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Research Article
Leachates Impact Assessment of Well Waters Surrounding the Upstream and Downstream of Abattoir

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Abstract

Background and Objective: Abattoir effluent, high in organic and inorganic matter, is known to have direct and indirect effects on water sources surrounding it. Thus, the quality of well water samples surrounding upstream and downstream of Onyearugbulem abattoir were investigated. **Materials and Methods:** The pre-surveillance study was carried out using the questionnaire method. Physico-chemical properties and bacteriological counts of well water samples were done using standard methods. **Results:** The pH downstream were slightly acidic with values ranging from 6.7-6.9 compared to 6.9-7.3 obtained from wells upstream. The temperature of water upstream and downstream ranged from 26-30°C. Both the temperature and pH upstream and downstream corroborates with WHO standard (30-32 and 6.5-8.5°C) for drinking water. The total bacteriological counts were higher downstream with about 2.0×10^4 CFU mL^{-1} as against the WHO limit of 1.0×10^2 CFU mL^{-1}. **Conclusion:** High concentration for most parameters especially the bacteria count is an indication of point pollution use through the affluent and non-point pollution from other domestic activities like washing of clothes and defecation in the area. This study provided an explicit revelation of pathogenic microorganisms in some of the wells located downstream of Onyearugbulem abattoir, resulting from the improper treatment of slaughterhouse wastewater discharge which pose a public health problem and environmental hazard to the residents of this community.

Key words: Bacterial, physico-chemical properties, water, slaughterhouses, antibiotics sensitivity, water pollution, effluent

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is a transparent fluid that forms the world's streams, lakes, oceans and rain, it is the major constituent of the fluids of living things. It is one of the most important, as well as one of the most abundant compounds on earth and it is vital to the survival of any organism[1]. Water in its purest form is odourless, colourless and tasteless, however, human and animal activities continue to challenge the wholesomeness of water. Water constitutes a sizeable percentage of our daily food intake as human bodies do not have a reserve supply and due to its natural abundance, it is considered a universal solvent[2,3]. An average man (of 53 kg to 63 kg b.wt.), requires about 3 L of water in liquid and food daily to keep healthy[4]. This fact accounts for why water is regarded as one of the most indispensable substances in life and like air, it is most abundant[5].

Natural impurities are present in all water milieu, predominantly inorganic impurities as a result of geological layers where water currents, then, mostly from human-generated pollution through microbes and chemicals[2]. The significance of water to human and other biological systems cannot be overemphasised and there are numerous scientific and economic facts that, water shortage or its pollution can cause a severe decrease in productivity and deaths of living species[6,7]. Reports by Food and Agricultural Organisation (FAO) of USA revealed that in African countries, particularly Nigeria, water-related diseases had been interfering with basic human development[8]. The common sources of water that are available to local communities in Nigeria are fast being severed by several anthropogenic factors, of which pollution remains the most dominant problem. The presence of faecal coliform tends to affect humans more than it does aquatic creatures, though not exclusively[9].

Water becomes unsafe for human consumption and usage when it contains pathogenic or disease-causing microorganisms. Pathogenic microorganisms and their associated disease(s) may include bacteria, such as *Salmonella typhi* (typhoid fever), *Vibrio cholerae* (cholera), *Shigella* sp. (dysentery, shigellosis) and viruses, such as Poliovirus or Hepatitis A virus and protozoa such as *Giardia lamblia* (giardiasis) or *Cryptosporidium parvum* (cryptosporidiosis)[10]. Various opportunistic pathogens that occur naturally in the environment may cause disease in humans. Those who are at greater risk of infection are infants and young children, people whose immune system is suppressed, the sick and the elderly. In such individuals, drinking water containing large numbers of opportunistic pathogens can occasionally produce infections[11].

Unsafe water, inadequate sanitation and insufficient hygiene account for an estimated 9.1% of the global burden of disease and 6.3% of all deaths, according to the World Health Organization[12]. This burden is disproportionately borne by children in developing countries, with water-related factors causing more than 20% of deaths of people under age 14, nearly half of all people in developing countries have infections or diseases associated with inadequate water supply sanitation[13]. A study by Wogu and Okaka[14] showed that 48% of the people in the Katsina-Ala Local Government Area of Benue State are affected by urinary schistosomiasis, due to an increase in the water pollution index. Some previous investigations indicate that 19% of the whole Nigerian population is affected, with some communities having up to 50% incidence. This has raised serious concerns to World Health Organisation, in an attempt to improve cultural and socio-economic standards of people in the tropical region[14-16].

