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Assessment of Bacteriological Quality and Determination of Antibiogram of Bacteria Isolated from Water Used in Selected Hospitals within Port Harcourt City and Obio/Akpor Local Government Area of Rivers State

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Abstract

Water is vital for life, and water void of bacterial contamination is vital for hospital consumption and use. This study aimed to determine the bacteriological quality and antibiogram of different water samples in some hospitals within Port Harcourt Metropolis. Water samples were collected in different hospitals in Port Harcourt and Obio/Akpor Local Government Area of Rivers State, Nigeria. Based on water dispensers, outdoor taps, washing hand faucets, and faucets in the toilet and theatre. The bacteriological quality of the water samples, coagulase, haemolysis, biofilm, starch, and antibiogram were determined using standard microbiological procedures. The mean range of the total heterotrophic bacterial, staphylococcal, faecal coliform, and total coliform counts of the water samples were $8.5\pm0.7\times10^5$ to $3.8\pm2.1\times10^7$, 1.2 ± 0.2 to $2.8\pm0.3\times10^5$, 0.0 ± 0.0 to $8.0\pm4.2\times10^3$ and 0.0 ± 0.0 to $1.1\pm0.1\times10^5$ CFU/mL, respectively. The prevalence of the isolated bacteria is Staphylococcus sp (17.5%), Bacillus sp (12.5%), Enterobacter sp. (12.5%), Klebsiella sp. (10%), Citrobacter sp. (5%), Escherichia coli (2.5%) and Siccibacter sp. (2.5%). Staphylococcus, Bacillus, Enterobacter, Klebsiella, Citrobacter, Escherichia coli, and Siccibacter sp. were positive for haemolysis and a-amylase production, 80% of Staphylococcus sp were coagulase positive while 46, 40, 57 and 25% of Staphylococcus, Bacillus, Enterobacter, and Klebsiella sp produced biofilm. The antibiogram showed multi-drug resistance (0.2-1.0). Levofloxacin was 60% effective against Staphylococcus sp, while susceptibility of Klebsiella and Citrobacter sp to ofloxacin, gentamycin, nalidixic acid, and levofloxacin 66.7%. The water samples from these hospitals might not be good for drinking. Thus, treatment of water before use is recommended. The high antibiotic resistance could imply the emergence of resistant isolates in hospital water.

Keywords: Hospital water sources, Bacteriological quality, Antibiogram

INTRODUCTION

Water is a substance that exists in gaseous, liquid, and solid phases and comprises the chemical elements hydrogen and oxygen (Iwamoto *et al.*, 2010). One of the most important substances on the planet is water. At ambient temperature, water is an odourless, tasteless liquid that can dissolve numerous other substances (Iwamoto *et al.*, 2010). Water is essential to the effectiveness of the healthcare sector especially as its use could range from washing surgical tools and equipment to creating a soothing environment for patients to have hydrotherapy (CDC, 2024). Water supply to healthcare facilities is frequently overlooked yet essential for safe patient care and can be a manageable source of infections. Numerous healthcare-associated outbreaks have been linked to contaminated water used for patient care, particularly maternal sinks, faucets, or shower heads associated with hand washing and cleaning of medical devices (WHO, 2017). Altin *et al.* (2017) documented that among the biggest water consumers in cities, hospitals are on the list and that the quality of water supplied to hospitals is very critical since they play important roles in the health of the patients as well as the hospital's daily operation. Because of the multiple exposure or use of water (Ferranti *et al.*, 2014; Suleyman *et al.*, 2018), water should be regarded as a significant source of infection. These scenarios include intricate hospital water systems and water-containing equipment and tools utilised in medical facilities (Yiek *et al.*, 2021). Waterborne pathogens are more likely to cause infection in healthcare facilities than in the general population due to patients' increased susceptibility to infections in hospitals, long-term care facilities, and rehabilitation centres (Bloomfield *et al.*, 2015).

The ability of bacteria to resist the effect of antibiotics designed to kill or inhibit their proliferation is termed antibiotic resistance (Prescot *et al.*, 2011). Even though antibiotic resistance is a natural process occurring due to genetic changes in the bacteria following exposure to antibiotics, it is being accelerated through the overuse and misuse of antibiotics (Amagliani *et al.*, 2012). Overuse of antibiotics causes susceptible bacteria to be killed and allows drug-resistant bacteria to proliferate (Barrett *et al.*, 2017). The resistance of microorganisms against antimicrobial drugs is a major problem of recent times, which is increasing day by day (Butt *et al.*, 2014). The rise in antibiotic-resistant bacteria in water sources has become significant to the health of humans. Public health action is required because borehole water, especially those found on hospital premises, contains microorganisms that produce biofilm and can form antibiotic resistance (Jordan and McAuliffe 2018). It has been noted that some human-related water diseases are recurrent, and the underlying cause is thought to be antibiotic-resistant bacteria (Jordan and McAuliffe 2018). More so, since moist environments and liquid solutions can create a favourable setting for the growth of many bacterial and some protozoal microbes, waterborne diseases can be spread (CDC, 2024). There hasn't been a robust literature review on studies on antimicrobial resistance bacteria (AMR) isolated from hospital water, the purpose of the study.

