**Antibiotic Resistance Profile Of Haemolytic and Biofilm-Forming Bacteria Isolated from Domestic Water Sources Around Choba, River State, Nigeria: A Public Health Concern.**

**Abstract**

This study investigated the antibiogram profiles, biofilm formation capabilities, and hemolytic activities of bacteria isolated from domestic water sources in off-campus student hostels around the University of Port Harcourt, Nigeria. Water samples were collected from six hostels in Choba, Aluu, Alakahia, and Rumuosi and analyzed for total heterotrophic bacteria count (THBC), as well as specific counts for Staphylococcus, Pseudomonas, Vibrio, and Salmonella species. Morphological and biochemical characterization revealed the predominance of eight bacterial genera: Salmonella, Pseudomonas, Proteus, Staphylococcus, Corynebacterium, Bacillus, Yersinia, Vibrio, and Micrococcus. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Among gram-positive isolates, LAN4 exhibited the highest resistance, being resistant to eight antibiotics, while CAM7 and CHI1 were susceptible to all tested antibiotics. TRO7 showed resistance to seven antibiotics for gram-negative isolates, whereas CHI2 was susceptible to all antibiotics tested. Biofilm formation assays identified six isolates capable of producing biofilms. Hemolysis testing revealed diverse patterns, with eleven isolates each demonstrating alpha and beta hemolysis, while fourteen exhibited gamma hemolysis. The THBC varied significantly among hostels, with FESTAC and CAM hostels showing the highest counts (1.77x10^4 and 1.86x10^4 CFU/ml, respectively). The study highlights the presence of potentially pathogenic bacteria with varying antibiotic resistance profiles in off-campus water sources. The coexistence of biofilm-forming abilities and hemolytic activities in some isolates raises concerns about their virulence potential. These findings underscore the importance of regularly monitoring and treating domestic water sources to ensure water safety and minimize public health risks associated with antibiotic-resistant and virulent bacteria. Furthermore, the study emphasizes the need for targeted interventions to address biofilm formation and prevent the spread of resistant bacterial strains in domestic water supply systems.

**Keywords:** Biofilm formation, Antibiogram, Haemolytic bacteria, Antibiotic resistance, Domestic water sources, Total heterotrophic bacteria count (THBC), Waterborne pathogens, Water safety, Public health

**Introduction**

Water is an essential resource for human survival and plays a crucial role in maintaining public health. However, the quality of water, particularly in domestic settings, remains a significant concern worldwide. In developing countries, where water treatment infrastructure may be inadequate or inconsistent, the risk of waterborne diseases is particularly high (WHO, 2019). This issue is further compounded in off-campus student housing, where water sources may not be subject to the same rigorous monitoring and treatment processes as those on university campuses (Oluseyi et al., 2020). Recent studies have underscored the need for vigilant monitoring of water quality, particularly in areas where domestic water sources are subject to potential contamination by pathogenic microorganisms (World Health Organization, 2017).

Accessibility to potable water supply has been a top priority of most developing societies in Sub-Saharan Africa like Nigeria. In Nigeria, the inability of the Government to provide pipe-borne water has led to the exploitation of groundwater for water supply by citizens. Groundwater is generally regarded as one of the pristine sources of water. However, recent studies have shown that the quality of groundwater in most urban areas in Nigeria is deteriorating fast (Ocheri et al., 2014). Mokuolu et al. (2017) reported that municipal water from treatment plants serves as the most secure drinking water source in many urban areas in Nigeria, however, the quality of pipe-borne water has been compromised due to the unsustainable demographic growth and breaches in the integrity of distribution pipe network (Eniola et al., 2015), which results from poor surveillance and maintenance practices by the government.

The presence of pathogenic bacteria in domestic water sources poses a substantial threat to human health. These microorganisms can cause a wide range of illnesses, from mild gastrointestinal disturbances to severe systemic infections. Among the most concerning are those capable of forming biofilms and producing hemolysins, as these traits can enhance their survival in water systems and increase their virulence potential (Flemming et al., 2016). Biofilms, in particular, provide a protective environment for bacteria, allowing them to persist in water distribution systems and resist disinfection efforts (Percival et al., 2015).