Antibiotics were discovered in the middle of the nineteenth century and brought down the threat of infectious diseases which had devastated the human race. The ability of microorganisms to become resistant to the major therapies used against them has long been recognized and is becoming increasingly apparent[17]. Resistance rates for many isolates are rising but are highly variable. It was also demonstrated that resistance to antimicrobial substances methicillin, gentamicin, kanamycin and tetracycline, can be acquired by the transfer of the relevant plasmid between Gram-positive microorganisms, among which *Bacillus cereus* falls[18]. Intraspecies, as well as the interspecies transfer of resistance, was established in the named organisms[19,20]. Increasing antimicrobial resistance presents a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality and health care expenditure[21].

The major issues of national and international interest are how these water pollution problems could be fully assessed and mitigated, proper knowledge and planning are thus essential. This paper, therefore, explores the pollution of water in the Onyearugbulem community. It emphasizes improper treatment of slaughterhouses wastewater discharge as the source of pollution to domestic water, which is mostly accessible to community inhabitants for their daily household applications. Associated consequences and the way forward had also been encompassed.

MATERIALS AND METHODS

Study area: The study area, the Onyearugbulem Community, is located in the southern part of Akure, Ondo State very close to where slaughterhouse effluent and wastewater are discharged directly to drainages. The slaughterhouse is the largest in the southern part of Akure with over 50-100 cows being killed per day, increasing the rate of effluent. The effluent from the abattoir is constantly discharged into drainages surrounding the abattoir at a different point along its course before finally discharging into a dam in the community. The well water samples collected is used for drinking and other domestic purposes. The study was carried out at the Research laboratory, Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Sterilization of materials: Glasswares were washed with detergent, rinsed with clean water to remove traces of residual washing compound and sterilized in a dry heat oven at 160°C for 1 hr. The workbench was disinfected with cotton wool soaked in 70% ethanol. All culture media were sterilized at 121°C for 15 min using the autoclave.

Sampling collection: Water samples were collected randomly with sterile bottles from 6 different wells downstream and 4 different wells upstream of the residential area surrounding Onyearugbulem abattoir, the bottles were coded W01 to W06 and W07 to W10, respectively. The pH and temperature were measured, appearance was noted whether clear or turbid and the water samples were taken to the laboratory for further analysis.

Physico-chemical analysis

pH analysis: The pH analysis of well water samples collected was done using a pH meter standardized using buffer solution of pH 6.4. After calibration, the electrode was rinsed with distilled water and cleaned with tissue paper. It was then dipped into each of the water samples collected for 2 min each and readings were recorded.

Temperature: A Mercury bulb thermometer was dipped into each well water sample and left for 3 min, it was withdrawn and the temperature on the thermometer was read for each water sample.

Bacteriological analysis

Bacterial load enumeration: The bacterial load enumeration was carried out at The Federal University of Technology, Akure (FUTA) Microbiology Laboratory I. This was done using the pour plate method. A 2-fold serial dilution was prepared for each well water sample (this involved adding 1 mL of the water sample in 9 mL of sterile deionized water and mixing thoroughly). One milliliter aliquot of the 10^{-2} serial dilution was drawn, inoculated into different sterile Petri dishes containing Nutrient, MacConkey and Eosin Methylene Blue Agar already cooled to 45°C using Pour Plating technique. The plates were incubated at 37°C for 24 hrs (this same procedure was carried out for each of the well water samples). The Colony-Forming Unit (CFU) was enumerated and the bacterial load was characterised using the Bergey's Manual (plating was done in duplicates).

Isolation of pure bacteria culture: Colonies from the incubated plates were picked and streaked on separate sterile plates of solidified Nutrient Agar. The plates were incubated at 37°C for 18 hrs. This was repeated until a satisfactory and evident pure culture was obtained. A loop full of the inoculum was preserved on Nutrient Agar slants of double strength and incubated at 37°C for 24 hrs. The slants were stored in the refrigerator at 4°C.

