribution pattern of enteric bacteria in river and stream vary significantly. *E. coli*, faecal coliforms, *Salmonella*, *Shigella* and *Campylobacter* with significance of 0.060, 0.200, 0.105 and 0.200 respectively were normally distributed, while enterococci, *Clostridium* and *Bifidobacterium* with significance of 0.000, 0.001 and 0.002 respectively were not normally distributed in the water samples from the river. On the other hand, *E. coli,* faecal coliforms, *Salmonella*, *Bifidobacterium*, *Clostridium* and *Campylobacter* with significance of 0.200, 0.200, 0.058, 0.139, 0.110, and 0.200 respectively were normally distributed; while both enterococci and *Shigella* with significance of 0.00 were not normally distributed in the water samples from the stream (Table 2).

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Table 2 | Kolmogorov–Smirnov[3] test for normality in water samples from river and stream

	Water samples from river			Water samples from stream		
Enteric bacteria	**Statistic**	**Df**	**Significance**	**Statistic**	**Df**	**Significance**
E. coli	0.237	12	0.060	0.148	12	0.200
Faecal coliforms	0.196	12	0.200	0.148	12	0.200
Enterococci	0.480	12	0.000	0.530	12	0.000
Salmonella	0.222	12	0.105	0.238	12	0.058
Shigella	0.171	12	0.200	0.350	12	0.00
Bifidobacterium	0.315	12	0.002	0.213	12	0.319
Campylobacter	0.225	12	0.094	0.197	12	0.200
Clostridium	0.331	12	0.001	0.221	12	0.110

Relationship between enteric bacteria, meteorological and physicochemical characteristics of water samples from river and stream

In water samples from the river, the Spearman's correlation showed that *Salmonella* had a positive relationship with rainfall at 48 hours before sampling ($r = 0.65$). *Bifidobacterium* exhibited positive relationships with electrical conductivity ($r = 0.56$), dissolved oxygen ($r = 0.56$) and salinity ($r = 0.50$). *Campylobacter* also exhibited a positive relationship with turbidity ($r = 0.58$) and dissolved oxygen ($r = 0.50$). *Clostridium* also exhibited a positive relationship with electrical conductivity ($r = 0.57$). *Clostridium* also exhibited a positive relationship with dissolved oxygen ($r = 0.54$). *Clostridium* also exhibited a positive relationship with dissolved oxygen ($r = 0.54$) and total dissolved solids ($r = 0.50$). *Shigella* also exhibited a negative relationship with salinity ($r = -0.50$) (Table 3). In water samples from the stream, the Spearman's correlation showed that temperature showed positive relationships with *Salmonella* ($r = 0.56$), faecal coliforms ($r = 0.72$). Temperature also showed positive relationship with *Campylobacter* ($r = 0.50$) and Enterococci ($r = 0.50$). *E. coli* had positive relationships with electrical conductivity ($r = 0.51$), Total Dissolved Solids ($r = 0.51$) and salinity ($r = 0.60$). *Clostridium* also had a positive relationship with pH ($r = 0.52$) (Table 4). Temperature showed a negative relationship with TDS, DO and turbidity in the river ($r = -0.55$, -0.69 and -0.64 respectively). The pH showed a negative relationship with EC and TDS in the river ($r = -0.57$ and -0.50 respectively) and also showed a negative relationship with EC, TDS, DO and salinity in the stream ($r = -0.54$, -0.54, -0.52 and -0.51 respectively). The EC showed a strong positive relationship with TDS, DO and salinity in both river and stream ($r = 0.74$, 0.83 and 0.63 respectively

Table 3 | Significant Spearman's correlation coefficient (r) between enteric bacteria, meteorological and physicochemical characteristics of water samples from the river

