Antibiogram of Enteric Bacteria in Pharmaceutical Industrial Wastewater and Surrounding Groundwater Sources

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Abstract: This study set out to determine the influence of microbial quality of wastewater from a pharmaceutical industry on surrounding groundwater sources and antibiotic susceptibility of isolated bacteria. Wastewater and water samples were collected from the study sites and the concentrations of enteric bacteria were determined using standard microbiological technique. Physicochemical characteristics of the wastewater and water samples were determined using standard methods. Relationships between the levels of enteric bacteria in the wastewater and water samples were determined using Pearson's correlation. Antibiotic susceptibility pattern of the isolates were determined using standard disc diffusion method. The results revealed that *E. coli* had the highest mean concentration in the wastewater and water samples. The levels of enterococci in the wastewater samples had the most correlations with the levels of other enteric bacteria in water samples from the boreholes. *Salmonella* was susceptible to perfloxacin, while enterococci was susceptible to zinacef and all the isolates showed resistance to multiple antibiotics. This study demonstrates that the microbial quality of the boreholes were influenced by the wastewater from the pharmaceutical industry. Water from the boreholes had poor microbial quality and potential pathogenic antibiotic-resistant bacteria that may pose significant risks to public health.
Key word: Antibiogram, enteric bacteria, water quality, microbial indicators, human health

INTRODUCTION

Water pollution may pose serious threat to the human and environmental health mainly because pollutant effects vary depending on their types, sources and effects. For instance, while heavy metals, dyes, and some other organic pollutants have been identified as carcinogens; hormones, pharmaceuticals, and cosmetics and personal care product wastes are known as endocrine disruptive chemicals (Adeogun *et al*., 2016). Complex and variable composition of organic and inorganic chemicals that are generated in wastewater represent a major source of surface and groundwater pollution (Bassin *et al.*, 2017). Most importantly, wastewater from industries can potentially alter the microbial ecological landscape, including the quality of receiving waters, and in general the aquatic ecosystem (Bassin *et al.*, 2017). Groundwater represent approximately 50% of drinking water in the world because it is usually less exposed to contamination, pathogen penetration and evaporation, thus making it more stable and suitable for supply. The water is usually abstracted through hand-dug wells; hand-pump operated shallow-wells and submersible pump operated deep well or boreholes (Ojo *et al.*, 2011; Olalemi *et al.*, 2023). Groundwater is often high in mineral content such as magnesium and calcium salts, iron and manganese depending on the chemical composition of the stratum through which the rock flows. Contamination of groundwater sources may occur through anthropogenic activities such as improper wastewater disposal, animal rearing and cross contamination from farmland, runoff through soil infiltration (Zhang *et al*., 2014; Beer *et al*., 2015).

Wastewater treatment facilities has three broad levels of treatment, designed to decrease its contamination effect on receiving waters. The primary treatment removes suspended and sinking solids by skimming and sedimentation, the secondary treatment removes residual organic compounds and suspended solids, while the tertiary treatment removes specific wastewater constituents like heavy metals, microorganisms, biodegradable and non-biodegradable materials (Edward *et al.*, 2021). Microorganisms in wastewater may include pathogenic bacteria, protozoa and viruses. The levels of contamination in wastewater may be determined using indicator organisms that are mostly non-toxic to human health (Ashbolt and Snozzi, 2001). Bacteria commonly utilized as indicator organisms in water and wastewater quality assessment include *E. coli* and faecal coliforms (Cabral, 2010). The occurrence of microorganisms such as *Clostridium, Shigella, Salmonella, Thiobacillus, Acinetobacter, Nitrosomonas, Nitrobacter, Achromobacter Alcaligenes, Bacillus, Flavobacterium, Micrococcus* and *Pseudomonas* have been detected in industrial wastewaters (Bassin *et al*., 2017). Interestingly, pharmaceutical industries may generate wastewaters that contain pathogens such as *Salmonella, Shigella, Thiobacillus, Clostridium*, and contaminants such as hydrocarbons, nitrogen, phosphorus, heavy metals. Microbial community composition and diversity in aquatic environments may be influenced by a combination of physicochemical parameters such as pH, dissolved oxygen and salinity, availability of nutrients, heavy metals, introduction of chemical contaminants (Quero *et al*., 2015).

Enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics (WHO, 2001). Multiple antibiotic resistance were detected in bacteria such as *Streptococcus faecalis, E. coli, Salmonella typhi* and *Shigella dysenteriae* that were isolated from biofilms of water obtained from boreholes and wells in Ado-Ekiti, Nigeria (Olalemi *et al.*, 2019). The rise in antibiotic resistance has been reported in the past two decades, and antibiotic resistance still remain a global challenge. Worldwide, approximately 700,000 deaths are recorded annually as a result of antibiotic resistant pathogens and this number is estimated to reach 10 million by 2050 (O'Neill, 2014; Ayodele *et al.*, 2019). It was estimated that over 56,000 mortalities of new born babies were recorded annually in India from infections by bacteria that are resistant to first-line antibiotics. The release of pharmaceutically active residues in low or high quantities from industrial wastewater into aquatic systems may induce multi-resistance in bacteria (Da Silveira *et al*., 2018; Bilal *et al*., 2019). Toxic and hazardous substances from industrial wastewaters may percolate to deeper layers of the soil and infiltrate underground aquifer when the wastewaters are discharged improperly into natural drains, sewer systems, septic tanks or surrounding field, thereby posing significant risk to human health. There are numerous reports on the microbial quality of industrial wastewaters and their perceived environmental effects, but there are limited reports on the bacterial diversity in groundwater sources sited around industries. The aim of this study was to determine the influence of microbial quality of wastewater from a pharmaceutical industry on surrounding groundwater sources and antibiotic susceptibility of isolated bacteria. The objectives of this study were to examine the levels and relationships between enteric bacteria in wastewater and water samples from the study sites; determine the physiochemical properties of wastewater and water samples; and examine the antibiotic susceptibility pattern of the bacterial isolates.

MATERIALS AND METHODS

Sampling sites and collection of samples: The wastewater sampling site was situated in a pharmaceutical manufacturing and marketing company in Akure, Ondo State, Nigeria. The company has major business interest in homecare and healthcare products that include; detergents, disinfectants, germicides, antiseptics, washing-up liquid, table water and some other antimicrobial products. The company process semi-finished products into finished products that are sold and distributed in Akure metropolis and its environs. All production activities are carried out following strict critical control points and wastewater that are generated are channelled into an open soak away system built solely for the storage and dispersal of the wastewater. The soak away system has a non-cemented floor to allow for percolation of the wastewater into the ground as they are not reused. It is also not covered, hence, subjected to drying by sunlight and frequent dilutions during rainfall events.

Three groundwater sources (boreholes) in close proximity to the pharmaceutical industry were selected. Borehole 1 (B1) was situated approximately 60 metres away from the wastewater sampling site, borehole 2 (B2) was about 95 metres directly opposite the wastewater sampling site and borehole 3 (B3) was approximately 130 metres from the wastewater sampling site. The water from the three boreholes are mainly used for drinking, washing and other domestic activities. Sampling activities were carried out every two weeks over a consecutive period of four months. On each sampling occasion, a sample of approximately one litre of wastewater and one litre of water from B1, B2 and B3 were collected in pre-sterilised plastic bottles in accordance with standard protocol (Anon. 2012). All wastewater (n=16) and water (n=48) samples were collected in duplicates and transported to the laboratory in cool boxes with ice packs and processed immediately (within less than one hour).

Enumeration of enteric bacteria in wastewater and water samples: The concentrations of *E. coli*, *Clostridium*, *Salmonella*, *Shigella* and intestinal enterococci in wastewater and water samples were determined using the membrane filtration following APHA (2012) standard methods. Wastewater samples were diluted serially and aliquots were filtered. Approximately, 100 ml of water samples were filtered through membranes filters (0.45 µm). The membrane filters were placed on freshly prepared selective media (membrane lauryl sulphate agar (MLSA), Eosin methylene blue (EMB) agar, membrane enterococci (*m*-Ent) agar, *Salmonella Shigella* agar (SSA) and *Clostridium perfringens* agar (*m*-CP). Agar plates were incubated aerobically at 37 ^{o}C for 24 hours (MLSA, EMB, SSA), 37 ^{o}C for 48 hours (*m*-Ent) and anaerobically at 37 ^{o}C for 24 hours (*m*-CP). The selective media used had components that enabled the growth and enumeration of the specific bacteria of interest. *Salmonella* had colourless colonies with black centre, whereas colonies of *Shigella* were clear, colourless and transparent. *Escherichia coli* had greenish metallic sheen in the confirmed test, *Clostridium* had yellow, opaque colonies and enterococci had brown colonies. All colonies were counted, calculated and expressed as colony-forming units (CFU) per 100 ml (APHA, 2012).

Determination of physicochemical properties of wastewater and water samples: The temperature of the wastewater and water samples were determined on-site (*in situ*) during sample collection using mercury-in-glass thermometer. The pH, electrical conductivity, salinity, total dissolved solids, turbidity, alkalinity and dissolved oxygen of the wastewater and water samples were determined using multi-parameter analyzer (HI98194, PH/ORP/EC/DO) (APHA, 2012).

Antibiotic susceptibility test of the bacterial isolates: The antibiotic susceptibility testing of the bacterial isolates was carried out using disc diffusion techniques as described by Clinical Laboratory Standard Institute (CLSI) (2012). Antibiotic discs used include pefloxacin 10 µg, zinacef 20 µg, amoxicillin 30 µg, ciprofloxacin 10 µg, streptomycin 30 µg, erythromycin 10 µg and chloramphenicol 30 µg. Values obtained were interpreted into resistant and sensitive, then transformed into percentage.

Statistical analysis: Data obtained were transformed to $\log_{10}$, then examined using general descriptive statistics. Further analyses were undertaken using Statistical Package for Social Sciences (SPSS) Version 20.0. All data were subjected to the Pearson's correlation analysis with test of significance at 0.05 to determine whether there were positive correlations between levels of enteric bacteria in wastewater and water samples from the study sites.