Biochemical tests

Gram staining: A smear was made by picking a loopful of the inoculum from 1 of the 24 hrs old culture plates, it was emulsified in a drop of water on a clean grease-free glass slide. The smear was allowed to air-dry before being heat-fixed by passing the slide over the blue-flamed Bunsen burner. The slide was placed on a staining glass rod and the smear was flooded with a few drops of crystal violet solution (primary stain) for 30 sec and immediately rinsed with water. The smear was flooded with Lugol's iodine (mordant) for 60 sec and rinsed with water. A few drops of 70% ethanol was applied to decolourize the smear and remove excess stain. The smear was rinsed with water and counterstained with a few drops of safranin for 1 min. The slide was rinsed with water, allowed to air-dry and a drop of immersion oil was placed on the smear. The slide was examined on the microscope using the oil immersion objective (×100).

Spore staining: Smears of the isolates were prepared on clean grease-free microscopic slides, the slides were placed on a slide rack and flooded with the malachite green solution and then steamed by placing over a beaker with boiling water for 10 min (the stain was added continuously to prevent drying up) before washing off under the running tap and allowed to air-dry. The smears were flooded with safranin for 20 sec and washed off under a running tap, allowed to air-dry and examined under oil immersion objective lens (×100). The spores appear green while the vegetative cells appear pink.

Motility test: The motility of the isolates was determined using the hanging drop method. A ring of vaseline was applied near the four corners of the clean grease-free cavity slide. A loop-full of bacteria suspension was taken from the 24 hrs old broth culture aseptically and a drop was placed at the centre of a coverslip laid on the bench. The drop was covered using the concave portion of the prepared cavity slide and pressed gently unto the coverslip in such a way that the cavity covers the drop. The slide was inverted quickly such that the drop hangs onto the cavity without spilling. The preparation was observed on the microscope with an ×40 objective lens. The movement of organisms in different specific directions indicates a positive motility test while a Brownian or no movement indicates a negative result.

Catalase test: The presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide was determined by this test. The test was carried out by making an emulsion of each 24 hrs cultured isolate on a clean grease-free microscopic slide and two drops of 3% hydrogen peroxide were added to the emulsion. The effervescence of oxygen evident by the production of bubbles showed a positive result.

Citrate test: An inoculum from a pure culture was transferred aseptically to a sterile tube of Simmons citrate agar. The inoculated tube was incubated at 35°C for 24 hrs and the results were determined. Abundant growth on the slant and a change from green to blue in the medium indicates a positive result.

Coagulase test: This test is used to determine the presence of cytochrome oxidase enzymes. The use of inoculating loop gives a false positive or negative result. A colony of test organism was emulsified in a drop of water on a clean grease-free microscopic slide. A sterile wire loop was then dipped into human plasma and added to the emulsion, visible clumping within 10 sec indicates the presence of coagulase enzyme and a positive result.

Oxidase test: Cytochrome oxidase is an enzyme that reduces oxygen. This enzyme, therefore, is an oxygen reductase. Cytochrome oxidase is the last step in the electron transfer system in most aerobic organisms. A drop of oxidase reagent was placed on a filter paper. An inoculum was picked with an applicator stick and dropped on the oxidase reagent. A purple colour change indicates a positive reaction.

Indole test: This test was carried out to determine bacteria's ability to split indole from the tryptophan molecule. Certain bacteria oxidize the amino acid, tryptophan, with tryptophanase enzymes to form three indolic metabolites. Sterile peptone water in test tubes was sterilized and allowed to cool. An inoculum was picked from the 24 hrs pure isolates, inoculated into the test tubes and incubated for 24 hrs at 37°C. Two milliliters of the incubated suspension was placed in a tube and 2 drops of Kovac's reagent was added. Red ring colouration at the top of the tube indicates a positive reaction.

Triple sugar iron test (TSI): Triple sugar iron agar was used to test for organisms' capability to ferment sugar and produce gas or hydrogen sulphide. As 6.5 g of the TSI agar was weighed in 100 mL of water and sterilized at 121°C for 15 min. About 15 mL was decanted into sterile test tubes, these tubes were bent to make a slant and allowed to solidify. An inoculum was picked from the 24 hrs old pure culture plate using a sterile inoculating loop and was introduced into the TSI agar slant. This was incubated for not more than 24 hrs to avoid false positive or negative results and observed changes were recorded.