MATERIALS AND METHODS

Study Area

The study was conducted in four different hospitals namely; IC (4O50'33.19188" N and 6^{O}58'55.704^{O}E), ELH (4^{O}52'58".14264N and 7^{O}0'3.32172E) MH (4°47'45.45023N and 6°59'13.37E) and LRS (4°47'44.5862N and 6°59'13.23924E) in Port Harcourt City and Obio/Akpor Local Government of Rivers State, Nigeria.

Collection of Samples

The water samples were taken at different point sources: water dispensers, outdoor taps, washing hand faucets, and faucets in the toilet and theatre. The main source of the water supplied to the different point source was from the storage tank (borehole) in each hospital, while the dispenser was bought as processed water for consumption. Before collection (fetching) of the water, the mouth of the tap was sterilized with cotton wool moistened with 70% ethanol and allowed to flow for 5 minutes. Ten millilitres (10mL) of each water sample was collected in sterile biological specimen bottles. The bottles were tightly covered and transported to the Microbiology Laboratory, Department of Microbiology, Rivers State University, in a cooler containing ice packs. A total of 32 water samples were collected.

Sample Preparation

The water samples were diluted by adopting the ten-fold serial dilution method (Prescott *et al.*, 2011) such that one millilitre (1ml) of the water sample was transferred aseptically into test tubes containing sterile 9ml normal saline with the aid of a sterile 1mL pipette to give an initial dilution of 10^{-1}. Subsequent dilutions were carried out consecutively by transferring 1 ml from the initial dilution to another test tube containing 9 ml sterile diluents until a dilution of 10^{-6} was obtained.

Determination of Bacterial Counts

The bacterial counts of the water sample were determined using the standard plate count method (Wilcox *et al.*, 2023) on nutrient agar. Aliquot (0.1mL) of 10^{-4} and 10^{-3} dilutions were inoculated on the surface of freshly prepared nutrient and mannitol salt agar in duplicates for enumeration and isolation of total heterotrophic bacterial and staphylococcal counts, respectively. While aliquots from 10^{-2} were inoculated onto the surface of Eosin methylene blue agar (EMB) for enumeration and isolation of faecal and total coliform. The media were evenly spread with the aid of a sterile bent glass rod and incubated at 37°C for 24 hours for the total heterotrophic bacteria, staphylococcal and coliform, while the media for faecal coliform were incubated at 44.5°C for 48 hours.

Isolation and Characterization of Bacterial Isolates

Immediately after incubation, distinct colonies from the various media for each water sample were picked with the aid of a sterile wire loop and subcultured onto the surface of newly prepared pre-dried nutrient agar media. The media were incubated at 37°C for 24 hours. On observation of pure cultures after incubation, cultures were preserved and served as a source for further tests while cultures with contaminants were subcultured again until pure culture was obtained. The isolates were identified based on Gram's reaction, motility, and biochemical tests (Catalase, Citrate, Oxidase, Methyl Red, Voges Proskauer, Indole, and Sugar Fermentations). The tests were carried out according to Cheesbrough (2006).

Phenotypic Testing for Virulence in Isolates

The virulence characteristics such as Coagulase, Biofilm Production, production of amylase and Haemolysis tests were carried out as described in a previous study. The methods are described below;

Haemolysis Test

To perform this test, pure culture of the isolate was inoculated on a blood agar media using a sterile wire loop and was incubated at 37°C for 24 hours. At the end of the incubation period, the media were observed for haemolysis (zone of inhibition/ clearing around the culture) and were interpreted as alpha partial haemolysis (greenish-grey discoloration around the colony), Beta haemolysis (clear zone of inhibition around the culture) and Gamma haemolysis (no lysis of red blood cells) (Prescott *et al.*, 2011).

Starch Hydrolysis Test

This test was carried out to determine the ability of the isolate to produce amylase and utilize starch as a carbon source. The pure culture of the isolate was inoculated on the prepared starch agar and incubated at 37°C for 24 hours. At the end of the incubation period, the starch agar media was flooded with iodine and observed for the formation of a halo on the colonies. The development of dark blue to purple-blue is indicative of a positive result, and no halo around the colonies is an indication of a negative result (Prescot *et al.*, 2011)

Biofilm Production Test

This test is carried out for the detection of biofilm-producing bacteria. To perform this test, the pure culture of the isolates was inoculated into a biofilm media (Congo red agar) using a sterile wire loop and was incubated at 37°C for 24 hours. A black colour formation on the agar media indicates a positive result, while no black colour formation indicates a negative result (Prescott et al., 2011).

Coagulase Test

A drop of water was placed on both ends of a clean, grease-free slide. The isolate colonies were smeared gently on both ends after which a drop of serum was transferred on one end of the prepared smear while the other part without serum served as control. Clumping reactions within 10 seconds indicated a positive result, while no clumping indicated a negative result (Prescott et al., 2011).