	Temp (°C)	**pH**	**EC (μS/cm)**	**TDS (mg/l)**	**DO (mg/l)**	**Sal (ppt)**	**Turb. (NTU)**	**Rain. 24 hrs (mm)**	**Rain. 48 hrs (mm)**
E. coli	−0.11	0.31	−0.31	−0.25	−0.22	−0.30	−0.12	0.06	0.08
Faecal coliforms	−0.39	0.10	0.25	0.13	0.22	0.35	0.24	−0.13	0.05
Enterococci	0.38	0.05	−0.18	−0.15	−0.25	−0.26	0.05	0.22	−0.31
Salmonella	−0.39	0.23	−0.05	−0.19	0.06	0.37	0.22	−0.25	**0.65**
Shigella	0.31	−0.06	−0.20	−0.31	−0.31	− **0.50**	0.02	**0.50**	−0.38
Bifidobacterium	−0.16	−0.01	**0.56**	0.30	**0.56**	**0.50**	−0.08	−0.25	−0.19
Campylobacter	−0.40	−0.14	0.29	0.28	**0.50**	0.13	**0.58**	0.39	−0.12
Clostridium	−0.18	−0.04	**0.57**	**0.50**	**0.54**	0.35	−0.06	−0.16	−0.15

Key: Values in bold figures indicate significant correlation; Temp – Temperature; EC – Electrical conductivity; TDS – Total Dissolved Solids; DO – Dissolved Oxygen; Sal – Salinity; Turb. – Turbidity; Rain – Rainfall; $n = 12$.

Table 4 | Significant Spearman's correlation coefficient (r) between enteric bacteria, meteorological and physicochemical characteristics of water samples from the stream

	Temp (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Sal (ppt)	Turb. (NTU)	Rain. 24 hrs (mm)	Rain. 48 hrs (mm)
E. coli	−0.28	0.05	**0.51**	**0.51**	0.32	**0.60**	−0.20	−0.36	0.10
Faecal coliforms	**0.72**	−0.08	−0.02	−0.24	−0.15	−0.27	−0.15	−0.01	−0.17
Enterococci	**0.50**	−0.31	−0.22	−0.22	−0.39	−0.35	−0.39	−0.28	−0.21
Salmonella	**0.56**	−0.15	0.10	0.10	0.01	−0.01	−0.32	−0.27	−0.05
Shigella	−0.10	−0.03	−0.16	−0.10	−0.24	−0.30	−0.36	−0.30	−0.11
Bifidobacterium	0.32	0.10	−0.05	−0.02	0.20	−0.03	0.28	0.30	0.25
Campylobacter	**0.50**	0.12	−0.20	−0.21	0.08	0.13	0.18	0.28	−0.10
Clostridium	−0.20	**0.52**	−0.22	−0.20	−0.03	−0.26	−0.04	−0.06	−0.03

Key: Values in bold figures indicate significant correlation; Temp – Temperature; EC – Electrical conductivity; TDS – Total Dissolved Solids; DO – Dissolved Oxygen; Sal – Salinity; Turb. – Turbidity; Rain – Rainfall; $n = 12$.

in the river; r = 0.99, 0.83 and 0.89 respectively in the stream). The TDS showed a strong positive relationship with DO (r = 0.87 and 0.84 in both river and stream respectively), it also showed a strong positive relationship with salinity (r = 0.89 in the stream). Dissolved oxygen showed a strong positive relationship with salinity (r = 0.78) in the stream (Tables 5 and 6).

Table 5 | Significant Spearman's correlation coefficient (r) between water quality parameters and rainfall data of the river

	Temp (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Sal (ppt)	Turb. (NTU)	Rain. 24 hrs (mm)	Rain. 48 hrs (mm)
Temp (°C)	1.00								
pH	−0.04	1.00							
EC (μS/cm)	−0.27	**−0.57**	1.00						
TDS (mg/l)	**−0.55**	**−0.50**	**0.74**	1.00					
DO (mg/l)	**−0.69**	−0.43	**0.83**	**0.87**	1.00				
Sal (ppt)	0.07	−0.33	**0.63**	0.02	0.33	1.00			
Turb. (NTU)	**−0.64**	0.19	−0.24	0.04	0.21	−0.33	1.00		
Rain. 24 hrs (mm)	**−0.58**	−0.03	−0.30	0.16	0.09	**−0.69**	**0.80**	1.00	
Rain. 48 hrs (mm)	**−0.50**	−0.02	−0.10	−0.20	0.10	0.24	**0.50**	0.33	1.00

Key: Values in bold figures indicate significant correlation; Temp – Temperature; EC – Electrical conductivity; TDS – Total Dissolved Solids; DO – Dissolved Oxygen; Sal – Salinity; Turb. – Turbidity; Rain – Rainfall; $n = 12$.