RESULTS

Escherichia coli had the highest mean concentration of 4.09 log_{10} cfu/100 ml in wastewater samples from the pharmaceutical industry, while *Shigella* had the least mean concentration of 2.88 log_{10} cfu/100 ml in wastewater samples. *Clostridium*, Enterococci and *Salmonella* had mean concentrations of 3.36, 3.83, 3.10 log_{10} cfu/100 ml, respectively (Figure 1). In addition, *E. coli* had the highest mean concentrations of 1.97, 1.93, 1.92 log_{10} cfu/100 ml in water samples from the boreholes (B1, B2 and B3), respectively, while *Salmonella* had the lowest mean concentrations of 1.30, 1.33, 1.27 log_{10} cfu/100 ml in water samples from the B1, B2 and B3, respectively (Figure 2).

The relationships between the levels of enteric bacteria in wastewater samples from the pharmaceutical industry and water samples from the three (3) boreholes were analysed using Pearson's correlation at $P < 0.05$ level of significance representing 95 % confidence interval (Table 1). Levels of enterococci in the wastewater samples showed a positive correlation with levels of *Shigella* ($r = 0.51$, $P < 0.05$) in water samples from borehole 1. Similarly, levels of enterococci in the wastewater samples exhibited positive correlations with levels of *Shigella* ($r = 0.70$, $P < 0.05$) and *Clostridium* ($r = 0.53$, $P < 0.05$), while it also showed negative correlations with levels of *E. coli* ($r = -0.58$, $P < 0.05$) and enterococci ($r = -0.58$, $P < 0.05$) in water samples from borehole 2. In addition, levels of *Clostridium* in the wastewater samples exhibited positive correlation with levels of *E. coli* ($r = 0.53$, $P < 0.05$) and negative correlation with levels of *Clostridium* ($r = -0.69$, $P < 0.05$) in water samples from borehole 2. Levels of *Shigella* in the wastewater samples showed a negative correlation with levels of *Clostridium* ($r = -0.54$, $P < 0.05$) in water samples from borehole 2. Furthermore, levels of enterococci in the wastewater samples showed positive correlations with levels of *E. coli* ($r = 0.64$, $P < 0.05$) and enterococci ($r = 0.63$, $P < 0.05$) in water samples from borehole 3.

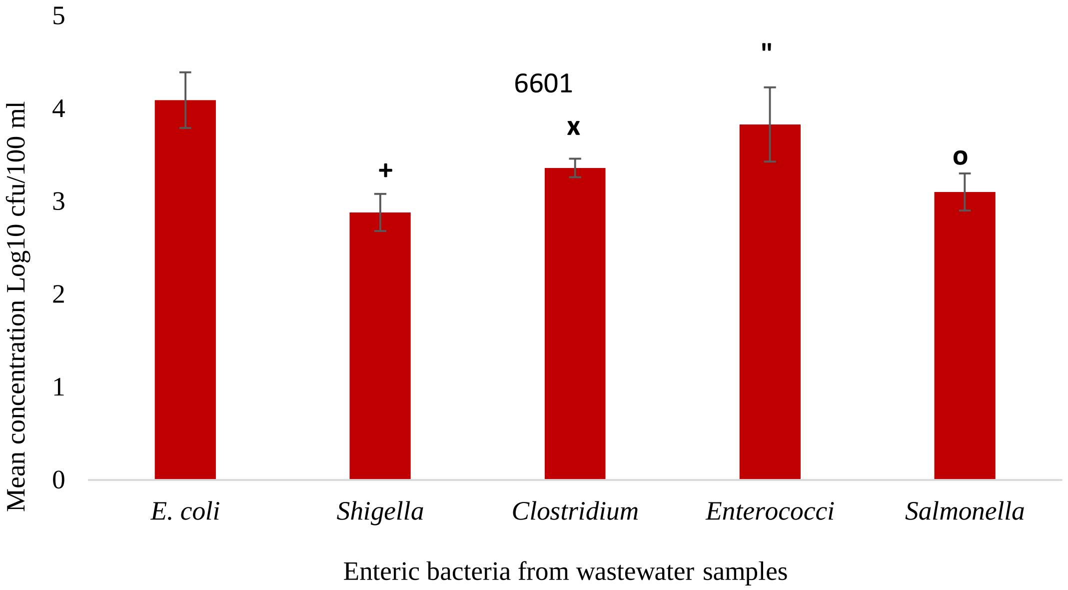

Figure 1: Mean concentration of enteric bacteria in wastewater samples from the pharmaceutical industry – The ANOVA was significant and a Bonferroni corrected Post-test (T-test) indicated that mean value of *E. coli* was significantly higher than those of *Shigella*, mean value of Enterococci was significantly higher than those of *Salmonella* and *Clostridium*. There was no significant difference in mean values of *Shigella* and *Clostridium*.