Antibiotic Susceptibility Test (Kirby-Bauer Disk Diffusion Method)

The antibiotics (Celtech Diagnostic) used were Augmentin (30µg), Cefotaxime (25µg), Imipenem (30µg), Ofloxacin (5µg), Gentamycin (10µg), Nalidixic Acid (30µg), Nitrofurantoin (300µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ampliclox (10µg), Cefixime (5µg), Levofloxacin (5µg), Azithromycin (10µg), Erythromycin(5µg) and Ciprofloxacin (5µg). The bacterial isolate was grown in nutrient broth for 18-24 hours, after which the turbidity was reduced by adding sterile normal saline until it matched the turbidity of the 0.5 McFarland Standard of the Clinical Laboratory Standard Institute (CLSI, 2022). A sterile swab stick was inserted into the standardized broth culture using an aseptic technique. The swab was pressed by the side of the tube just above the broth in the test tube to remove excess liquid. After which, the surface of the Mueller–Hinton agar media was gently swabbed horizontally and vertically to form a bacterial lawn. Disks containing specific antibiotics were applied to the media using an antibiotic dispenser. Flame-sterilized forceps were used to gently press each disk onto the agar and ensure it was attached to the surface of the medium. Medias were then incubated for 24 hours at 37°C. The diameter of each zone of inhibition was measured in mm from the edge of the dish to the end of the clear zone, and results were interpreted as Resistant, Susceptible, or intermediate (European Committee on Antimicrobial Susceptibility Testing, EUCAST, 2021).

Statistical Analysis

The results were expressed as mean + standard deviation. A two-way Analysis of variance (ANOVA) was carried out to check for significant differences, and mean values were separated using the Duncan multiple range test (DMRT) at $P<0.05$. The percentage occurrence of antibiotic susceptibility was determined. All analyses were carried out using SPSS (version 27.0)

RESULTS

The bacterial counts of the water sample in IC showed that the total heterotrophic bacterial counts, staphylococcal, faecal coliform, and total coliform counts of the water samples ranged from 0.085±0.7 - 2.4±0.1x10^7, 1.7±1.5 - 2.8±0.3x10^5, 0.0±0.0 - 2.0±0.1x10^5, 1.3±0.1 - 6.7±0.9x10^6 CFU/mL, respectively (Table 1). In the result, the THB of the water sample from the faucet in the toilet was higher than the THB of the water dispenser, outdoor tap, and washing hand water (faucet) in the reception. More so, the total staphylococcal counts of the water dispenser, outdoor tap, and toilet water were similar and higher than those observed for the water used in handwashing in the reception. In contrast, the faecal coliform counts of the tap outdoors were higher than the faecal coliform counts from the other water sources.

The bacterial counts of the water sample in LRS presented in Table 2 showed that the total heterotrophic bacterial counts, staphylococcal, faecal coliform, and Total coliform count of the water samples ranged from 8.5±0.3 x10^5 - 1.7±0.2x10^7, 1.4±0.2 - 2.7±0.2x10^5, 0.0±0.0 - 1.1±0.2x10^5, 1.4±0.2 - 4.5±0.6x10^4 CFU/mL, respectively.

The bacterial counts of the water sample in MH presented in Table 3 showed that the total heterotrophic bacterial counts, staphylococcal, faecal coliform, and total coliform counts of the water sample ranged from 0.9±0.6 - 3.7±3.3x10^6, 1.4±0.1 - 2.2±2.1x10^5, 0.0±0.0 - 4.0±1.4x10^3, 1.4±0.2 - 9.0±1.2x10^4 CFU/mL, respectively. The THB of the water sample from the toilet was significantly higher (P<0.05) than THB counts from tap outdoors and washing hand water in the convenience.

The results of the bacterial counts of the water sample in ELH presented in Table 4 showed that the range of the total heterotrophic bacterial counts, staphylococcal, faecal coliform, and total coliform counts was 1.1±0.2 - 3.8±2.1x10^7, 1.2±0.2 - 2.8±0.2x10^5, 0.0±0.0 - 8.0±4.2x10^3 and 0.0±0.0 - 11.5±0.1x10^4CFU/mL, respectively. The THB of the water collected from the convenience (toilet water) was higher than the THB of the washing hand water at the reception, the tap outdoors, and the water flowing in the theatre. The total staphylococcal counts of the water flowing in the theatre and washing hand water in the reception were higher than the counts observed for water samples from the outdoor tap and toilet (convenience), while the faecal coliform counts of the water from the outdoor tap was higher than counts observed in a water sample from the faucet in the toilet.

