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Emergence Of Pathogenic Bacterial And Physicochemical Properties Of Drinking Water Sources In Sagbama Local Government Area

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ABSTRACT
Water has a significant impact on human health, and the quality of the water supplied is critical in determining individual and community health. Safe drinking water is a crucial concern in terms of public health, as the human race's health is inextricably linked to the quality of water utilized. Rapid population growth, rising living standards in cities, and industrialization have resulted in increased demand for high-quality water, while pollution of water sources has been continuously increasing on the other. The prevalence of multidrug resistance bacterial and physicochemical quality of water source from Sagbama Local Government Area of Bayelsa state, Nigeria was investigated. Water samples (Sachet water, borehole and river) from different sources were collected and analyzed using standard microbiological and physicochemical methods. Isolate were tested for for drug sensitivity using Kirby Bauer disc diffusion method. The results of the study revealed that the different water sources were contaminated with one or more pathogenic bacteria species which includes *Salmonella* sp. *Klebsiella aerogenes*, *Escherichia coli Brevundimonas olei* and *Klebsiella pneumoniae*. The bacterial isolates recorded Multiple Antibiotic Resistance (MAR) index that range from 0.2 - 0.9 and Multidrug Resistant (MDR) positives resisting more than two antibiotics. All the bacterial isolates were susceptible and resistant to Levofloxacin and Cefuroxime respectively. The physico-chemical parameters in majority of the water sampled were within the WHO water standards for domestic use. On the contrary, the ordour for Toru-Orua river water had objectionable properties. The present study indicated that drinking water sources in Sagbama Local Government Area are contaminated with various pathogenic bacteria and unfit for drinking.
Keywords:

INTRODUCTION
The most vital renewable natural resource that all living beings require is water, and the presence of microbes in drinking water raises issues for public health (Eniolorunda, 2021), aswater that is sufficient, safe, and clean is necessary for human survival as well as the wellbeing of ecosystems, societies, and

ed [illegible] public health, thus water from different water sources should be treated before being used for any domestic purposes (Alex & Kpormon, 2023). But the common experience in developing countries is drinking of untreated water, even in the most developed countries (Amatobi & Agunwamba, 2022; Azuonwu, 2020). Bacterial parameters indicating faecal contamination have historically been the primary technique of monitoring the microbiological safety of drinking water: these factors are associated with gastrointestinal disorders, arise from faecal contamination (Babič *et al*., 2017).The primary sources of residential water supply are either closed water supply lines, such as tap water and ground water from boreholes, or open waterways, also known as surface waters, such rivers, streams, and lakes. Dead animals, animal faeces (distinct natural sources of pollutants found in surface water) (Dike-Ndudim *et al*., 2022) and human bacterial pathogens pose a global threat to drinking water supplies due to increasing demand and decreasing raw water quality, resulting from climate change negative impact on freshwater resources,which have necessitatedadvances in molecular detection technologies that could improve water safety by accurately detecting and identifying pathogen s(Brettar & Höfle, 2008).Sachet water became a primary drinking source in Nigeria due to surface and borehole water issues, potentially posing health risks due to contamination rather than body replenishment (Tenebe *et al*., 2023).According to (Ma *et al*., 2020);(Nwaiwu *et al*., 2020)and (Nwand1kor & Okolo, 2016), there is a significant public health risk associated with Nigeria's ongoing sales growth and careless consumption of packaged drinking waterthat does not meet the WHO standard for potability due faecal pollution arising from poor hygiene and failure of treatment processes.

Isolation and characterization of bacterial pathogens from drinking water sources have been reported by several researchers (Angnunavuri *et al*., 2022; Bahago *et al*., 2019; Emerenini, 2007; Miner *et al*., 2015; Onajite *et al*., 2018; Onyango *et al*., 2018). Biofilm forming and biofouling agents like faecal indicator bacteria (*Escherichia coli*), obligate bacterial pathogens of faecal origin (*Campylobacter* spp.) opportunistic bacteria of environmental origin (*Legionella* spp., *Pseudomonas aeruginosa*),*Salmonella* spp., *Enterococcus* sp., *Shigella* sp., and *Clostridium* sp. wereisolated as contaminants from drinking water sources (Olalemi *et al*., 2020; Wingender & Flemming, 2011). The genera Exiguobacterium, Delftia, Kocuria, and Lysinibacillus (Pindi *et al*., 2013),Bacillus, Salmonella, Escherichia, Pseudomonas, Micrococcus, Staphylococcus, Vibrio, Enterobacter and Streptococcus(Ekanem & Ottong, 2021) and *Acinetobacter* sp. (Egberongbe *et al*., 2021; Kalu *et al*., 2024; Kumar *et al*., 2022) were reported predominantly in drinking water.