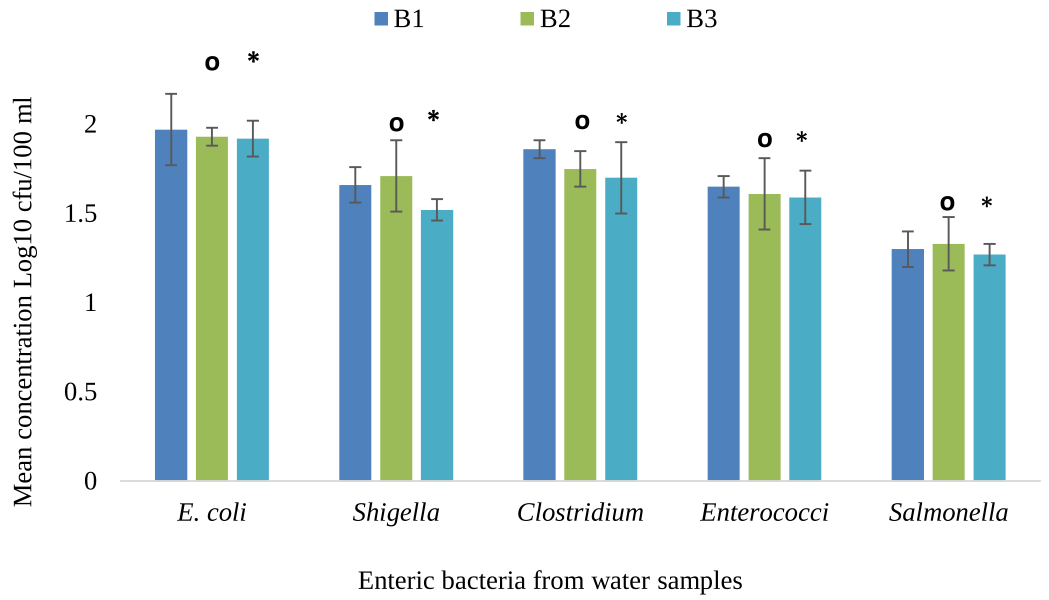

Figure 2: Mean concentration of enteric bacteria in water samples from the boreholes (B1, B2, B3) sited close to the wastewater from the pharmaceutical industry – The ANOVA was significant and a Bonferroni corrected Post-test (T-test) indicated that the mean values of *E. coli*, *Shigella*, Enterococci and *Salmonella* in B1 were not significantly higher than those in B2 and B3; mean value of *Clostridium* in B1 was significantly higher than those in B2 and B3.

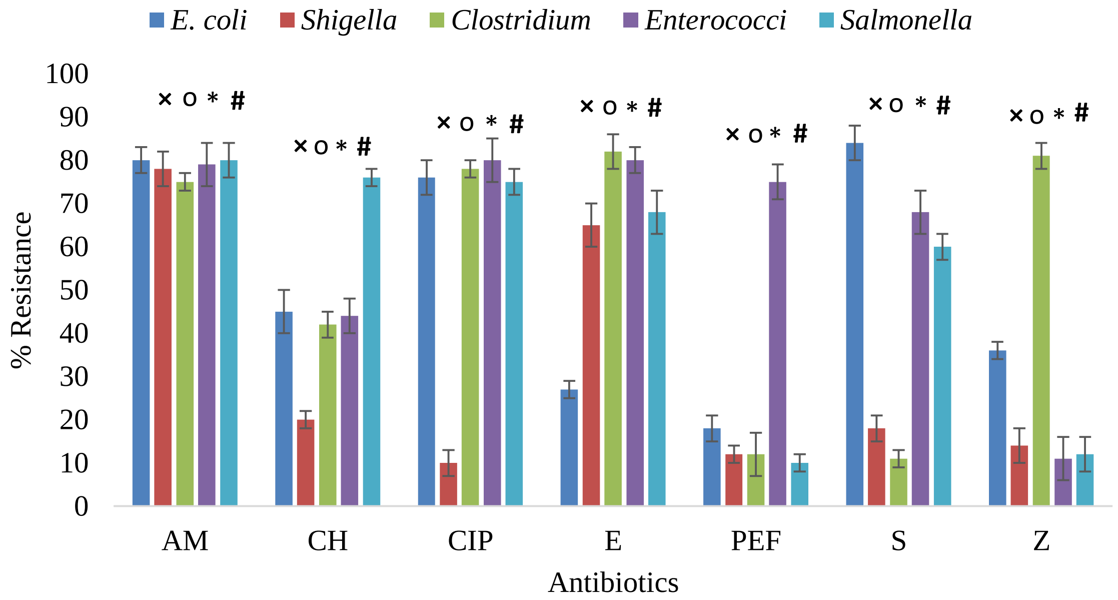

Figure 3: Percentage resistance of enteric bacteria in water samples from the boreholes (B1, B2, B3) to different antibiotics (AM – Amoxillin 30 µg, CH – Chloramphenicol 10 µg, CIP – Ciprofloxacin 10 µg, E – Erythromycin 10 µg, PEF – Perfloxacin 30 µg, S – Streptomycin 30 µg, Z – Zinacef 20 µg) – The ANOVA was significant and a Bonferroni corrected Post-test (T-test) indicated that the percentage resistance of all enteric bacteria to amoxillin had no significant difference, percentage resistance of *Salmonella* to chloramphenicol was significantly higher than the other bacteria, percentage resistance of *Shigella* to ciprofloxacin was significantly lower than the other bacteria, percentage resistance of Enterococci to perfloxacin was significantly higher than the other bacteria, percentage resistance of *E. coli* to streptomycin was significantly higher than the other bacteria, and the percentage resistance of *Clostridium* to zinacef was significantly higher than the other bacteria.