Table 1: Bacterial Counts (Cfu/ml) of Water Sample in IC

Samples	THB (x10^7)	TSC (x10^5)	FC (x10^5)	TCC(x10^6)
WD	1.5±0.2^a	2.8±0.2^a	1.9±0.5^b	1.4±0.2^a
OT	1.3±0.2^a	2.8±0.3^a	2.0±0.1^b	6.7±0.9^a
WHW	0.085±0.7^a	1.7±1.5^a	0.0±0.0^a	1.3±0.1^a
TW	2.4±0.1^a	2.8±0.5^a	0.03±0.02^a	1.5±1.3^a

Keys: WD- Water Dispenser, OT-Outdoors tap, WHW- Washing hand water, TW- Toilet Water
*Means with similar superscripts shared no significant difference (P>0.05)

Table 2: Bacterial Counts (Cfu/ml) of Water Sample in LRS

Samples	THB (x10^7)	TSC (x10^5)	FC (x10^5)	TCC(x10^4)
OT	0.085±0.3^a	1.5±0.2^a	0.0±0.0^a	4.5±0.6^a
TW	1.7±0.2^a	1.5±0.2^a	0.0±0.0^a	4.5±0.7^a
WD	1.3±0.2^a	1.4±0.2^a	0.0±0.0^a	1.4±0.2^a
WHW	0.15±0.2^a	2.7±0.2^a	1.1±0.2^b	2.0±0.3^a

Keys: OT- outdoors tap, TW- Toilet water, WD- Water Dispenser, WHW- Washing hand water
*Means with similar superscripts shared no significant difference (P>0.05)

Table 3: Bacterial Counts (Cfu/ml) of Water Sample in MH

Samples	THB (x10^6) (Cfu/ml)	TSC (x10^5) (Cfu/ml)	FC (x10^3) (Cfu/ml)	TCC(x10^4) (Cfu/ml)
OT	1.3±0.9^a	1.5±0.2^a	3.5±2.1^a	4.5±0.6^a
TW	3.7±3.3^b	1.4±0.1^a	4.0±1.4^a	2.5±0.4^a
WD	2.0±1.3^{ab}	2.2±2.1^a	0.0±0.0^a	9.0±1.2^a
WHW	0.9±0.6^a	1.3±0.2^a	0.0±0.0^a	1.4±0.2^a

Keys: OT- outdoors tap, TW- Toilet Water, WD- Water Dispenser, WHW- Washing hand water
*Means with similar superscripts shared no significant difference (P>0.05)

Table 4: Bacterial Counts (Cfu/ml) of Water Sample in ELH

Samples	THB (x10^7) (Cfu/ml)	TSC (x10^5) (Cfu/ml)	FC (x10^3) (Cfu/ml)	TCC(x10^4) (Cfu/ml)
OT	1.4±0.0^a	1.2±0.2^a	8.0±4.2^a	3.5±0.5^a
THW	1.9±1.2^a	2.8±0.2^a	0.0±0.0^a	0.0±0.0^a
WHW	1.1±0.2^a	2.8±0.06^a	0.0±0.0^a	4.0±0.6^a
TW	3.8±2.1^b	2.0±0.7^a	6.0±2.8^a	11.5±0.1^a

*Means with similar superscripts shared no significant difference (P>0.05)
Keys: OT- Outdoors tap, THW- Theatre Water, WHW- Washing Hand Water, TW- Toilet Water.

Forty bacteria such as *Staphylococcus* sp, *Enterobacter* sp, *Escherichia coli*, *Bacillus* sp, *Siccibacter* sp, *Klebsiella* sp, *Sacchrobacter* sp, and *Citrobacter* sp were isolated from the water samples. The morphology and biochemical characteristics of representative isolates are presented in Table 5.

The percentage occurrence of *Staphylococcus* sp, *Enterobacter* sp, *Escherichia coli*, *Bacillus* sp, *Siccibacter* sp, *Klebsiella* sp, *Sacchrobacter* sp and *Citrobacter* sp was 37.5, 17.5, 12.5, 12.5, 10, 5, 2.5 and 2.5 %, respectively (Figure 1). *Staphylococcus* sp had the highest prevalence while *Citrobacter* sp and *Sacchrobacter* sp had the least prevalence.

The distribution of the bacterial isolates in Table 6 showed that the isolates were not uniformly distributed across the various water samples. *Staphylococcus* sp and *Enterobacter* were isolated from the theatre water, while most of the outdoor tap of the respective hospitals was characterised by the presence of *Citrobacter*, *Klebsiella*, *Sacchrobacter*, *Staphylococcus* sp, and *Bacillus* sp. *E. coli* were only isolated from the outdoors of water samples from MH.

The virulence attributes of the bacterial isolates are presented in Table 7. Eighty (80) percent of the *Staphylococcus* sp were coagulase positive. For amylase production, 80 and 60% of *Staphylococcus* sp. and *Bacillus* sp were positive, respectively, while 46, 40, 57 and 25 % of *Staphylococcus* sp, *Bacillus* sp *Enterobacter* sp, and *Klebsiella* sp were positive for biofilm formation.