Table 6 | Significant Spearman's correlation coefficient (r) between water quality parameters and rainfall data of the stream

	Temp (°C)	pH	EC (μS/cm)	TDS (mg/l)	DO (mg/l)	Sal (ppt)	Turb. (NTU)	Rain. 24 hrs (mm)	Rain. 48 hrs (mm)
Temp (°C)	1.00								
pH	−0.32	1.00							
EC (μS/cm)	−0.24	**−0.54**	1.00						
TDS (mg/l)	−0.28	**−0.54**	**0.99**	1.00					
DO (mg/l)	−0.11	**−0.52**	**0.83**	**0.84**	1.00				
Sal (ppt)	−0.22	**−0.51**	**0.89**	**0.89**	**0.78**	1.00			
Turb. (NTU)	0.17	−0.16	−0.05	−0.01	0.26	0.01	1.00		
Rain. 24 hrs (mm)	−0.51	0.38	−0.61	−0.59	−0.30	−0.52	0.56	1.00	
Rain. 48 hrs (mm)	0.30	−0.35	0.33	0.33	0.23	0.28	0.59	0.03	1.00

Key: Values in bold figures indicate significant correlation; Temp – Temperature; EC – Electrical conductivity; TDS – Total Dissolved Solids; DO – Dissolved Oxygen; Sal – Salinity; Turb. – Turbidity; Rain – Rainfall; $n = 12$.

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DISCUSSION

This study investigated the level and distribution pattern of enteric bacteria in two surface water sources (river and stream) commonly used for domestic, recreational and agricultural purposes in Akure, Nigeria, and examined the effect of meteorological (rainfall) and physicochemical factors on the occurrence of enteric bacteria in the water samples. About 1% of the bacterial biomass in the gastrointestinal tract (GIT) of humans and other warm-blooded animals is made up of *E. coli*, which is a normal flora in the intestines of animals (Ashbolt *et al.* 2001; Leclerc *et al.* 2001). The mean total viable count of *E. coli* in the river and stream obtained in this study appeared to be higher than those obtained by Andrea & Thomas (2009), where the authors observed that the concentration of *E. coli* ranged from 1.77 to 4.43 $\log_{10}$ cfu/100 ml in eight sampling points of the River Swist in North-Rhine Westphalia, Germany. The presence of *E. coli* in the river and stream was most likely due to faecal contamination. The stream appeared to be more faecally polluted than the river because of its higher concentration of *E. coli.* In addition, the concentration of faecal coliforms in the river and stream in this study were higher than those observed by Olalemi (2019), where the author observed that the concentration of faecal coliforms ranged from 4.23 to 4.51 $\log_{10}$ cfu/100 ml in the River Owena, Akure, and also higher than those reported by Kavka & Poetsch (2002), where the authors observed that the concentration of faecal coliforms ranged from 1.3 to 4.6 $\log_{10}$ cfu/100 ml at 79 sampling sites in the Danube River. Similarly, enterococci is best used as an indicator for swimming illnesses, this has been helpful in the management of recreational waters (EPA 2003; Wade *et al.* 2003). Intestinal enterococci are very effective in determining water quality as they survive longer in water than *E. coli*; they do not multiply in water and also resist chlorination and drying (WHO 2006). In this study, the stream had higher concentration of enterococci than the river. Nevertheless, the mean viable count of enterococci in the river and stream appear to be lower than those observed by Lukasz *et al.* (2016) where the authors reported an average concentration of enterococci between 1.88 $\log_{10}$ cfu/100 ml and 2.20 $\log_{10}$ cfu/100 ml in the River Ganga, India.