Table 1: Pearson's correlation between levels of enteric bacteria in wastewater samples from the pharmaceutical industry and water samples from the boreholes

		Wastewater from pharmaceutical industry				
		E. coli	*Shigella*	*Clostridium*	Enterococci	*Salmonella*
Water from B1	*E. coli*	-0.22	-0.05	0.18	-0.15	-0.06
	Shigella	-0.01	-0.27	-0.29	**0.51**	-0.09
	Clostridium	-0.22	0.28	0.44	-0.40	0.21
	Enterococci	-0.23	-0.03	0.20	-0.16	-0.06
	Salmonella	-0.09	-0.03	0.09	-0.30	-0.16
Water from B2	*E. coli*	-0.19	0.38	**0.53**	**-0.58**	0.13
	Shigella	0.13	-0.40	-0.44	**0.70**	0.13
	Clostridium	0.46	**-0.54**	**-0.69**	**0.53**	0.00
	Enterococci	-0.09	0.42	0.47	**-0.58**	0.08
	Salmonella	0.22	-0.11	-0.13	-0.28	-0.13
Water from B3	*E. coli*	0.31	-0.34	-0.44	**0.64**	0.37
	Shigella	-0.12	0.11	0.16	0.22	0.25
	Clostridium	-0.30	0.27	0.45	-0.03	0.38
	Enterococci	0.25	-0.31	-0.39	**0.63**	0.39
	Salmonella	0.07	0.18	0.26	-0.45	0.07

Key: Borehole 1 = B1; Borehole 2 = B2; Borehole 3 = B3; values in bold indicate significant correlation at $P < 0.05$.

Table 2: Physicochemical characteristics of wastewater samples from the pharmaceutical industry and water samples from the boreholes in comparison with standards (FEPA, 1991; WHO, 2017)

Parameter	**Wastewater (Mean ± SD)**	**Wastewater from Pharmaceutical industry (FEPA, 1991)**	**Borehole water (Mean ± SD)**	**Drinking water guidelines (WHO, 2017)**
Temperature (°C)	26.5 ± 5.1	23 – 29	29.5 ± 1.5	
pH	7.2 ± 0.2	6 – 9	6.8 ± 0.6	6.5 – 8.5
Electrical conductivity (µS/cm)	1256.1 ± 184.1	927 – 1455	696.5 ± 278.9	
Turbidity (NTU)	34.0 ± 8.6	23 – 51	3.1 ± 2.7	< 0.2
Dissolved oxygen (mg/l)	6.9 ± 1.5	4 – 10	5.8 ± 1.7	
Salinity (mg/l)	0.3 ± 0.1	0.1 – 0.5	0.4 ± 0.1	
Alkalinity (mg/l)	255.0 ± 45.9	170 – 320		
Total dissolved solids (mg/l)	527.9 ± 142.6	324 – 854	441.3 ± 145.9	< 600
Biological oxygen demand (mg/l)	6.0 ± 1.2	4 – 8	3.7 ± 1.3	

Key: Values are expressed as Mean ± Standard Deviation; Wastewater (n = 16); Water from boreholes (n = 48)

The mean temperature of the wastewater from the pharmaceutical industry was 25.6°C and the mean pH was 7.2. The mean electrical conductivity of the wastewater was 1256.1 µS/cm, while the mean value of total dissolved solids was 527.9 mg/l. In addition, the mean biological oxygen demand in the wastewater was 6.0 mg/l, whereas the mean value of dissolved oxygen was 6.9 mg/l. Salinity of the wastewater was 0.3 mg/l and turbidity was 34.0 NTU. All the physicochemical characteristics of the wastewater were within the FEPA recommended standards stated in Table 2 for wastewaters from pharmaceutical industry (FEPA, 1991). Furthermore, the mean temperature of the water samples from the boreholes was 29.5°C and the mean pH was 6.8. The mean electrical conductivity of the water samples was 696.5 µS/cm, while the mean value of total dissolved solids was 441.3 mg/l. Also, the mean biological oxygen demand in the water samples was 3.7 mg/l, while the mean value of dissolved oxygen was 5.8 mg/l. Mean salinity of the water samples was 0.4 mg/l and turbidity was 3.1 NTU. The physicochemical characteristics of the water samples except turbidity were within the WHO recommended standards stated in Table 2 for drinking water (WHO, 2017).