There is catalogue of reports of antibiotics resistant bacterial isolated from drinking water sources (Onohuean *et al*., 2022). (Alalade *et al*., 2020), reported *Escherichia coli* isolated from sachet water and borehole (tap) water that are multi drug resistant. (Gugu *et al*., 2023) reveal that antibiotic resistance was recorded for *Salmonella* spp., *E. coli*, and *Pseudomonas* spp. against ceftazidime, Ampicillin, and ceftazidime, respectively.*Escherichia coli* showed high resistance to polymyxin B, colistin, cefotaxime, ceftazidime, ciprofloxacin, tetracycline, and imipenem, while meropenem showed moderate resistance and gentamicin showed low resistance. *Klebsiella pneumoniae* also showed high resistance to these antibiotics. Antibiotic resistance genes (ARGs) offer resistance against a wide range of antibiotics, including tetracyclines, beta-lactams, and fluoroquinolones. This highlights the possible hazards linked to the dissemination of ARGs in drinking water sources and their possible conversion to dangerous bacteria(Akinola *et al*., 2022; Iloghalu *et al*., 2020; Wilcox *et al*., 2023). The present study seeks to evaluate the physicochemical and multidrug resistance bacteria in drinking water sources in Sagbama Local Government Area.

MATERIALS AND METHODS

Sample Collection

Water samples were collected from different water sources (Sachet water, bore hole and river) in Sagbama Local Government Area, using clean sterilized 250 ml bottles from 20 to 30 cm depth (to avoid floating materials) according to Mgbemena *et al*. (2012). Water for dissolved oxygen (DO) and biological oxygen demand (BOD) determination were collected in 250 ml dark glass containers. The bottles were

carefully closed and transported on ice and stored at 4ºC in a refrigerator until the analysis of microbiological and physicochemical parameters.

Isolation of Pure Bacterial Culture

The water samples from different sites were handled separately. Water culture samples were prepared by streak and spread plate techniques. A sterile wire loop was used to collect a loop full of each of undiluted water sample and inoculated on the surface of nutrient agar, MacConkey agar, Eosine methylene blue agar and *Salmonella Shigella* agar, respectively. The inoculated culture were sub-cultured on fresh nutrient agar, MacConkey agar, Eosine methylene blue agar and *Salmonella Shigella* agar plates to obtain pure cultures which were used to further study their morphological and biochemical characteristics. The pure culture isolates were sub-cultured in nutrient agar and incubated at 37oC for 24 hours for bacterial enumeration.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk plate method was employed following EUCAST (2017) and Bauer, (1966) guidelines to determine the susceptibility of isolates to selected reference antibiotics. Ten microlitres of 24 h broth culture of bacterial isolates of turbidity equivalent to a 0.5M MacFarland standard was aseptically spread on 20 mL of freshly prepared sterile Mueller-Hinton agar (Thermo-Fisher, London, UK). Antibiotic discs were then put on the surface of the Mueller-Hinton agar after drying for 10 min at room temperature of 25˚C prior to incubation at 37˚C for 24 h.

Determination of physicochemical parameters

Water temperature

This was done according to Keupers and Wilems (2017) and WHO (2017) where temperature was determined *in situ* at all sampling sites, by suspending a thermometer about 10 cm below water surface for at least 2 minutes before taking the readings.

Water pH

The pH was determined *in situ* using a combined meter (model MI 806) as described by Keupers and Wilems (2017). The pH meter was calibrated by inserting its probe in standard buffer solutions with pH 4.0 and 7.0 then being rinsed with distilled water and inserted in water samples for 2 minutes before taking the readings. It was done in triplicates per site.

Water turbidity

Turbidity was determined *in situ* according to Keupers and Wilems (2017) and WHO (2017) at different sites using turbidimeter (Nephelometer) (model HACH 2100P). Before analysis, the turbidimeter was calibrated using prepared standards in the desired range for accuracy as indicated in the manufacturer's operating instruction. After calibration, standardized readings were taken in Nephelometric turbidity units (NTU).

Water Dissolved Oxygen

This was done as described by Vaidya and Labh (2017). The dissolved oxygen was determined *in situ* by use of dissolved oxygen (DO) meter (model MW-600). During determination, pre-rinsed probe was immersed approximately 1.25 cm into water samples and stabilized readings taken.

Water Biological Oxygen Demand

Biological oxygen demand (BOD) was calculated by the use of values obtained from dissolved oxygen according to Obed (2012) as follows; BOD (mg/l) = D1-D2, Where: D1= initial DO measurement (dissolved oxygen demand) of water samples on the first day was done *in situ* D2= final DO measurement of the samples at the 5th day was taken *ex situ* after storing water samples in dark place for five days, D2 which was the value of DO taken.

Water Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined according to procedure by Reece (2017), using dichromate reflux method. Ten ml of 0.25 M of potassium dichromate K2Cr2O7 and 30 ml of H2SO4+ Ag2SO4 reagent were added into 20 ml of diluted water samples diluted by distilled water. The mixture were refluxed for 2 hours and cooled to room temperature. The solutions were diluted to 150 ml using distilled water and excess K2Cr2O7 was titrated with ferrous ammonia sulfate (FAS). Ferrous ammonia sulfate was first used in blank (A) and finally used in sample. The difference between (A-B) was then

multiplied by the normality of ferrous ammonia sulfate (N). The samples from different sites were treated independently. The COD values were determined using the equation: COD= ((A-B) * N*100*8)/ Volume of sample, where: A- is the ml of FAS used as blank B- is the ml of FAS used for sample N- is the normality of FAS 8- is milli equivalent of oxygen

Analysis of water nitrates

The procedure described by Obed (2012) was employed in nitrates determination. Ten ml of the water samples obtained from collected water samples was pipetted into test tubes and one ml of 1.3 M NaOH was added and gently mixed, followed by one ml of reducing mixture and gently mixed. The mixture was heated for ten minutes at 60°C in a water bath and allowed to cool at room temperature. One ml of colour developing reagent was added to the mixture and shaken and the absorbance read at 520 nm using spectrophotometer (UV-1650 PC-UV-VIS, Shimadzu).