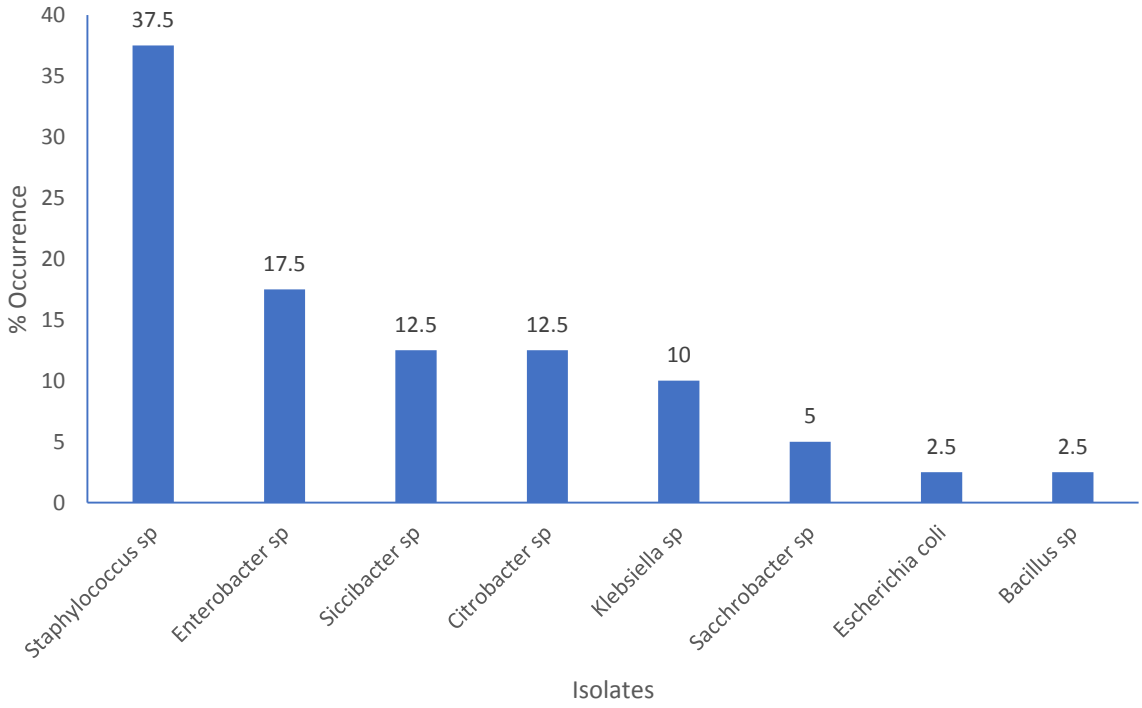

Fig. 1: Prevalence of the Isolate from the water samples

The antibiotic susceptibility pattern of the Gram-Negative Isolate presented in Table 8 showed that *Enterobacter* sp, *E. coli*, and *Citrobacter* sp were 100% resistant to Amoxicillin/Clavulanate, Cefotaxime, Imipenem, Ofloxacin, Gentamycin, Nalidixic Acid, Nitrofurantoin, Cefuroxime, Ceftriaxone, Ampliclox, Cefixime, and Levofloxacin while *Klebsiella* sp despite being completely (100%) resistance to Amoxicillin/Clavulanate, Cefotaxime, Imipenem, Nitrofurantoin, Cefuroxime, Ceftriaxone, Ampliclox, and Cefixime displayed 66.7% susceptibility to Ofloxacin, Gentamycin, Nalidixic Acid and Levofloxacin. *Sacchrobacter* sp, completely resisted all the antibiotics except ofloxacin and gentamycin with a percentage susceptibility of 100% recorded for both antibiotics. Nalidixic acid (NA), was 100% less susceptible and GN and OFX was 100% susceptible. The percentage susceptibility of *Siccibacter* sp to ofloxacin and levofloxacin was 50%, respectively, while other antibiotics had no effect on the isolate.

The antibiotic susceptibility pattern of *Staphylococcus* sp. (Table 9) showed that the isolates of *Staphylococcus* sp displayed 6.7, 60, 40, 13.3, 40, 26.7, 33.3 and 46.7% susceptibility to Amoxicillin/ Clavulanate, levofloxacin, ciprofloxacin, cefuroxime, ofloxacin, erythromycin, gentamycin and azithromycin, respectively. Thus, levofloxacin was the most potent antibiotic against the staphylococcal isolates, while the least potent antibiotic was Amoxicillin/ Clavulanate.

The multiple antibiotic-resistant (MAR) index of the isolates showed that they were within the range of 0.2 to 1.0 (Table 10).