Enteric bacterial isolates from water samples from the three boreholes showed varying susceptibility pattern to antibiotics as indicated by different zones of inhibition (mm). *Shigella* was susceptible to chloramphenicol (80%), ciprofloxacin (90%), perfloxacin (88%), streptomycin (82%) and zinacef (86%), while *Clostridium* was susceptible to perfloxacin (88%), streptomycin (89%), and *Salmonella* was susceptible to perfloxacin (90%) and zinacef (88%). Enterococci was susceptible to zinacef (89%), while *E. coli* was susceptible to erythromycin (73%). In addition, all the isolates showed resistance to multiple antibiotics, *E. coli* was resistant to three antibiotics; amoxillin (80%), ciprofloxacin (76%), streptomycin (84%), while *Shigella* was resistant to two antibiotics; amoxillin (78%), erythromycin (65%), and *Clostridium* was resistant to four antibiotics; amoxillin (75%), ciprofloxacin (78%), erythromycin (82%), zinacef (81%). Similarly, enterococci was resistant to five antibiotics; amoxillin (79%), ciprofloxacin (80%), erythromycin (80%), perfloxacin (75%), streptomycin (68%), while *Salmonella* was resistant to five antibiotics; amoxillin (80%), chloramphenicol (76%), ciprofloxacin (75%), erythromycin (68%), streptomycin (60%) (Figure 3).

DISCUSSION

This study investigated the influence of microbial quality of wastewater generated from a pharmaceutical industry on surrounding groundwater sources, determined the physiochemical properties of wastewater and water samples, and examined the antibiotic susceptibility pattern of enteric bacteria isolated from the surrounding groundwater sources. Improperly disposed industrial wastewaters may percolate to deeper layers of the soil and infiltrate underground aquifer. Pollutants from wastewater may have potential to exert selective pressure on microbial communities of receiving environments (Bassin *et al*., 2017). Its immediate impacts, including the degradation of aquatic ecosystems and waterborne illness from contaminated water supplies, may have far-reaching implications on human health. Widespread occurrence of enteric bacteria in water contaminated with wastewater of various quality constitutes a public health problem. Several instances of waterborne infections have been attributed to environmental pollution with wastewater. Gastrointestinal infections are amongst the most common infections caused by ingestion of enteric bacterial pathogens in contaminated water. These infections generally may include diarrhoea, dysentery, typhoid, human enteritis and so on (Liang *et al.,* 2006; Olalemi and Akinwumi, 2022). In this study, *E. coli* had the highest mean concentration in wastewater samples from the pharmaceutical industry, while *Shigella* had the least mean concentration in wastewater samples. In water samples from the boreholes, *E. coli* had the highest mean concentrations, while *Salmonella* had the lowest mean concentrations in water samples. The high levels of enteric bacteria detected in the wastewater samples from the pharmaceutical industry may have contributed to the levels observed in the water samples from the surrounding boreholes. This may be due to the percolation and infiltration of underground aquifer by the wastewater. The levels of *E. coli* in the wastewater and water samples suggest faecal contamination. The concentration of *Clostridium* in the wastewater and water samples may indicate intermittent faecal pollution. This may be as a result of the ability of the organism to form spores that can survive in environmental samples for several months (Wilson, 2005).

Faecal indicator bacteria such as *E. coli*, faecal coliforms and enterococci have been used as indicator organisms to detect faecal contamination and monitor the level of pollution of water and wastewater. The detection of these indicator organisms in drinking water is an indication that the water is not microbially safe for ingestion (Sinclair *et al*., 2012; Olalemi *et al*., 2021). Of all the targeted enteric bacteria in the wastewater samples from the pharmaceutical industry, enterococci had the most correlations with the levels of other enteric bacteria in water samples from the boreholes, followed by *Clostridium* and *Shigella*. Levels of *E. coli* and *Salmonella* in the wastewater samples had no correlations with the levels of other enteric bacteria in water samples from the boreholes. Enterococci are non-spore formers and have a temperature growth range from 10 to 45°C. Members of the genus have the ability to survive and grow at high pH of about 9.6 and in the presence of 6.5% sodium chloride (Devane *et al*., 2020). They are also resistant to bile acids, desiccation and appear to be more persistent than other enteric bacteria in water systems (Staley *et al*., 2014). These attributes may likely be responsible for the high correlations observed between the levels of enterococci in the wastewater samples from the pharmaceutical industry and other enteric bacteria in water samples from the boreholes. It is important to note that the most correlations between the levels of enterococci in the wastewater samples and other enteric bacteria were detected in water samples from borehole 2 (B2). The B1 was situated approximately 60 metres away from the wastewater sampling site, B2 was about 95 metres directly opposite the wastewater sampling site and B3 was approximately 130 metres from the wastewater sampling site. The location of B2 may likely be connected with this observation.