Determination of water Phosphates

This was carried out as previously described by Ouma (2015). Phosphates (PO4-) were analyzed by use of ascorbic acid followed by turbidimetric analysis. Twenty five (25) ml aliquot of the water sample was measured in a 50 ml graduated tube. Four (4) ml of combined reagent prepared according to manufacturers‟ specification consisting of ascorbic acid, ammonium molybdate, potassium antimonyl tartarate and sulphuric acid was added. The tube was covered with parafilm, shaken well and left to stand for 10-30 minutes to develop stable blue colour thereafter reading was taken at 880 nm absorbance using spectrophotometer. The photometer was zeroed with a blank solution.

RESULTS

Agarose gel electrophoresis is a molecular biology technique used to analyse DNA fragments. It helps verify PCR products, determine DNA and RNA purity, and determine size. The agarose gel eletrophoresis shown in Figure 1 illustrates the presence of genes extracted from 10 bacterial isolates. Using the ladder (M) as a yardstick, the bacterial DNA sizesare 1.5 kilobase pairs. The gel shows the amplification of the 16S rRNA gene, with bands indicating DNA quantity. Brighter bands indicate higher DNA, fainter bands indicate lower DNA, and no bands indicate no DNA.

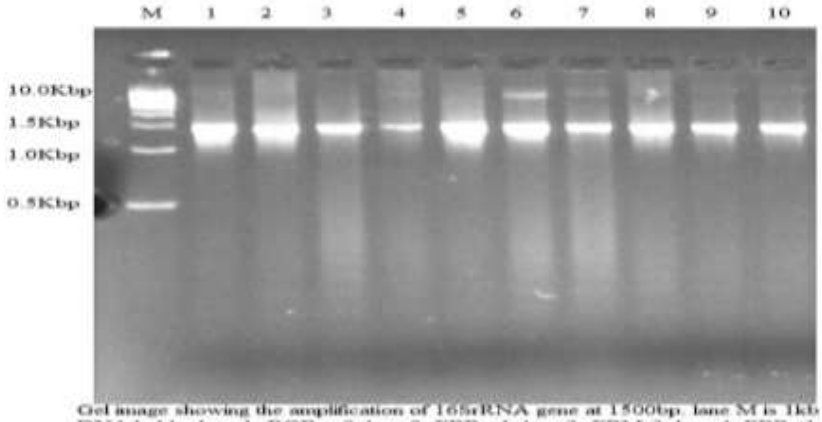


Figure 1. Agarose gel electrophoresis showing the size of the amplifiedgenesof bacteria isolates.

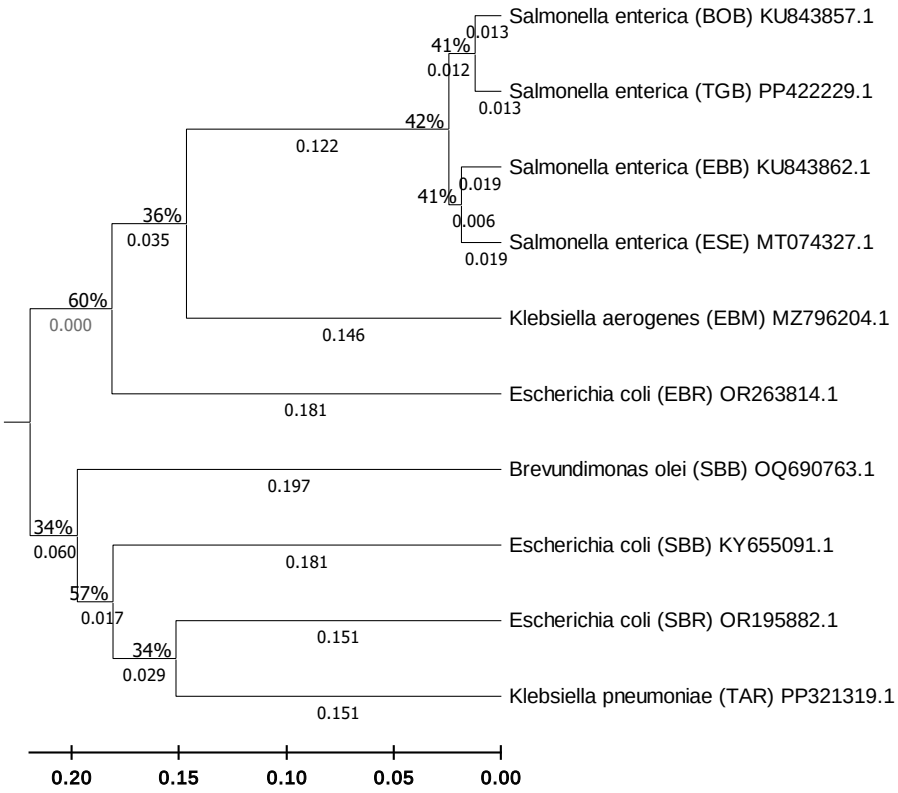
The results of bacterial isolates isolated from drinking water samples taken from 6 communities within the Sagbama local government area are shown in Table 1, along with a comparison of the isolates' DNA sequences to reference sequences in the National Centre for Biotechnology Information (NCBI) database. *Salmonella* sp., *Klebsiella* sp., Escherichia coli, and *Brevundimonasolei* were the four genera of bacteria identified. *Salmonella enterica subspecies SA49, Enteritidis strain NS5, and Klebsiella aerogenes*