Susceptibility test: The probable isolates were screened for antimicrobial resistance using the disc diffusion method[22] as approved by the National Committee for Clinical Laboratory Standard Guidelines (CLSI document VET01-A4, 2013). The disc diffusion method is widely recognized to work well with rapidly growing, facultatively anaerobic and aerobic organisms such as Enterobacteriaceae. Mueller-Hinton agar was prepared according to the manufacturer's specification, it was sterilized, allowed to cool to 45°C, poured into sterile Petri dishes and allowed to solidify. The inoculum was emulsified in peptone water and transferred to the Mueller-Hinton agar plate using the lawn method to obtain an even distribution of the isolates. It was allowed to soak the agar for some minutes and the excess was drained. The antibiotic disc was placed on it and incubated for 24 hrs at 37°C, the sensitivity plates were observed for clear zones of inhibition after 24 hrs of incubation. The zone diameter was measured with a ruler to determine the most susceptible and effective drug of choice.

RESULTS

Table 1 and 2 contain surveillance data and physical descriptions of studied well waters around the abattoir. Water samples collected had varying pH and temperature. The pH of water samples downstream (W01-W06) ranged from 6.7-6.9 with W05 and W06 having the highest pH (6.9) and a temperature ranging from 28-30°C, W06 had the highest

Table 1: Pre-surveillance data of residences around Onyearugbulem abattoir

	Domestic water source		Water purification		Public access		Position to sewer		Common disease occurrence		
Age group	Well	Borehole	T	NT	SF	MF	OPP	DIG	Diarrhea	Typhoid	Non
1											
0-15	1	0	1	0	1	0	1	0	0	1	0
Percentage (%)	100	0	100	0	100	0	100	0	0	100	0
2											
16-24	10	1	9	2	7	4	8	3	2	6	3
Percentage (%)	90.9	9.1	81.8	18.2	63.6	36.4	72.7	27.3	18.2	54.5	27.3
3											
25 and above	8	4	9	3	8	4	10	2	6	1	5
Percentage (%)	66.7	33.3	75.0	25.0	66.7	33.3	83.3	16.7	50.0	8.3	41.7

T: Treated, NT: Not treated, SF: Single fetcher, MF: Multiple fetcher, OPP: Opposite and DIG: Diagonal

Table 2: Description of study well water samples

Sample code	Description	Depth (ft)	Sewer to well	Distance from flow
W01	Household well, T/SF	12	Diagonal	200 m from abattoir 20 m from flow
W02	Well for piggery purposes, T/SF	9	Diagonal	250 m from abattoir 20 m from flow
W03	Household well for laundry purposes, NT/MF	15	Opposite	150 m from abattoir 20 m from flow
W04	Household well, T/SF	12	Opposite	200 m from abattoir 30 m from flow
W05	Household well, T/SF	9	Diagonal	300 m from abattoir 20 m from flow
W06	Church well, T/MF	12	Nil	250 m from abattoir 15 m from flow
W07	Household well, T/MF	15	Diagonal	Upstream
W08	Household well, T/SF	12	Diagonal	Upstream
W09	School well, T/MF	15	Nil	Upstream
W10	Hospital well, T/MF	15	Diagonal	Upstream

T: Treated, NT: Not treated, SF: Single fetcher and MF: Multiple fetcher

Table 3: Physico-chemical parameters of well water samples

Sample code	pH	Temperature (°C)	Appearance
W01	6.8	28.0	Clear
W02	6.8	29.0	Turbid
W03	6.7	29.0	Turbid
W04	6.8	28.0	Clear
W05	6.9	28.0	Clear
W06	6.9	30.0	Clear
W07	7.2	27.0	Clear
W08	7.2	26.0	Clear
W09	6.9	26.0	Clear
W10	7.3	28.0	Clear

Table 4: Total bacterial count (CFU mL^{-1}) of well water samples

Sample code	Total bacterial count (CFU mL^{-1})
W01	3.0×10^{2}
W02	1.38×10^{4}
W03	2.0×10^{4}
W04	7.2×10^{3}
W05	1.28×10^{4}
W06	8.9×10^{3}
W07	3.8×10^{3}
W08	7.0×10^{3}
W09	3.2×10^{3}
W10	6.8×10^{3}

temperature (30°C). The pH of samples collected upstream (W07-W10) ranged from 6.9-7.3 with W10 having the highest pH (7.3) and a temperature ranging between 26 and 28°C, with 28°C being the highest temperature. The W02 and W03 had turbid appearance while W01, W04, W05, W06, W07, W08, W09 and W10 had a clear appearance as shown in Table 3.