The temperature of the wastewater samples were within the temperature range of mesophilic organisms. Temperature is an important parameter because of its effect on chemical reaction rates, microbial growth and solubility of essential gases such as oxygen in water. The hydrogen-ion concentration is an important quality parameter of water and wastewater. To protect microorganisms and their viability, pH should remain between 6 and 9 (Gökse *et al*., 2015). The observed mean value of turbidity in the water from the boreholes was high and above the acceptable limit of less than 0.2 for drinking water (WHO, 2017). Meteorological condition such as rainfall and storm events may increase the turbidity of groundwater (Pticek Sirocic´ *et al*., 2023). The high electrical conductivity values in the wastewater and water samples may likely be as a result of dissolved chemicals and other pharmaceutically active compounds in the samples (Heloui *et al.*, 2015). Salinity has a significant influence on the species composition of aquatic systems. The mean values of salinity in the water samples were higher than those observed in the wastewater samples, but were considerably normal to support proliferation of microorganisms. Total dissolved solids in the wastewater and water samples may be as a result of the presence of dissolved ions, organic and inorganic solvents. The discharge of wastewater with high levels of BOD may deplete the concentration of dissolved oxygen aquatic systems (Aniyikaiye *et al*., 2019). All the physicochemical characteristics of the wastewater samples were within the FEPA recommended standards including temperature, pH, turbidity of wastewaters from pharmaceutical industry (FEPA, 1991).

The wastewater that are generated from the pharmaceutical industry are channelled into an open soak away system that has a non-cemented floor to allow for percolation of the wastewater into the soil. One of the most efficient natural filter is the soil that may protect groundwater from contamination, but the extent of contamination prevention is highly dependent on the nature and concentration of the pollutants (Bilal *et al*., 2019; Lu *et al*., 2019). Studies have shown that groundwater sources may be contaminated with traces of pharmaceutically active products (Mahmood *et al*., 2019). In this study, the detection of pharmaceutically active products in water samples from the boreholes were not carried out, but all the targeted enteric bacterial isolates showed resistance to multiple antibiotics and this may likely be connected to inducement by the presence of pharmaceutically active products contaminating the groundwater (Da Silveira *et al*., 2018; Bilal *et al*., 2019). In addition, the observed antibiotic resistance in enteric bacteria in water samples from the boreholes may have been acquired through horizontal gene transfer from bacteria that had developed antibiotic resistance in wastewater from the pharmaceutical industry infiltrating the underground aquifer. The quality of the wastewater from the production of pharmaceuticals varies as a result of the variety of basic raw materials, working processes and waste products. Maintaining minimum safe distance between water supply from groundwater sources and potential sources of pollution such as industrial wastewater is important in order to eliminate the risks of contamination with enteric pathogens. The use of household water treatment may be required to prevent occurrence of waterborne infections. In addition, the use of antibiotics must be based strictly on doctor's prescription to reduce the menace of antibiotic resistance.

CONCLUSION

The findings from this study revealed that water from the three groundwater sources had poor microbial quality and potential pathogenic antibiotic-resistant bacteria that may pose significant risks to public health. The microbial quality of the water samples were influenced by the wastewater from the pharmaceutical industry. The physicochemical characteristics of the wastewater samples were within the recommended standards for wastewaters from pharmaceutical industry, and those of the water samples except turbidity were within the recommended standards for drinking water. Adequate treatment of the water from the boreholes must be encouraged to prevent occurrence of waterborne infections caused by the bacterial isolates.

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REFERENCES

Adeogun, A. O., Ibor, O. R., Adeduntan, S. D. and Arukwe, A. (2016). Intersex and alterations in the reproductive development of cichlid, *Tilapia guineensis*, from a municipal domestic water supply lake (Eleyele) in south western Nigeria. *Science of the Total Environment*, 18(1): 28-30.

Aniyikaiye, T., Oluseyi, T., J. O. Odiyo, Joshua N. and Edokpayi, (2019). Physicochemical analysis of wastewater discharge from selected paint industries in Lagos, Nigeria. *International Journal of Environmental Research and Public Health*, 16(7): 23-35.

APHA (2012). Standard Methods for the Examination of Water and Wastewater, 22nd edn. APHA/AWWA/WEF, Washington, DC, USA.

Ashbolt, N. J. and Snozzi, M. (2001). Indicators of microbial water quality. Water quality-guidelines, standards and health. *Assessment of risk and risk management for water-related infectious disease*, 289-316.

Ayodele, O., Olalemi, A. and Ogundare, A. (2019). Phytochemical and antibacterial properties of

Andrographis paniculata (Burn.f.) on enteric bacteria isolated from a surface water in Owena, Nigeria. *Journal of Advances in Microbiology*, 19(3): 1-26.

Bassin, J. P., Rachid, C. T., Vilela, C., Cao, S. M., Peixoto, R. S. and Dezotti, M. (2017). Revealing the bacterial profile of an anoxic-aerobic moving-bed biofilm reactor system treating a chemical industry wastewater. *International Biodeterioration and Biodegradation*, 120: 152-160.

Beer K. D., Gargano J. W., Roberts V. A., Hill V. R., Garrison L. E., Kutty P. K., Hilborn E. D., Wade T. J., Fullerton K. E. and Yoder J. S. (2015). Surveillance for waterborne disease outbreaks associated with drinking water—United States 2011–2012. *Morbidy and Mortality Weekly Report*, 64: 842–848.

Bilal, M., Ashraf, S. S., Barceló, D., and Iqbal, H. M. (2019). Biocatalytic degradation/redefining "removal" fate of pharmaceutically active compounds and antibiotics in the aquatic environment. *Science of The Total Environment*, 691: 1190-1211.