The ability of bacteria to form biofilms is a significant factor in their persistence within water distribution systems. Biofilms are complex communities of microorganisms that adhere to surfaces and are encased in a self-produced extracellular polymeric substance (EPS) matrix. This matrix provides protection against environmental stressors, including disinfectants and antibiotics, making biofilm-associated bacteria particularly challenging to eradicate (Hall-Stoodley et al., 2004). In the context of domestic water sources, biofilms can form on the inner surfaces of pipes, storage tanks, and other water infrastructure, creating reservoirs of potentially pathogenic bacteria that can periodically detach and contaminate the water supply (Wingender and Flemming, 2011).

Hemolytic bacteria, capable of lysing red blood cells, represent another category of concern in water quality assessments. The production of hemolysins is often associated with increased virulence, as these enzymes can damage host tissues and facilitate the spread of infection (Alonzo and Torres, 2014). In water systems, the presence of hemolytic bacteria not only indicates potential pathogenicity but also suggests the possibility of more severe health outcomes if these organisms are ingested or come into contact with broken skin (Ramírez-Castillo et al., 2015).

The rise of antibiotic resistance among waterborne bacteria has further complicated the landscape of water safety and public health. The overuse and misuse of antibiotics in human medicine and agriculture have led to the emergence and spread of antibiotic-resistant strains, which can find their way into water sources through various routes, including human and animal waste (Pruden et al., 2013). When these resistant bacteria persist in domestic water supplies, they pose a dual threat: not only are they potentially pathogenic, but they also carry the risk of transferring resistance genes to other bacteria, including those that are typically commensal or opportunistic pathogens (Martinez, 2009).

The study of antibiotic resistance patterns, or antibiograms, of bacteria isolated from water sources provides valuable insights into the potential risks associated with these microorganisms. Antibiograms not only inform treatment strategies for infections caused by waterborne pathogens but also serve as indicators of the overall prevalence of antibiotic resistance in the environment (Jorgensen and Ferraro, 2009). In the context of off-campus student housing, where large numbers of young adults live in close proximity and often share water sources, the spread of antibiotic-resistant bacteria could have significant public health implications (Collignon et al., 2018).

The intersection of biofilm formation, hemolytic activity, and antibiotic resistance in bacteria isolated from domestic water sources presents a complex challenge for water quality management and public health. Biofilms can act as reservoirs for antibiotic resistance genes, facilitating their transfer between bacterial species and potentially leading to the emergence of multi-drug resistant strains (Balcázar et al., 2015). Moreover, the protective environment of biofilms can enhance the survival of hemolytic bacteria, allowing them to persist in water systems and potentially cause infections in susceptible individuals (Kostakioti et al., 2013).

In developing countries, where water treatment and distribution infrastructure may be less robust, the risks associated with biofilm-forming, hemolytic, and antibiotic-resistant bacteria in domestic water sources are particularly pronounced. Limited access to clean water and inadequate sanitation facilities can exacerbate the spread of waterborne pathogens, leading to a higher incidence of waterborne diseases (Ashbolt, 2015). In such settings, off-campus student housing may be especially vulnerable, as it often falls outside the purview of university-managed water quality control measures (Onyango et al., 2018).

The assessment of water quality in off-campus student housing is crucial for several reasons. First, students represent a significant portion of the young adult population, and their health and well-being have broader societal implications. Second, the communal nature of student housing can facilitate the rapid spread of waterborne pathogens if present in the water supply. Third, students may come from diverse geographical backgrounds, potentially introducing or being exposed to novel bacterial strains (Saxena et al., 2015). Therefore, understanding the microbiological profile of water sources in these settings is essential for implementing targeted interventions and preventing outbreaks of waterborne diseases.

The use of multiple analytical approaches, including culture-based methods, biochemical characterization, and molecular techniques, provides a comprehensive understanding of the bacterial communities present in water sources. Culture-based methods allow for the isolation and enumeration of viable bacteria, while biochemical tests help in the identification of specific genera and species (Rompré et al., 2002). Molecular techniques, such as PCR and sequencing, can provide further insights into the genetic basis of virulence factors and antibiotic resistance (Ramírez-Castillo et al., 2015).

The investigation of total heterotrophic bacteria count (THBC) serves as an important indicator of overall water quality and the effectiveness of treatment processes. While not all heterotrophic bacteria are pathogenic, high THBC can indicate conditions favorable for bacterial growth and may suggest the presence of nutrients that could support the proliferation of pathogenic organisms (Allen et al., 2004). In the context of off-campus student housing, where water storage and distribution systems may vary in quality and maintenance, monitoring THBC is crucial for identifying potential hotspots of bacterial contamination.