EGBON 118 were isolated from drinking water samples in Bolu-Orua and Ebedebiri, respectively. The isolates showed high DNA similarity to the reference sequence, with a 99.27% match and 100% similarity to the reference sequence. *Escherichia coli and Salmonella enterica* were isolated from river water samples from Ebedebiri and sachet water, with perfect (100%) DNA sequence matching and significant similarity. Two *Escherichia coli* AG93 and KPD4 strains isolated from Sagbama town borehole water, had 83.78% and 100% similarity matches to the reference sequence respectively. The *Brevundimonasolei* strain showed 87.16% similarity, while the Typhi strain R1 from the Tungbo river water sample showed a high 98.07% similarity to the reference sequence.

Table 1. Molecular identification of bacteria isolates from drinking water sources.

Location/brand of sachet	Water sample	Bacterial isolate	Subspecies	Serovar/strain	Accession number	% Similarity
Bolu-Orua	Borehole	*Salmonella enterica*	enterica	Paratyphi C strain SA49	KU843857.1	97.52
Ebedebiri	Borehole	*Salmonella enterica*	enterica	Enteritidis strain NS5	KU843862.1	99.27
EBM	Sachet	*Klebsiella aerogenes*	-	EGBON 118	MZ796204.1	100
Ebedebiri	River	*Escherichia coli*	-	EB4	OR263814.1	100
ESE	Sachet	*Salmonella enterica*	-	BH67	MT074327.1	99.39
Sagbama town	Borehole	*Escherichia coli*	-	EGE 4460515-62	KY655091.1	100
Sagbama town	Borehole	*Brevun dimonasolei*	-	113-2	OQ690763.1	87.16
Sagbama town	River	*Escherichia coli*	-	AG93	OR195882.1	83.78
Toru-Angiama	River	*Klebsiellap neumoniae*	-	KPD4	PP321319.1	100
Tungbo	River	*Salmonella enterica*	enterica	Typhi strain R1	PP422229.1	98.07

Figure 2 shows the phylogenetic tree depicting the evolutionary history of of the bacterial isolates from drinking water sources from communities in Sagbama Local Government Area. Ten bacteria isolates identified were divided into two clades. The first clade include *Salmonella* sp.$^{(BOB)(TGB)(EBB)(ESE)}$, *Klebsiella aerogenes*$^{(EBM)}$ and *Escherichia coli*$^{(EBR)}$, and the second clade consist of *Brevundimonas olei*$^{(SBB)}$, *Escherichia coli*$^{(SBR)(SBB)}$, and *Klebsiella pneumoniae*$^{(TAR)}$. The two clades are polyphyleticwith low percentage similarity ranging from 34 – 60 %and variation in nucleotide sequence ranging from 0.006 – 0.197.*Klebsiella aerogenes*$^{(EBM)}$ and *Escherichia coli*$^{(EBR)}$showed distance relationship with *Escherichia coli*$^{(SBR)(SBB)}$, and *Klebsiella pneumoniae*$^{(TAR)}$. The *Salmonella entericas*howed two clades (groups) with substitution rate per nucleotide sequence of 0.013 and 0.019, respectively.*Escherichia coli*$^{(SBR)}$and *Escherichia*$^{(SBB)}$ recorded substitution rate of 0.151 and 0.181, respectively. *Escherichia coli*$^{(SBR)}$ and *Escherichia coli*$^{(EBR)}$ recorded the same substitution of 0.181 rate per nucleotide sequence. *Brevundimonas olei*$^{(SBB)}$ recorded the highest value of substitution rate per nucleotide sequence of



0.197.

Figure 2. Evolutionary history (phylogenetic tree) inferred using the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) method

Table 2 illustrates the estimates of evolutionary divergence (base substitutions per site) between Sequences using the Maximum Composite Likelihood model.The number of modifications (base substitutions) that have happened between two sequences per site (nucleotide location) is represented by the estimations of evolutionary divergence, sometimes referred to as genetic distance. The rate and pattern of evolutionary changes can be understood, as well as the evolutionary relationships between sequences and their similarities and differences, by using these estimates. Low distance values (0.01 - 0.10) indicate a recent common ancestor or high sequence similarity.Moderate distance values (0.10 - 0.50) imply a more distant common ancestor or moderate sequence divergence, andsignificant sequence divergence or a reasonably old common ancestor are indicated by high distance values (0.50 - 1.00).The distance recorded

among the 10 bacteria isolates range from 0.025 – 0.655. The *Salmonella enterica* bacteria low distance that range from 0.025 – 0.054, among the *Escherichia coli* distance of 0.315 and 0.655 was recorded.