Cabral J. P. S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, 7 (10): 3657-3703.

CLSI (2012). Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard-eight Edition. Clinical and Laboratory Standards Institutes, 27(2):1-35.

Da Silveira, A. T., Maranho, L. A., Torres, N. H., Ferreira, L. F. R., de Salles Pupo, M. M., Bharagava, R. N., and Tornisielo, V. L. (2018). Assessment of 14C-sulfadiazine on Danio rerio (zebrafish). *Journal of Radioanalytical and Nuclear Chemistry*, 318(2): 1001-1008.

Devane, M. L., Moriarty, E., Weaver, L., Cookson, A. and Gilpin, B. (2020). Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring. *Water Research*, 185: 116202.

Edward, R. J., Michelle, T. H., Van Vliet, M. Q. and Marc F. P. B. (2021). Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth System Science Data*, 13(2): 237-254.

FEPA (1991). Guidelines and standards for environmental pollution control in Nigeria. Federal Environmental Protection Agency (FEPA), Lagos, Nigeria.

Gökse, E. T., Abdullah, E. T., Serpil, E. and Serpil, E. (2015). Prediction of wastewater treatment plant performance using multi-linear regression and artificial neural networks. *International Symposium on Innovations in Intelligent Systems and Applications* (*INISTA*), 15(3): 34-45.

Heloui, M. E., Rachida, M. and Fatima, H. (2015). Impact of Treated Wastewater on Groundwater Quality in the Region of Tiznit (Morocco), *Journal of Water Reuse and Desalination*, 6(3): 61-68.

Liang, J. L., Dziuban, E. J., Craun , G. F., Hill, V., Moore, M. R., Gelting, R. J., Calderon, R. L., Beach, M. J. and Roy, S. L. (2006). Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking. *Center for Disease Control/Morbidity and Mortality Weekly Report*, 55(12): 31-58.

Lu, T., Zhu, Y., Ke, M., Peijnenburg, W., Zhang, M., Wang, T. and Qian, H. (2019). Evaluation of the taxonomic and functional variation of freshwater plankton communities induced by trace amounts of the antibiotic ciprofloxacin. *Environment International*, 126: 268-278.

Mahmood, A. R., Al-Haideri, H. H., and Hassan, F. M. (2019). Detection of antibiotics in drinking water treatment plants in Baghdad City, Iraq. *Advances in Public Health*, 7851354.

O'Neill, J. (2014). Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. *Review on Antimicrobial Resistance*, 1–16.

Ojo, O. I., Ogedengbe, K., and Ochieng, G. M. (2011). Efficacy of solar water disinfection for well waters: Case study of Ibadan slums, Nigeria. *International Journal of Physical Sciences*, 6(5): 1059-1067.

Olalemi, A. O., Akinruli, A. T. and Oluwasusi, V. O. (2019). Antibiotic resistance pattern of bacteria isolated from biofilms in water from groundwater sources in Ado-Ekiti, Nigeria. *South Asian Journal of Research in Microbiology*, 3(4): 1-9.

Olalemi, A., Atiba, R., Weston, S. and Howard, G. (2023). Sanitary inspection and microbial health risks associated with enteric bacteria in some groundwater sources in Ilara-Mokin and Ibule-Soro, Nigeria. *Journal of Water and Health*, 21(12): 1784-1794.

Olalemi, A. O., Ige, O. M., James, G. A., Obasoro, I. F., Okoko, F. O. & Ogunleye, C. O. (2021). Detection of enteric bacteria in two groundwater sources and associated microbial health risks. *Journal of Water and Health*, 19: 322– 335.

Olalemi, A. O. and Akinwumi, I. M. (2022). Microbial health risks associated with rotavirus and enteric bacteria in River Ala in Akure, Nigeria. *Journal of Applied Microbiology*, 132(5): 3995-4006.

Pticek Sirocic´, A., Ojdanic´, K., Dogancic´, D. and Plantak, L. (2023). Water quality for human consumption from the public water supply system. *Environmental Sciences Proceedings*, 25: 21-27.

Quero, G. M., Cassin, D. and Botter, M. (2015). Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Frontiers in Microbiology*, 6:115.

Sinclair, R. G., Rose, J. B., Hashsham, S. A., Gerba, C. P. and Haas, C. N. (2012). Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Applied and Environmental Microbiology*, 78: 1969–1977.

Staley, C., Dunny, G. M., Sadowsky, M. J. (2014). Environmental and animal-associated enterococci. *Advances in Applied Microbiology*, 87: 147-186.

WHO (2001). Global strategy for containment of antimicrobial resistance. Geneva, Switzerland. World Health Organization.

WHO (2017). Guidelines for drinking- water quality. Geneva, Switzerland: World Health Organization.

Wilson, M. (2005). Microbial inhabitants of humans their ecology and role in health and disease. *Cambridge University Press; Cambridge*, UK 20-25.

Zhang Y., Kelly W. R., Panno S. V. and Liu W. T. (2014). Tracing fecal pollution sources in karst groundwater by Bacteroidales genetic biomarkers, bacterial indicators, and environmental variables. *Science of the Total Environment*, 490: 1082–1090.