The specific focus on genera such as Staphylococcus, Pseudomonas, Vibrio, and Salmonella in water quality assessments is warranted by their potential to cause a range of infections and their ability to persist in water environments. Staphylococcus species, particularly S. aureus, are known for their ability to form biofilms and produce hemolysins, making them formidable pathogens in both clinical and environmental settings (Archer et al., 2011). Pseudomonas, especially P. aeruginosa, is notorious for its intrinsic antibiotic resistance and its capacity to form robust biofilms in water systems (Drenkard and Ausubel, 2002). Vibrio species, including V. cholerae, pose a significant threat to water sources due to their ability to cause severe gastrointestinal infections (Lutz et al., 2013). Salmonella, a leading cause of foodborne illness worldwide, can also persist in water environments and poses a risk of waterborne transmission (Winfield and Groisman, 2003).

The study of antibiotic resistance patterns in these bacterial isolates is particularly relevant in the context of off-campus student housing. Students may come from diverse backgrounds with varying exposure to antibiotics, and the communal living environment can facilitate the exchange of resistant bacteria or resistance genes. Moreover, the potential for students to seek medical care independently and possibly receive antibiotic treatments without proper oversight could contribute to the selection and spread of resistant strains (Collignon et al., 2018).

In conclusion, the investigation of hemolytic and biofilm-forming bacteria isolated from domestic water sources in off-campus settings, coupled with the analysis of their antibiotic resistance profiles, addresses a critical gap in our understanding of water quality and its implications for public health. This comprehensive approach not only provides insights into the current state of water safety in these environments but also informs strategies for monitoring, treatment, and prevention of waterborne diseases. By elucidating the complex interplay between bacterial virulence factors, persistence mechanisms, and antibiotic resistance, this study aims to contribute to the development of more effective water management practices and, ultimately, to the protection of student health in off-campus housing environments.

**2. Materials and Methods**

**2.1 Study Location**

The water samples used in this study were sourced from different student hostels off campus. Locations around the University of Port Harcourt including Choba, Aluu, Alakahia, and Rumuosi were all part of areas where borehole samples were collected.

**2.2 Collection of Samples**

To obtain borehole samples, the nozzle of each of the taps was sterilized with cotton wool soaked in ethanol and the taps were left to run for two minutes to avoid water left on the pipe from being used as samples. Samples were collected using sterile containers from all the sampling points. The samples were transported to the laboratory for analysis.

**2.3 Media Preparation**

**Nutrient Agar**

Nutrient agar is a general-purpose media that is used in the laboratory to culture a variety of bacteria and can be used to obtain the total heterotrophic count of bacteria in a sample. The nutrient agar was prepared according to the manufacturer’s specifications by dissolving 28 g of nutrient agar powder in 1 L of distilled water, mixing properly, and autoclaved at 121oC for 15 min at 15 psi, allowed to cool a little before dispensing into sterile Petri dishes and allowed to solidify.

**Salmonella Shigella Agar**

The Salmonella Shigella Agar media was used to culture the growth salmonella species. 63 g of SS agar powder was dissolved into 1000 ml of distilled water according to the manufacturer’s specifications. The agar was boiled by heating for a few minutes and allowed to cool at 42-45oC before dispensing into Petri plates.

**Mannitol Salt Agar**

The mannitol salt agar is a medium used for the isolation of Staphylococcus sp and was prepared according to the manufacturer’s specifications. 111 g of the agar was suspended in 1000 ml of distilled water, swirled, and boiled by heating to dissolve completely. After which it was sterilized by autoclaving at 121oC for 15 min and at 15 psi.

**Preparation of Eiosin Methylene Blue Agar**

The EMB agar media was used to culture the growth of Escherichia coli. 36 g of EMB agar powder was dissolved into 1000 ml of distilled water in accordance to the manufacturer’s specifications. The agar was subjected to heating for a few minutes and was sterilized in an autoclave at 121 ˚C for 15 min at 15 psi. The medium was allowed to cool before dispensing into Petri plates.