Table 2. Estimates of Evolutionary Divergence (base substitutions per site) between Sequences using the Maximum Composite Likelihood model

	Salmonella enterica BOB)	*Salmonella enterica* (EBB)	*Klebsiella aerogenes* (EBM)	*Escherichia coli* (EBR)	*Salmonella enterica* (ESE)	*Escherichia coli* (SBB)	*Brevundimonasolei* (SBB)	*Escherichia coli* (SBR)	*Klebsiella pneumoniae* (TAR)	*Salmonella enterica* (TGB)
Salmonella enterica (BOB)	1									
Salmonella enterica (EBB)	0.053	1								
Klebsiella aerogenes (EBM)	0.314	0.280	1							
Escherichia coli (EBR)	0.339	0.331	0.462	1						
Salmonella enterica (ESE)	0.054	0.037	0.285	0.340	1					
Escherichia coli (SBB)	0.391	0.388	0.307	0.472	0.391	1				
Brevundimonasolei (SBB)	0.492	0.430	0.350	0.586	0.436	0.370	1			
Escherichia coli (SBR)	0.436	0.436	0.383	0.655	0.447	0.315	0.434	1		
Klebsiella pneumoniae (TAR)	0.442	0.431	0.307	0.559	0.438	0.409	0.380	0.303	1	
Salmonella enterica (TGB)	0.025	0.044	0.293	0.339	0.045	0.395	0.483	0.436	0.439	1

The table illustrates nucleotide variations and substitution between two sequences per site. When $p \leq 0.05 \pm SE$, as measured by the number of variances in base composition biases between sequences, sequences have developed with the same pattern of substitution.

Table 3 depicts the antibiogram of the 10 bacterial isolates isolated from the drinking water sources of the 6 communities in Sagbama Local Government Area. The codes "S", "I" and "R" means Susceptible, Intermediate and Resistance. Susceptibility infers that antibiotics drug A is potent for treatment of infection or disease caused by Z bacteria, intermediate implies that the antibioticswill be administered for a longer period to achieve potency and resistance means the antibiotics cannot be used for treatment of infection or disease caused by a resistant bacterium. The bacterial isolates recorded Multiple Antibiotic Resistance (MAR) index that range from 0.2 - 0.9and Multidrug Resistant (MDR) positives. The MAR index recorded are as follows *Salmonella enterica*$^{(BOB)}$0.2; *Brevundimonasolei*$^{(SBB)}$ 0.5; *Salmonella enterica*$^{(EBB)}$, *Klebsiella aerogenes*$^{(EBM)}$, *Escherichia coli*$^{(SBB)}$ 0.6; *Escherichia coli*$^{(EBR)}$, *Salmonella enterica*$^{(ESE)}$, *Escherichia coli*$^{(SBR)}$ 0.7; *Salmonella enterica*$^{(TGB)}$ 0.8 and *Klebsiella pneumoniae*$^{(TAR)}$ 0.9. The antibiotics recorded the following percentage susceptibility patterns (S, I, R): Streptomycin (50, 20, 30), Ciprofloxacin (0, 30, 70), Amoxil (10, 10, 80), Augmentin (0, 10, 90), Gentamicin (10, 30, 60), Pefloxacin (20, 0, 80), Ofloxacin (30, 30, 40), Azithromycin (10, 0, 90), Levofloxacin (100, 0, 0), and Cefuroxime (0, 0 100). All the bacterial isolates were susceptible and resistant to Levofloxacin and Cefuroxime, respectively.

Table 3. Showing percentage susceptibility pattern of antibiotics against bacterial isolates.

Bacterial isolate	Streptomycin	Ciprofloxacin	Amoxil	Augmentin	Gentamicin	Pefloxacin	Ofloxacin	Azithromycin	Levofloxacin	Cefuroxime	MAR Index	MDR
Salmonella enterica(BOB)	S	R	I	I	S	S	S	R	S	R	0.2	Positive
Salmonella enterica (EBB)	S	I	R	R	R	S	R	R	S	R	0.6	Positive
Klebsiella aerogenes(EBM)	I	R	R	R	I	R	I	R	S	R	0.6	Positive
Escherichia coli (EBR)	R	I	R	R	R	R	I	R	S	R	0.7	Positive
Salmonella enterica(ESE)	S	R	R	R	I	R	R	R	S	R	0.7	Positive
Escherichia coli (SBB)	I	I	S	R	R	R	R	R	S	R	0.6	Positive
Brevundimonasolei(SBB)	S	R	R	R	I	R	I	S	S	R	0.5	Positive
Escherichia coli (SBR)	S	R	R	R	R	R	S	R	S	R	0.7	Positive
Klebsiella pneumoniae (TAR)	R	R	R	R	R	R	R	R	S	R	0.9	Positive
Salmonella enterica(TGB)	R	R	R	R	R	R	S	R	S	R	0.8	Positive
% Susceptible	**50**	**0**	**10**	**0**	**10**	**20**	**30**	**10**	**100**	**0**		
% Intermediate	**20**	**30**	**10**	**10**	**30**	**0**	**30**	**0**	**0**	**0**		
% Resistant	**30**	**70**	**80**	**90**	**60**	**80**	**40**	**90**	**0**	**100**		

Performance standard for antibiotic susceptibility test using Clinical and Laboratory Standards Institute (CLSI) 2020.