Table 5: Characterization of Bacteria Isolate from the Sample

Isolate Code	Macroscopy	Microscopy	Motility	Catalase	Oxidase	Methyl red	Voges Proskauer	Indole	Citrate	Glucose	Lactose	Probable Identity
EH OT	Pink punctiform and smooth	+ve cocci in clusters	-	+	+	-	+	-	+	AG	AG	*Staphylococcus* sp
EH OT	Pink, round & large	-ve rods	-	+	+	+	-	-	+	AG	A	*Enterobacter* sp
EH OT	Milky, moderate & mucoid	-ve rods	+	+	+	+	-	-	+	AG	A	*Siccibacter* sp
EH OT	Pink, small & round	+ve cocci in clusters	-	+	+	+	-	-	+	AG	AG	*Staphylococcus* sp
EH THW	Milky, small & round	+ve cocci in clusters	-	+	+	-	+	-	+	AG	A	*Staphylococcus* sp
EH THW	Purple, moderate & round	-ve rods	+	+	+	-	-	-	+	A	A	*Enterobacter* sp
EH THW	Milky, moderate & punctiform	+ve cocci in clusters	-	+	+	-	+	-	+	AG	A	*Staphylococcus* sp
IC OT	White, large & round	-ve rods	+	+	+	+	-	-	+	AG	A	*Cirobacter* sp
IC OT	Pink, large & umbonate	-ve rods	-	+	-	+	+	-	+	AG	A	*Klebsiella* sp
IC OT	Purple, large & umbonate	-ve rods	-	+	-	+	+	-	+	AG	AG	*Sacchrobacter* sp
IC WD	Pink, small & punctiform	+ve cocci in clusters	-	+	+	-	+	-	+	A	AG	*Staphylococcus* sp
MH OT	Pink, small & punctiform	+ve cocci in chains	+	-	-	-	+	-	-	AG	A	*Staphylococcus* sp
MH WD	milky, small & punctiform	+ve cocci in clusters	-	-	-	-	-	-	-	A	AG	*Staphylococcus* sp
MH WHW	milky, small & punctiform	+ve cocci in chains	+	+	+	+	-	-	+	AG	A	*Staphylococcus* sp
MH TW	Pink, moderate & round	-ve rods	+	+	+	-	-	-	+	AG	A	*Enterobacter* sp
MH TW	Milky, small & irregular	-ve rods	+	+	+	+	-	-	+	AG	AG	*Siccibacter* sp
LH OT	Purple, punctiform & round	-ve rods	-	+	+	+	-	-	+	AG	AG	*Enterobacter* sp
LH WHW	Metallic sheen green, small and round	-ve rods	+	+	--	+	-	-	-	AG	AG	*Escherichia coli*
LH TW	Pink, small and punctiform	+ve cocci in clusters	-	-	+	-	+	-	+	AG	AG	*Staphylococcus* sp
LH WHW	Yellow, small and smooth	+ve cocci in clusters	-	+	+	-	+	+	+	AG	A	*Staphylococcus* sp
IC TW	Pink, small and punctiform	+ve cocci in clusters	+	-	-	-	+	-	-	AG	AG	*Staphylococcus* sp
LH WD	Yellow, small and smooth	+ve cocci in clusters	+	+	+	+	-	-		AG	AG	*Staphylococcus* sp
MH WD	Milky, smooth and punctiform	+ve cocci in chains & clusters	-	-	-	+	-	-	+	AG	A	*Staphylococcus* sp
LH WHW	Yellow, small & puntiform	+ve cocci	-	+	-	+	+	-	+	AG	AG	*Staphylococcus* sp
IC WHW	Milky, small and punctiform	+ve cocci in clusters	-	-	+	-	-	-	-	AG	AG	*Staphylococcus* sp
IC OT	White, large and dry	+ve rod	+	+	-	-	+	-	+	AG	A	*Bacillus* sp

Table 6: Distribution of the bacterial Isolates Across the Study Location

Bacteria Species	A OT	A WD	A WHW	A TW	D OT	D WHW	D THW	D TW	C WD	C OT	C WHW	C TW	B OT	B WD	B WHW	B TW
Staphylococcus sp	-	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+
Enterobacter sp	-	-	-	-	+	-	+	-	-	-	+	+	+	-	+	+
Siccibacter sp	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
Citrobacter sp	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella sp	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Sacchrobacter sp	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	+
Bacillus sp	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-

Keys: OT- Outdoors tap, THW- Theatre Water, WHW- Washing Hand Water, TW- Toilet Water, WD – water dispenser, A- IC, B- LRS, C- MH, D- ELH

Table 7: Virulence attributes of the Isolates from the water samples

Bacteria Species	β Haemolysis	α Haemolysis	δ Haemolysis	Coagulase	Starch Hydrolysis	Biofilm
Staphylococcus sp	12(80)	3(20)	0	12(80)	12(80)	7(46)
Bacillus sp	2(40)	2(40)	1(10)	0	3(60)	2(40)
Enterobacter sp	2(29)	4(57)	1(14)	0	3(42)	4(57)
Klebsiella sp	2(50)	1(25)	1(25)	0	1(25)	1(25)
Citrobacter sp	1(100)	0	0	0	1(100)	0
Sacchrobacter sp	0	1(100)	0	0	0	0
Escherichia coli	4(80)	1(20)	0	0	1(20)	0
Siccibacter sp	1(100)	0	0	0	1(100)	0

Table 8: Antibiotic Susceptibility Pattern of Gram-Negative Isolates from the Water Samples