Total bacterial count (CFU mL^{-1}) for downstream well samples W01 to W06 were 3×10^{2}, 1.38×10^{4}, 2.0×10^{4}, 7.2×10^{3}, 1.28×10^{4} and 8.9×10^{3}, respectively with W02 and W03 having the highest count while upstream well samples W07 to W10 had a total bacterial count of 3.8×10^{3}, 7.0×10^{3}, 3.2×10^{3} and 6.8×10^{3}, respectively with W08 having the highest count as shown in Table 4.

Table 5: Morphological characteristics of bacterial isolates from well water samples

Colour	Elevation	Colony formed	Surface	Opacity	Grain stain	Cellular morphology	Arrangement	Presumptive identity
	Umbonate		Mucoid		-	Rod	Straight rod	*Pseudomonas aeruginosa*
Creamy white	Raised	Cir	Wet	Tsl	-	Rod	Straight rod	*Klebsiella* spp.
Cream white	Raised	Cir	Wet	Opq	-	Rod	Straight rod	*Salmonella* spp.
Yellow	Flat	Cir		Opq	+	Cocci	Clusters	*Micrococcus* spp.
Creamy yellow	Slightly flat	Cir	Dry	Opq	+	Cocci	Clusters	*Staphylococcus aureus*
Cream	Flat	Cir	Dry	Opq	+	Rod	Straight rod	*Bacillus* spp.
Grayish white	Raised	Cir	Dry	Opq	+	Cocci	Clusters	*Staphylococcus epidermidis*
Cream		Regular			+	Cocci	chain	*Enterococcus faecalis*

Table 6: Biochemical identification of isolates

Motility	Spore	Coagulase	Catalase	Oxidase	Indo	Citrate	Glucose	Sucrose	Lactose	Gas prod.	H_2S	TSI INT	Presumptive identity
+	NT	+	+	+	-	+	-	-	-	-	-	K/NC	*Pseudomonas aeruginosa*
-	NT	-	+	-	-	+	+	+	+	+	-	A/AG	*Klebsiella* sp.
M	NT	-	+	-	-	+	+	-	-	-	+	K/A	*Salmonella* sp.
NT	NT	+	+	+	NT	-	-	-	-	-	-	K/NC	*Micrococcus* spp.
NT	NT	+	+	-	NT	-	+	+	+	+	+	A/AG	*Staphylococcus aureus*
NT	+	-	+	+	-	+	+	-	-	+	+	K/AG	*Bacillus* sp.
NT	NT	-	+	-	NT	-	+	+	+	+	+	A/AG	*Staphylococcus epidermidis*
NT	NT	+	-	-	-	-	+	+	+	-	-	A/A	*Enterococcus faecalis*

-: Negative, +: Positive, M: Motile, NM: Non motile, NT: Not tested, A/A: Production of acid acidic but no gas, A/AG: Production of acid and gas, K/A: Production of alkaline/acid but no gas, K/AG: Production of alkaline/acid but gas produced and K/NC: Alkaline slant/alkaline but no reaction

Table 7: Occurrence of bacterial isolates in the sampled well waters

Suspected isolates	W01	W02	W03	W04	W05	W06	W07	W08	W09	W10
Staphylococcus aureus	+	-	-	-	-	+	-	+	-	-
Salmonella sp.	+	+	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	+	-	-	+	-	-	-	-	-
Bacillus sp.	-	+	-	-	-	+	+	+	-	+
Staphylococcus epidermidis	-	+	+	-	-	-	-	-	-	+
Enterococcus faecalis	-	-	+	-	-	-	-	-	+	-
Micrococcus sp.	-	-	-	+	-	-	-	-	+	-
Klebsiella sp.	-	-	+	-	+	-	-	-	-	-

-: Negative and +: Positive

Table 8: Antibiotics sensitivity pattern for Gram-negative isolates

	Zone of inhibition (mm)					
Gram-negative bacteria isolates	PEF	CN	CPX	CH	S	AU
Pseudomonas aeruginosa	R	15	20	4	3	R
Klebsiella sp.	R	14	16	14	2	R
Salmonella sp.	R	17	14	18	R	R