Table 4 below, shows the physicochemical properties of drinking water sources in Sagbama Local Government Area of Bayelsa State. The temperature of the drinking water samples from the various communities range from 27.30 – 28.30 (°C); Bolu-Orua borehole water recorded the highest temperature of 28.30 (°C). Although, there is no specific guidelines for temperature of potable water (drinking water sources) by WHO. The pH ranges from 6.88 - 7.56 and the pH values of the water sample are within the WHO permissible limit range of 6.50 – 8.50. Total dissolved solids (TDS) values are below the WHO permissible limit of 1000 (mg/L), but Tungbo borehole water and EBM sachet water had the highest value of 130 and 101, respectively. Electrical conductivity (EC) ranges from 22 – 205 (µS/cm), while Tungbo borehole water and EBM sachet water had the highest value of 205 and 114, respectively: which are below the permissible limit of 250 (µS/cm). The chlorine content (mg/L) of the water samples are < 0.01, which is below the permissible limit of 5 (mg/L). Alkalinity (mg/L) of the water samples ranged from 0.84 – 7.28. Tungbo borehole water and EBM sachet water had the highest value of 7.28 and 5.656, respectively: which are below the permissible limit of 600. Hardness of water samples recorded, ranged from 1.03 – 8.88 (mg/L), the highest value of water hardness (8.88 and 6.91) was recorded against Tungbo borehole water and EBM sachet water, respectively, which are below the permissible limit of 500 (mg/L). Turbidity (NTU) of the water samples ranged from 0.170 – 4.33, the values of turbidity recorded were below the permissible limit of ≤ 5. DO and COD (mg/L) ranged from 3.10 – 5.30 and 4.058 – 8.624, although, there is no specific guideline. BOD (mg/L) ranged from 2.30 – 5.00, the sachet water recorded lower BOD value, the values were below the permissible limit of 6 (mg/L). Nitrate, sulphate and phosphate (mg/L) ranged from 1.216 – 1.737, 0.212 – 0.255, and 0.027 – 0.069, and the values recorded are below the permissible limit of 50, 500 and 100, respectively. The colour of the river water samples and Toru-Orua borehole were not clear, and only Toru-Orua borehole water samples recorded objectionable in term of odour.

Table 4: Physicochemical properties of drinking water sources in Sagbama Local Government Area of Bayelsa State

Location/sachet water brand	Temperature (°C)	pH	Total Dissolved Solids (mg/L)	Electrical Conductivity (µS/cm)	Chlorine (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Nitrate (mg/L)	Sulphate (mg/L)	Phosphate (mg/L)	Colour	Odour
Toru-Angiama river	27.4	7.36	51	59	<0.01	2.856	3.49	0.383	3.8	3.7	6.512	1.737	0.249	0.061	NotClear	Unobjectionable
Sagbama town river	27.5	7.33	51	59	<0.01	2.856	3.49	0.429	3.8	3.8	6.688	1.366	0.239	0.047	NotClear	Unobjectionable
Tungbo river	27.4	7.44	47	53	<0.01	2.632	3.21	0.433	5	5	8.8	1.35	0.241	0.056	NotClear	Unobjectionable
Ebedebiri river	27.6	7.38	47	60	<0.01	2.632	3.21	0.321	5	4.9	8.624	1.682	0.247	0.069	NotClear	Unobjectionable
Bolu-Orua river	27.5	7.15	49	58	<0.01	2.744	3.34	0.365	5.3	5	8.8	1.184	0.245	0.049	NotClear	Unobjectionable
Toru-Orua river	27.5	7.28	49	60	<0.01	2.744	3.34	0.33	5.2	4.9	8.624	1.729	0.255	0.048	NotClear	Objectionable
Bolu-Orua borehole	28.3	7.52	74	96	<0.01	4.144	5.05	0.172	3.1	2.3	4.058	1.318	0.222	0.034	Clear	Unobjectionable
Tungbo borehole	27.4	7.23	130	205	<0.01	7.28	8.88	0.19	5.1	5	8.8	1.547	0.245	0.044	Clear	Unobjectionable
Sagbama town borehole	27.5	7.49	47	57	<0.01	2.632	3.21	0.251	5	4.8	8.448	1.224	0.235	0.034	Clear	Unobjectionable
Ebedebiri borehole	27.6	7.19	31	46	<0.01	1.736	2.12	0.199	5.2	4.8	8.624	1.429	0.243	0.043	Clear	Unobjectionable
Toru-Orua borehole	27.6	6.88	49	58	<0.01	2.744	3.34	0.267	5.2	4.6	8.096	1.39	0.246	0.046	NotClear	Unobjectionable
EBM	27.6	7.47	101	114	<0.01	5.656	6.91	0.222	3.3	2.6	4.576	1.713	0.245	0.027	Clear	Unobjectionable
GG	27.4	7.56	64	83	<0.01	3.584	4.37	0.249	3.3	2.7	4.752	1.342	0.235	0.043	Clear	Unobjectionable
AQB	27.3	7.45	15	22	<0.01	0.84	1.03	0.217	3.4	3	5.28	1.287	0.212	0.034	Clear	Unobjectionable
PA	27.5	7.20	66	67	<0.01	3.696	4.51	0.175	3.5	3.2	5.632	1.232	0.224	0.028	Clear	Unobjectionable
ESE	27.4	7.24	25	32	<0.01	1.4	1.71	0.17	4	3.8	6.688	1.216	0.226	0.03	Clear	Unobjectionable
WHO permissible limit	**NS**	**6.5-8.5**	**1000**	**250**	**5**	**600**	**500**	**≤ 5**	**NS**	**6**	**NS**	**50**	**500**	**100**	**clear**	**Unobjectionable**