Antibiotics	*Enterobacter Sp.* (n=4)			*Klebsiella Sp.* (n=3)			*Escherichia coli* (n=5)			*Sacchrobacter sp.* (n=1)			*Citrobacter Sp.* (n=1)			*Siccibacter sp.* (n=2)		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	S	I	R	S	I	R
AUG	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
CTX	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
IMP	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
OFX	0	0	4(100.0)	2(66.7)	0	1(33.3)	0	0	5(100.0)	1(100.0)	0	0	0	0	1(100.0)	2(50.0)	0	2(50.0)
GN	0	0	4(100.0)	2(66.7)	0	1(33.3)	0	0	5(100.0)	1(100.0)	0	0	0	0	1(100.0)	0	0	2(100.0)
NA	0	0	4(100.0)	2(66.7)	0	1(33.3)	0	0	5(100.0)	0	1(100.0)	0	0	0	1(100.0)	0	0	2(100.0)
NIF	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
CXM	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
CRO	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
AEX	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
ZEM	0	0	4(100.0)	0	0	3(100.0)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	0	0	2(100.0)
LBC	0	0	4(100.0)	2(66.7)		1(33.3)	0	0	5(100.0)	0	0	1(100.0)	0	0	1(100.0)	2(50.0)		2(50.0)

Keys: AUG - Amoxicillin/ Clavulanate, CTX - Cefotaxime, IMP - Imipenem, OFX- Ofloxacin, GN-Gentamycin, NA-Nalidixic Acid, NF-Nitrofurantoin, CXM-Cefuroxime, CRO - Ceftriaxone, ACX - Ampliclox, ZEM - Cefixime, LBC - Levofloxacin.

Table 9: Antibiotic Susceptibility Pattern of *Staphylococcus* sp from the water Samples

Antibiotics	*Staphylococcus* sp. (n=15)		
	S	I	R
AUG	1(6.7)	0	14(93.3)
CTX	0	0	15(100.0)
CRO	0	1(6.7)	14(93.3)
ZEM	0	1(6.7)	14(93.3)
LBC	9(60.0)	4(26.7)	2(13.3)
CIP	6(40.0)	0	9(60.0)
IMP	0	0	15(100.0)
CXM	2(13.3)	0	13(86.7)
OFX	6(40.0)	0	9(60.0)
ERY	4(26.7)	0	11(73.3)
GN	5(33.3)	1(6.7)	9(60.0)
AZN	7(46.7)	1(6.7)	7(46.7)

Keys: AUG - Amoxicillin/ Clavulanate, CTX - Cefotaxime, IMP- Imipenem, OFX- Ofloxacin, GN- Gentamycin, AZN - Azithromycin, ERY - Erythromycin, CXM- Cefuroxime, CRO - Ceftriaxone, ZEM - Cefixime, LBC - Levofloxacin, CIP- Ciprofloxacin.

Table 10: Multiple Antibiotic Resistance (MAR) Index of the Isolates

MAR INDEX	*Staphylococcus* sp. (n=15)	*Enterobacter* sp. (n=7)	*Escherichia coli* (n=5)	*Citrobacter sp.* (n=1)	*Klebsiella Sp.* (n=4)	*Siccibacter sp.* (n=2)	*Sacchrobacter sp.* (n=1)
0.1	0	0	0	0	0	0	0
0.2	1(6.8)	0	0	0	0	0	0
0.3	0	0	5(100)	0	0	0	0
0.4	0	0	0	0	0	0	0
0.5	2(13.3)	0	0	0	0	0	0
0.6	3(20)	0	0	0	0	0	0
0.7	2(13.3)	0	0	0	0	0	0
0.8	2(13.3)	0	0	0	0	1(50)	0
0.9	3(20)	0	0	0	0	0	0
1.0	2(13.3)	7(100)	0	1(100)	4(100)	1(50)	1(100)

DISCUSSION

In the present study, the bacteriological quality of the various water samples could be considered poor, especially since they were high and exceeded the WHO permissible limits for drinking water. This agreed with the findings of Jordan and McAuliffe (2018), who reported a high microbial count in their study. The standard for drinking water, as highlighted by the World Health Organisation, states that the total heterotrophic bacteria, faecal coliform, and coliform should not exceed 1.0×10^2, 0/100mL, and 0-10 CFU/mL, respectively (WHO, 2017). The high bacterial counts recorded in the various toilets could be attributed to the inadequate cleaning and sanitization of the toilet environment or contamination with the channel through which the water was conveyed. If the toilet and bathroom facilities are not cleaned and sanitized regularly and effectively, it can accumulate microbial contaminants including coliform bacteria (Hsia *et al.* 2019). More so, the high total coliform counts may also be linked to cross-contamination since poor hygiene practices by hospital staff or patients could lead to cross-contamination (Woolhouse *et al.*, 2020). Patients may inadvertently transfer bacteria from contaminated surfaces or hands to the toilets and surrounding areas. This agreed with Singh *et al.* (2016) who reported similar observations in their study. The absence of faecal and total coliform in the water samples from the theatre and washing hand water from ELH could be attributed to the high effectiveness of the hospital water treatment and purification system (Landers *et al.*, 2012). Proper disinfection, filtration, and chlorination processes can eliminate faecal coliform bacteria in water supply systems. More so, hospital staff likely adhere to strict hygiene and infection control practices which could prevent contamination of the water sources with faecal matters (Mulani *et al.*, 2019). The high total heterotrophic bacteria count observed in the Meridian Water Dispenser could be attributed to infrequent cleaning and maintenance of the water dispenser (Kumar *et al.*, 2013). Inadequate cleaning of the water dispenser can result in the building of bacterial biofilms and contamination (Koonse *et al.*, 2015).