PEF: Pefloxacin (30 µg), CN: Gentamicin (10 µg), CPX: Ciprofloxacin (10 µg), CH: Chloramphenicol (30 µg), S: Streptomycin (30 µg) and AU: Augmentin (30 µg)

Table 9: Antibiotics sensitivity pattern for Gram-positive isolates

	Antibiotics/zone diameter (mm)					
Gram-negative bacterial isolates	AM	CPX	S	E	CN	APX
Staphylococcus aureus	10	14	3	21	18	R
Staphylococcus epidermidis	8	7	R	16	14	R
Micrococcus sp.	3	10	8	17	R	R
Enterococcus faecalis	5	7	8	18	14	R
Bacillus sp.	R	17	9	12	R	R

PEF: AM: Amoxicillin (30 µg), CPX: Ciprofloxacin (10 µg), S: Streptomycin (30 µg), E: Erythromycin (15 µg), CN: Gentamicin (10 µg) and APX: Ampiclox (30 µg)

Eight bacterial species from 7 genera were presumptively identified using morphological and biochemical properties as detailed in Table 5 and 6 and compared to Bergey's manual. These bacteria are *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Staphylococcus epidermidis*, *Klebsiella* sp., *Enterococcus* sp. and *Micrococcus* sp. Table 7 showed the frequency occurrence of bacterial isolates in the sampled well waters. Well W02 had the highest variety of bacteria isolated and *Bacillus* sp., was most represented in the well water samples. Table 8 and 9 revealed

antibiotic sensitivity patterns for Gram-negative and Gram-positive isolates, respectively. Hundred percent of the isolates were susceptible to ciprofloxacin. The Enterobacteriaceae group, *Klebsiella*, *Pseudomonas* and *Salmonella* have the highest zone diameter of 16, 20 and 18 mm for ciprofloxacin, ciprofloxacin and chloramphenicol, respectively. Ciprofloxacin had the highest zone of inhibition on *Klebsiella* sp. and *Pseudomonas aeruginosa* while chloramphenicol had the highest zone of inhibition on *Salmonella* sp. as the antibiotics corroborate with the performance standards for antimicrobial disc susceptibility test. Erythromycin had the highest zone of inhibition with zone diameter of 21 and 16 mm for *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively followed by gentamycin, ciprofloxacin and amoxicillin with zone diameters of 18, 14 and 10 mm, respectively (Table 9). Augmentin had no zone of inhibition, as such, 100% of tested bacterial isolates were resistant to the antibiotics.

DISCUSSION

In this study, it was observed that the pH values of the water samples were close to neutral. Tsega[23] reported that the pH of water plays an important role in the rate of survival of microorganisms in an aquatic environment. Generally, bacteria are known to grow well within a range of pH values between 6 and 9[22]. The pH of water samples collected varied from 6.7 to 6.9 downstream and 6.9 to 7.3 upstream which corroborates with the standard pH range (6.0 to 9.0) for drinking water[24]. The range of temperature of the water samples obtained upstream and downstream was between 26 and 30°C which also agrees with the temperature of growth for mesophilic bacteria (20-40°C)[25], these temperature values were within the normal range for aquatic lives and will have minimal effects on acidity[26].

Table 4 shows the total bacteria count for well water samples obtained upstream and downstream which ranged from 3×10^2 to 2.0×10^4 CFU mL^{-1}. Samples collected downstream had a higher bacterial count with W03 having the highest (2.0×10^4 CFU mL^{-1}). Table 3 shows the turbidity observed in W01 and W02 downstream implied a high presence of impurities in the water while the high bacterial count obtained from well downstream implied that there was a possibility of a high quantity of the abattoir leachates getting into the water of hand-dug wells within the abattoir. Thus, the water samples from the wells downstream the abattoir are heavily polluted and those from the wells upstream the abattoir are sparingly polluted, this result agrees with the work done by Adeyemo *et al.*[27]. Bacteria count obtained for wells downstream and upstream do not corroborate with WHO standard bacterial count limit for drinking water (1.0×10^2)[28], this implied contamination from an abattoir, domestic activities and lifestyle. Bacteria count of wells upstream was much lower than that obtained downstream which implied that the abattoir had little or no impact on the water, contamination or pollution of such water results from domestic activities and lifestyle of residents. Leachates of effluents which have a large number of microbes to a great extent, pollute shallow and hand-dug wells[29]. The organic nutrients added to groundwater from leachates of this effluent which has a large number of microbes, account for the excessive microbial growth, causing unpleasant taste and odours of water from this source[30,31].