NS = None specified guideline. Coloured words indicate parameters that did not meet the guidelines

DISCUSSION

Water that is clean and safe is essential for good health and a productive life. Water has a significant impact on human health, and the quality of the water supplied is critical in determining individual and community health. Safe drinking water is a crucial concern in terms of public health, as the human race's health is inextricably linked to the quality of water utilized. Rapid population growth, rising living standards in cities, and industrialization have resulted in increased demand for high-quality water, while pollution of water sources has been continuously increasing on the other. Fresh water availability and access have become one of the world's most pressing natural resource concerns in recent years. The prevalence of multidrug resistance bacterial and physicochemical quality of water source from Sagbama Local Government Area of Bayelsa state, Nigeria revealed that the different water sources were contaminated with one or more pathogenic bacteria species (table 1) which renders the water unfit for use as portable water. Although most of the contaminated water were from surface water (river sources), some of the bore hole and sachet water were also contaminated with bacteria of questionable sources. The observed finding in the present study can be attested to that reported by several other researches Biiton, (1994); Okonko *et al*., (2008); Likambo, (2014); Okoro *et al*., (2017), Onuoha-Elvis *et al.,* (2024) and Enaregha, (2024) who reported similar findings in their study. The presence of these organisms signifies contamination of water from anthropogenic activities, dept of bore hole, closeness to potential source of contamination, production line and production material and others. Human activities around river such as runoff from farm land, bathing, washing etc are major sources of river contaminations.

According to WHO (2017), total microbial counts should not be more than 1.0×10^2 cells/ ml, and zero MPN count per 100 ml of a water sample. Moreover, different study by Biiton, (1994) and Okoro *et al*., (2017) stated that diverse unfriendly environmental human activities in the vicinity of underground water; poor borehole construction and unhygienic production method for sachet water contributed greatly to their pollutions and poor water qualities**.** In addition, it was observed that some of the domestic and water factory boreholes are pumped into pipes for distribution and storage in plastic and metal tanks with little or no purification methods. Rusty pipes and tanks affect the quality of water by allowing seepage of microbial contaminants into the water (Ibe *et al*., 2002). Some of these tanks are equally long overdue for wash which may allow the growth and formation of microbial biofilm in the tank. The presence of some of these enteric bacterial especially *E. coli* is an indication of recent feacal contamination of the water either of human or animal origin. Other more dangerous microorganisms could be present (Richman, 1997). This result compared favourably with the report of Banwo (2006) who reported similar findings. The detection of *Escherichia coli*, *Salmonella* species and *Klebsiella* species (Table 5) in borehole water that is intended for human consumption was a cause for concern. These isolates may pose severe health complications to humans especially if they harbour virulence gene determinants.

Escherichia coli is a gram negative rod. It forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar. These are the most widely adopted indicator of faecal pollution and they can also be isolated and identified simply, with their numbers usually being given in the form of faecal coliforms/100 ml of wastewater (De Boer *et al*., 2000) Outbreaks of these diseases can occur as a result of, drinking water from taps polluted by a combination of different wastewater microorganism species, eating contaminated fish, or indulging in recreational activities in polluted water bodies containing water borne pathogen. E. coli cause urinary tract infection and diarrhea (Fine *et al*., 1996). *Klebsiella pneumonia* is a gram negative rod and is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrohea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia. *Salmonella* infections typically cause clinical manifestations: gastroenteritis, bacterium or septicemia, typhoid fever/enteric fever and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* sp. Can be divided into two fairly distinct groups: the typhoidal species/serovars i.e. *Salmonella typhi* and *S. paratyphi* and the remaining non-typhoidal species/serovars (WHO, 2004).