The EU-Bathing Water Quality Directive 2006/7/EEC categorized the rate of faecal pollution of surface waters into five different classes based on the concentration of faecal coliforms (equal to that of *E. coli*) and enterococci as: Class 1 – little pollution, where the concentration of faecal coliforms (*E. coli*) and enterococci is $\leq$2 $\log_{10}$ cfu/100 ml and 1.6 $\log_{10}$ cfu/100 ml respectively, Class 2 – moderately polluted, where the concentration of faecal coliforms (*E. coli*) and enterococci is >2–3 $\log_{10}$ cfu/100 ml and >1.6–2.6 $\log_{10}$ cfu/100 ml respectively, Class 3 – critically polluted, where the concentration of faecal coliforms (*E. coli*) and enterococci is >3–4 $\log_{10}$ cfu/100 ml and 2.6–3.6 $\log_{10}$ cfu/100 ml respectively, Class 4 – strongly polluted, where the concentration of faecal coliforms (*E. coli*) and enterococci is >4–5 $\log_{10}$ cfu/100 ml and 3.6–4.6 $\log_{10}$ cfu/100 ml respectively, Class 5 – excessively polluted, where the concentration of faecal coliforms (*E. coli*) and enterococci is >5 $\log_{10}$ cfu/100 ml and 4.6 $\log_{10}$ cfu/100 ml respectively (Kavka & Poetsch 2002). To this end, the level of faecal pollution in the river may be classified as 'strong' while those in the stream may be classified as 'excessive'. Furthermore, the World Health Organization (2001) classified water for drinking and bathing into four categories based on coliform count, namely: Class 1 – conformity, where the count is <0 $\log_{10}$ cfu/100 ml, Class 2 – low, where the count is 0–1 $\log_{10}$ cfu/100 ml, Class 3 – intermediate, where the count is 1.04–2 $\log_{10}$ cfu/100 ml, Class 4 – high, where the count is >2 $\log_{10}$ cfu/100 ml. In this present study, water samples from the river and stream may be categorized as 'Class 4'. The usage of these water sources for drinking or bathing without adequate treatment may be of significant public health risk.

Outbreak of diseases caused by *Salmonella* may occur as a result of drinking faecally impacted water and this may lead to great economic losses and eventual death among the human population (Momba *et al.* 2006; WHO 2006). In this study, water from the river possesses greater risk associated with *Salmonella* than the stream, as it had a higher concentration of the bacteria. In the same vein, *Shigella* are most commonly known to cause acute dysentery and higher percentage of diarrhea

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diseases worldwide (Thapar & Sanderson 2004; Kotloff *et al.* 2013). The presence of *Shigella* in waters indicates recent human faecal pollution (WHO 2006). In this study, water from the stream possesses greater risk associated with *Shigella* than the river. *Bifidobacterium* constitutes a larger percentage of the microflora in GIT of humans and animals (Biavati & Mattarelli 2003; Wilson 2005), its presence in water is also an indication of faecal contamination. The reports of Biavati & Mattarelli (2003) and Wilson (2005) suggested that *Bifidobacterium* are usually lower than other coliforms in water due to the condition of low temperature (especially temperatures lower than 30°C). This is not in agreement with the findings of this study; despite lower water temperatures, the mean viable count of *Bifidobacterium* in the river and stream were considerably high. Similarly, *Campylobacter* has been reported as one of the most common bacteria responsible for gastroenteritis and there is evidence of the presence of *Campylobacter* in polluted surface waters (Hattaka *et al.* 2003; Andrea & Thomas 2009; Schönberg-Norio *et al.* 2014). According to WHO (2006), infections associated with *Campylobacter* are on the increase as the pathogen is associated with faecal materials and is also resistant to disinfectants. The concentration of *Campylobacter* in the river and stream were slightly close to those obtained by Andrea & Thomas (2009), where the authors reported *Campylobacter* counts of 4 $\log_{10}$ cfu/100 ml in water from the Jungbach tributary, North Rhine Westphalia, Germany. *Clostridium* is an indicator for enteric viruses and protozoa, it can survive environmental stress and resist disinfection (WHO 2006). In this present study, the stream had a higher concentration of *Clostridium* than the river, thus suggesting the potential presence of enteric viruses and protozoa in the water sources.