Regular cleaning and disinfectants reduce bacterial growth. The present study agreed with Kapoor *et al.* (2017), who reported high microbial counts (19.4±10.7 to 17.4±9.7 and 18.4±12.8 to 18.4±11.8) in drinking water supply.

Most of the bacterial isolates such as *Staphylococcus* sp, *Enterobacter* sp, *Escherichia coli*, *Bacillus* sp, *Klebsiella* sp, and *Citrobacter* sp in the present study have been reported as water contaminants in previous studies (Pant *et al.*, 2016; Onuorah *et al.*, 2019). The uneven distribution of the bacterial isolates across the samples could be attributed to contamination from external sources (like the environment) or the carrier system (pipes) through which the water flowed. For instance, *E. coli* was mostly isolated from the water samples from the toilet and rarely from other water samples. The high prevalence of *Staphylococcus* sp and *Enterobacter* sp could be attributed to contamination of the water and the inadequate treatment of the water sources (Jordan and McAuliffe 2018). Insufficient water treatment and disinfection processes could fail to eliminate or control *Staphylococcus* sp in the hospital water supply (Deepanjali *et al.*, 2015). While most bacterial isolates in the present study have been implicated in different diseases, including gastrointestinal diseases, *E. coli* is regarded as an indicator microorganism (Prescot *et al.*, 2011). Thus, its presence signified the availability of pathogenic bacteria in the water. More so, possessing virulent attributes such as coagulase, amylase (starch hydrolysis), and haemolytic ad biofilm could be important since these attributes aid bacteria in causing diseases. This agreed with Vandenesch *et al.* (2012), who reported that the potential of bacteria to cause disease is linked to a vast range of virulence factors that allow colonisation and persistence spread within the host and immune system evasion.

The present study revealed that most bacterial isolates were highly resistant to the tested antibiotics. Thus, the susceptibility of the isolates to the antibiotics was very low except for levofloxacin, which displayed higher potency against staphylococcal isolates, and nalidixic acid, ofloxacin, and levofloxacin which were highly effective against *Klebsiella* sp and *Citrobacter* sp. The resistance of the bacterial isolates in the present study could be attributed to the overuse and misuse of antibiotics (Albuquerque *et al.*, 2017). Widespread and inappropriate use of antibiotics can exert selective pressure on bacteria, favouring the survival and proliferation of antibiotic-resistant strains (Albuquerque *et al.*, 2017). The susceptibility of some bacterial isolates to the tested antibiotics could be attributed to the genetic characteristics of the bacteria (David *et al.*, 2017), especially since the genetic makeup of bacteria plays a crucial role in antibiotic susceptibility.

Abdollahzadeh *et al.* (2016) reported that Staphylococcus sp were resistant to Amoxacillin/Clavulanate, which agreed with the present study. The present study contradicts the study of Abdollahzadeh *et al.* (2016), who reported that the *E. coli* in their study was susceptible to gentamycin. Antibiotic resistance is a major public health threat, and resistant organisms in water are an emerging concern worldwide. However, the high incidence of antibiotic-resistant bacteria in this study region appeared analogous to what was predicted in a previous study (Gopal *et al.*, 2015). Indiscriminate use of antibiotics, lack of proper knowledge, and negligence toward disease increase the occurrence of antibiotic-resistant bacteria isolates in the hospital water. More so, the high level of multi-drug resistance exhibited by these isolates is high and a course for concern.

CONCLUSION

The study on the bacteriological quality and antibiogram of bacteria isolated from water samples in some hospitals in River State showed that the presence of high total heterotrophic bacterial counts in the range of 8.5×10^5 to 2.4×10^7 and faecal coliform counts in the range of 3.5×10^3 to 2.0×10^5 of tap outdoors, theatre water, washing hand water and water dispenser of the hospitals implied they could be contaminated by bacteria from faecal matter or environment. Detection of virulent strains of *Staphylococcus*, *Bacillus*, *E. coli*, *Enterobacter*, and *Klebsiella* sp in the water could be of serious concern, especially to consumers. This underscores the importance of water treatment before washing, cleaning equipment, and drinking. Furthermore, *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Staphylococcus* sp exhibited high levels of antibiotic resistance.

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