The percentage susceptibility pattern of antibiotics against bacterial isolates (table 3) showed that almost all the isolated were resistant to more than two antibiotics except for Levofloxacin that showed 100% sensitivity to the test isolates. The results of the study can be attested to that reported by Thung *et al*. (2016), Kinge *et al*. (2020) and Ogunleye *et al*. (2008) ho reported similar findings from different water sources. This high percentage of multi-drug resistant bacterial isolates can be attributed to the constant increase in the misuse of antibiotics in most developing countries, especially in animal husbandry, human and agriculture. Isolates of *E. coli* were multi-drug resistant as most isolates were resistant to more than six of the reference antibiotics. The findings of this study confirm the pattern of antibiotic resistance in *E. coli* isolates obtained from water similar to a study by Sayah *et al*. (2005) which reported that *E. oli* isolates from surface water, domestic and wild animals and faecal samples, were found to be resistant to antibacterial agents such as ampicillin, chloramphenicol, tetracycline, sulphamethoxazole/trimethoprim, cephalothin, neomycin and streptomycin. These findings are also consistent with a report by Wose-Kinge *et al*. (2010) on the antibiotic resistance profiles of *E. coli* isolated from different water sources in the Mmabatho locality in South Africa, where these isolates were identified to be resistant to antibiotics such as erythromycin, tetracycline, ampicillin and chloramphenicol. There is a need to determine the possible mechanism(s) of resistance for these multi-drug resistant bacterial strains.

Table 4 shows the physico-chemical analyses determined. Generally, most of the physico-chemical parameters in the majority of the water sampled were within the WHO water standards for domestic use. On the contrary, the ordour for Toru-Orua river water had objectionable properties. Temperature is one of the most important ecological and physical factor which has a profound influence on both the living and non-living components of the environment, thereby affecting organisms and the functioning of an ecosystem. Although temperature generally influences the overall quality of water (physico-chemical and biological characteristics), there are no guideline values recommended for drinking water. In the present study, all water samples collected had pH values within the recommended ranges for WHO drinking water standards. The pH of water is important because many biological activities can occur only within a narrow range, thus any variations beyond an acceptable limit could be fatal to a particular organism. Therefore, the pH of the water in the study area could be classified as suitable for Portable water.

Turbidity results for the water were within WHO standards. Turbidity is defined as the measure of the clarity or cloudiness of water and the values are attained by measuring the scattering and absorbing effect that suspended particles have on light. Conductivity is defined as the ability of a substance to carry electrons (electric current). In water, this capacity is influenced by the amount of dissolved salts and the temperature. This means that at higher salt content, higher conductivity. In this way, this property can be used to measure the content of salts in a sample of water. All sampled water were within the limit recommended by WHO.

Alkalinity is the ability to react with the hydrogen ions of water, being mainly caused by the carbonate ($CO^{-}3$) and bicarbonate ($HCO^{-}3$) ions, although it is also influenced by the content in others such as borates, phosphates, silicates and oxydriles (Aznar-Jimenez, 2004). The alkalinity is influenced by the pH, the general composition of the water, the temperature and the ionic strength (Aznar-Jimenez, 2004).

The water samples evaluated showed values that did not exceed the allowed limits of WHO. The Nitrates ($NO^{-}3$) are very soluble salts, derived from nitrogen, which can be found in food and drinking water. They derive mainly from the use of nitrogen fertilizers, animal excreta, discharges from sanitary and industrial waste, and from the use as food additives (preserved fish and meat). If a water resource receives domestic wastewater discharges, nitrogen will be present as organic ammoniacal nitrogen, which, in contact with dissolved oxygen, will be transformed by oxidation into nitrites and nitrates. All evaluated water sample showed acceptable values.

COD constitutes the amount of chemical oxidant that is needed to be able to oxidize the materials contained in the water. COD is one of the most effective parameters in the control of water quality; Quantifies the amount of total organic matter susceptible to chemical oxidation (biodegradable and non-biodegradable) in a liquid sample and is used to establish the level of contamination (Ohanu *et al*., 2012)

Our findings of the physico-chemical analyses, although within the acceptable limit of WHO vary with that reported by Taiwo *et al*. (2014), Olorode *et al* . (2015), Shittu *et al*. (2008), Choudhury *et al*. (2016), Fatombi *et al*. (2012). These observed differences can be attributed to the sources of the water sample, sampling season and methods used.

CONCLUSION

The results of the present study indicated that drinking water sources in Sagbama Local Government Area are contaminated with various pathogenic bacteria and unfit for drinking. The study also revealed large-scale incidence of multi drug resistance bacteria in the water sources. All necessary strict measures should be taken by relevant stakeholders involved in the use, regulation and monitoring of antibiotics in human and animal husbandry. For this reason, it is recommended that groundwater for human consumption is treated before distribution to users. Detailed and continuous monitoring and assessment of other chemical species in the area such as total phosphorus concentrations which are indicative of pollution from human and animal waste is highly recommended. Increasing the frequency of sampling and analysis is also needed to effectively monitor the quality of the borehole water. Early detection of possible contamination can lead to faster implementation of corrective measures, preventing an imminent waterborne disease outbreak. Communities using borehole water as their source of water should be educated of the possible risks associated with consumption of contaminated water. Education should also include possible means of treatment of water such as boiling and use of chlorination tablets so as to prevent possible adverse health effects.

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