Studies have shown that the microbial load of surface waters is directly proportional to the amount of rainfall. This is likely due to runoff waters from the land carrying soil microbes into the water and wastes being washed into poor drainage systems. Rainwater moves microorganisms into rivers but may also dilute the concentration of intrinsic microbes in the rivers (Amah 2015; Kostyla *et al.* 2015). In this study, the maximum amount of rainfall (76.80 mm) was observed at 24 hours before sampling on the second week and constant rainfall was observed at 24 hours and 48 hours during the last four weeks of sampling. This may partly be responsible for the high levels of enteric bacteria in the water sources. Biochemical reactions of water are moved by the intrinsic temperature of the water (Gangwar *et al.* 2012).

The water temperature observed in the river and stream falls within the maximum limit of 40°C given by FEPA (1991). Temperature measures the average kinetic energy of molecules and atoms (Jamshed & Amit 2015), the molecules and atoms have low kinetic energy in the river and stream, the values increase the DO holding capacity of the water bodies as temperature tends to lower DO in water (Perlman 2013); a negative relationship was observed between the temperature and DO in the river. The temperature of water determines the tolerance ability of aquatic life (Bhadja & Vaghela 2013), the river and stream have temperature values that are less toxic to the bacterial pathogens.

The pH of the river and stream conform to the range permitted for drinking water, which is 6.5–8.5 (Wang *et al.* 2002; WHO 2004, 2017; Fakayode 2005). The pH gives information about the acidity and alkalinity of water, the mean pH values of the river and stream tends around neutral, which supports the survival of aquatic life. High or low pH of water impacts the solubility and toxicity of heavy metals (USGS 2013), the pH of the river and stream showed a negative relationship with the total dissolved solids as the neutral pH level of the river and stream makes it less toxic for bacterial pathogens and supports their survival.

The EC of the river and stream were below the 1,000 μS/cm value of WHO recommendation limits. Electrical conductivity shows the current conductance ability of water and also has a direct relationship with TDS in water (Mihir *et al.* 2015), this is also observed as the EC showed a strong correlation with TDS in both river and stream. EC also provides information about the concentration of salt ions, which is observed with the large positive correlation with salinity in both river and stream (Mihir *et al.* 2015; Meride & Ayenew 2016). Potable water is less conductive, the low EC values of the river and stream shows they are less ionized and contain less ionic compounds.

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TDS in water is a reflection of turbidity and ionic deposition of water that supports the growth of bacteria and other pathogens (Singh *et al.* 2010). The TDS in both river and stream were lower than the 500 mg/l standard of EPA (2002). TDS measures the amount of organic matter, inorganic ions and other dissolved solids in water (Phyllis *et al.* 2007); it has a strong relationship with salts concentration, as this is observed in the stream. Moderate values of TDS in the stream and river indicate moderate quantity of ionic compounds and toxicity of ions to bacterial pathogens. The mean values of DO obtained from the river and stream were lower than the permitted limit of 5 mg/l for drinking, irrigation and domestic use. Dissolved oxygen is a major parameter in assessing water quality as it impacts the survival rate of aquatic life (MPCA 2009). High or low DO affects the growth of organisms in water, the values of DO observed in the river and stream are moderately sufficient for bacterial pathogens' survival.

The turbidity in both water sources exceeded the maximum value of 10 NTU required for drinking water (AWWA 1998; WHO 2004); turbidity reduces the ability of light to penetrate into the water and also reflects the rate of pollution of the water bodies (Meride & Ayenew 2016). High values of turbidity in the river and stream show a lesser rate of photosynthesis and high rate of pollution, the two water sources have low quality and are unfit for consumption and other human uses.

In summary, the mean values of water temperature, dissolved oxygen and salinity were higher in the river than those in the stream while the mean values of turbidity, pH, electrical conductivity and total dissolved solids were higher in the stream than those in the river.

Rainfall contributes to the reduction of water quality as it is observed to increase the turbidity and also to have negative impacts on the salinity, temperature and TDS in both river and stream, it influences these parameters to be moderately conducive for the growth and survival of the bacteria pathogens.