The bacteria species (*Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Klebsiella* sp., *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Micrococcus* sp.) presumptively identified from well water upstream and downstream of Onyearugbulem abattoir have been associated with water milieu[26]. The presence of *Salmonella*, *Klebsiella* and *Pseudomonas* in water samples obtained downstream, indicated pollution with faecal matter from warm-blooded animals, which is contained in effluent from the surrounding abattoir. The organisms are pathogenic causing diseases such as typhoid, diarrhoea and pneumonia. These pathogenic organisms are members of the Enterobacteriaceae which are commonly found in sewage. Blood from the slaughtering of cows, which is contained in the effluent, is an enriched medium that supports the growth of these microorganisms, especially the haemolytic bacteria (*Pseudomonas aeruginosa*).

Several species of *Pseudomonas* have been considered as opportunistic pathogens that can colonize animal and human tissues resulting in serious infections[32,33]. *Klebsiella* sp., isolated might be from faecal material deposit around the abattoir since they are natural inhabitants of the bowel of man. The presence of *Staphylococcus*, *Enterococcus* and *Micrococcus* sp., indicated bacterial contamination from human activities and physical description as described in this study (some wells were well covered while some were not, some use a single fetcher while others use multiple fetchers which increases the risk of a non-point source of contamination). The presence of these pathogenic organisms pose a health and ecological hazard in water and it increases the risk of waterborne disease outbreak. According to Mulamattathil *et al.*[1], the risk posed to source water quality from faecal waste discharged from a point source such as the slaughterhouse is especially high, as it can result in very high contamination variability which corroborates with the result

obtained in this work. Managing faecal contamination of groundwater surrounding the abattoir is made challenging by the continuous activities in the abattoir and continuous wash of effluent to drainages leaching down to groundwater. This is significantly high during the rainy season.

These results obtained were consistent with the findings of Olusola-Makinde *et al.*[34] that pathogenic microorganisms were isolated from waters surrounding the abattoir. Reported cases of water-borne diseases through the pre-surveillance data obtained from residents of Onyearugbulem community could also be a pointer to contaminated water sources. The differences in the prevalence of these diseases is based on various factors such as treatment, mode of access to water, sewer to well etc. (Table 1).

Ciprofloxacin was the most effective drug for the *Klebsiella* sp. and *Pseudomonas aeruginosa* isolates while chloramphenicol is the most effective drug for the *Salmonella* sp., isolates as the antibiotics corroborate with the performance standards for antimicrobial disc susceptibility test[25].

This is an indication that leachates from Onyearugbulem abattoir effluents increase incidence of pathogenic microorganisms and influence physico-chemical parameters in studied wells located downstream, even though, the isolated bacteria and fungi were susceptible to conventional antibiotics. Therefore, there is need for efficient treatment of abattoir waster water before releasing to the immediate environment and close sensitization of surrounding residents about in-house water treatment to prevent water-borne disease and other related public health concerns.

CONCLUSION

The study revealed that some of the wells located downstream of the Onyearugbulem abattoir were loaded with pathogenic microorganisms from slaughterhouse wastewater discharge which pose a public health problem and environmental hazard to the inhabitants of this community. Therefore, well water in this region or community should be made portable through simple water treatments such as filtration, the addition of alums and boiling before it is used for drinking, washing or cooking to prevent outbreaks in the area. The quality of effluents from the slaughterhouse did not meet the WHO minimum required standard, there is, therefore, the need to treat wastewater before discharging it into surface water to prevent contaminating groundwater and subsequently preventing water-borne diseases in the study area.

SIGNIFICANCE STATEMENT

This study discovered that leachates from abattoir effluents in Akure municipal have distorted the bacterial load and physico-chemical properties of receiving groundwater making them unsafe. The pollution level of the groundwater will help researchers to uncover risks associated and understand water-borne related infectious agents.

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