The positive relationship observed between TDS and the concentrations of *Clostridium* in the river and *E. coli* in the stream may be because TDS helps in maintenance of the aquatic ecosystem and its values represent an increase in ionic deposition in the water, thus creating a favourable environment for the organisms to grow and multiply (Singh *et al.* 2010). The value of TDS obtained in the river and stream provides support for the growth of *Clostridium* in the river and *E. coli* in the stream, as it was reported that TDS helps in maintenance of the aquatic ecosystem and its values represent an increase in ionic deposition in the water, thus creating a favourable environment for the organisms to grow and multiply (Singh *et al.* 2010). EC is related to hardness of water, which is associated with the presence of dissolved ions in water. EC is directly proportional to TDS and it has been demonstrated that an increase in TDS may lead to an increase in salinity (Istifanus *et al.* 2013). This may be responsible for the positive relationship observed between *E. coli*, EC, TDS and salinity in water samples from the stream. TDS reflects the dissolved organic and inorganic salts that support the growth of microbial cells in water, this makes it responsible for the positive correlation observed between TDS and *E. coli*, and it is also responsible for the increase in the level of electrolytes and salinity of the water. *Campylobacter* exhibited a positive relationship with turbidity and this is in agreement with Shittu *et al.* (2008), where the authors reported that high turbidity of water is often associated with higher levels of pathogenic bacteria such as *Campylobacter*. Olalemi *et al.* (2020) also demonstrated that counts of enteric bacteria increased with increase in water turbidity. The positive relationship between *Bifidobacterium*, *Clostridium* and EC in this study does not agree with the findings of Mijin *et al.* (2019), where the authors demonstrated a negative correlation between electrical conductivity and some enteric bacteria in the Nakdong River, South Korea. The value of EC in this study supports the growth of *Bifidobacterium*, *Clostridium*, which makes it responsible for the positive correlation observed, Mijin *et al.* (2019) reported a low value of EC in Nakdong River, South Korea, which was suggested to be as a result of high rainfall data obtained in the sampling area leading to a low amount of electrolytes in the water, thereby resulting in negative correlation between EC and the enteric bacteria. DO is essential for growth and survival of aquatic organisms (EPA 2013), it is

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interesting to note that the positive relationship between *Bifidobacterium*, *Clostridium* and DO in this study despite the low or no oxygen requirement of the organisms may likely be as a result of the excessive faecal pollution of the water sources. In addition, the concentrations of faecal coliforms, enterococci, *Salmonella* and *Campylobacter*, which demonstrated a positive relationship with water temperature in the stream, do not agree with studies that have shown that water temperature is related to solar radiation levels and a major factor influencing the inactivation of enteric bacteria in aquatic systems (Olalemi *et al.* 2016, 2020). This study reveals differences in the response of enteric bacteria in water with respect to temperature on the basis of variation in seasons of the year and ecological diversity in regions of the world, as the concentrations of faecal coliforms, enterococci, *Salmonella* and *Campylobacter* that demonstrated a positive relationship with water temperature in the stream, and the report of a relationship of water temperature to solar radiation levels, which influences inactivation of enteric bacteria in aquatic systems (Olalemi *et al.* 2016, 2020). However, this observation is in agreement with Mijin *et al.* (2019) where the authors reported positive correlation between coliform bacteria and temperature in three out of eight weir stations in the Nakdong River, South Korea. Similarly, Sanindhar & Nitin (2014) suggested that certain levels of temperature of water favour the growth of aquatic microorganisms.

CONCLUSION

The various anthropogenic activities taking place in and around the river and stream, including animal rearing, direct defecation and proximity to sewer channels, contributed to the level of faecal pollution of the water sources. The concentrations of *E. coli*, faecal coliforms, *Salmonella* and *Campylobacter* were normally distributed while those of enterococci were not normally distributed in the water samples from the river and stream. Rainfall and physicochemical factors influenced the concentration of enteric bacteria in the water samples from the two water sources. Based on microbiological water quality categories, the findings from this study demonstrated that the level of enteric bacteria in the river and stream suggests strong and excessive faecal pollution that may pose significant risks of diarrheal diseases to humans and a potential source of environmental hazard to the residents of the community. Water from these two surface water sources must be treated before use in order to protect human health.

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DATA AVAILABILITY STATEMENT

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

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