**Preparation of Thiosulphate Citrate Bile Salt Agar**

The TCBS agar media was used to culture the growth Vibrio species and was prepared following the manufacturer’s specifications. 89 g of TCBS agar powder was dissolved into 1000 ml of distilled water. The agar was boiled by heating for a few minutes and allowed to cool at 42-45 ˚C before dispensing onto Petri plates

**2.4 Isolation, Cultivation, and Counting of Bacteria**

Ten-fold serial dilution was performed on the samples. 10ml of the water Sample was suspended in 90ml sterile normal saline (0.85% w/v, Nacl) to make a stock solution. 1ml of the aliquot was pipetted into a test tube containing 9ml sterile normal saline to make 10-1,10-2 and 10-3. Using a sterile 1ml pipette (syringe), 0.1ml of each of the dilutions was inoculated into nutrient agar plates by the spread plate technique (Standard Method for the evaluation of water and wastewater,1985). The inocula was spread evenly using a sterile glass rod. Incubation of the petri dishes was carried out in an inverted position at 370C for 24 hours. The number of colonies was enumerated after incubation of the plates that yielded between 30 to 300 colonies The inoculation was done in duplicates to minimize error and the average was taken afterwards. The number of colonies formed was calculated by multiplying the number of colonies by the dilution factor and the inverse of the volume plated. Colonies were sub-cultured to obtain pure colonies which were stored in bottles for further tests.

**2.5 Colonial Characterization and Identification of Isolates**

Isolates were identified based on their morphological (cultural characteristics) on growth media and biochemical tests. Identification protocol according to Cheesebrough, (2000) was used to identify discrete colonies from the bacteriological media of sub-cultured isolates. Morphological Characterization of bacterial isolates was based on cell morphology which includes shape, size, opacity, colour, edge, elevation, Gram stain, and biochemical tests while the biochemical identification includes the citrate, catalase, motility, etc. tests carried out.

**2.6 Gram Staining**

This test was employed to differentiate the bacterial isolates into two groups of Gram-positive and Gram-negative organisms. Gram staining differentiates bacteria by the chemical and physical properties of their cell wall by detecting peptidoglycan which is present in a thick layer in Gram-positive bacteria. (Beveridge., 2001). The test was performed as described by Holts et al, (1994). A smear of the test bacterial culture was prepared and heat-fixed in a grease-free slide. The slide was flooded with crystal violet for one minute gently washed off with tap water and then dried off. The slide was exposed to gram's iodine for one minute and then washed with 75% alcohol for 30 seconds. The slide was washed with tap water and air dried then 0.25% safranin was used to counter-stain the slide for 30 seconds. The slide was washed, drained dried, and examined under an oil-immersion microscope (x100). A purple colouration indicated a gram-positive organism as a result of retention of the primary stain (crystal violet). In contrast, pink colouration, arising from the colour of the counterstain (safranin) as a result of the inability to retain the primary stain indicated a gram-negative organism (Rohde et al., 2019).

**2.7 Biochemical Test to Identify Bacterial Isolates**

**Oxidase Test**

This test was carried out to determine the ability of the isolates to produce oxidase enzymes. The dry filter paper method as described by McFadden (2000) was employed. A piece of filter paper was soaked with the reagent solution, allowed to dry and further smeared with a Colony of the isolates and observed for the formation of purple colour. The purple colour within 2 minutes indicated a positive result while isolates with no purple colour within 2 minutes were adjudged negative (Yehia et al., 2015).

**Catalase test**

This test was carried out to identify catalase-producing bacteria and non-productive bacteria as described by Macfidden (2000). The slide test method was carried out. A drop of hydrogen peroxide solution was placed on a sterile glass slide using a sterile wire loop after which a Colony of each of the isolates was collected and placed in another sterile glass slide which served as a cover slip. This was inverted and placed on the hydrogen peroxide solution and observed for 10 seconds. The formation of bubbles showed a catalase-positive result whereas the non-production of bubbles showed a negative result (Nakashima et al., 2015)

**Methyl Red Test**

During mixed acid fermentation, a variety of end products would be elicited. These include ethanol, succinate, lactate, acetate, molecular hydrogen, and carbon dioxide. The products vary depending on the bacterial species. In a situation where mixed acid fermentation is the primary fermentation pathway for bacteria species, acidic products will accumulate a sufficient amount. This will drastically reduce the pH of the spent broth.MR-VP medium measuring 15g was dissolved in one liter of distilled water and 10 ml was dispensed into each tube and autoclaved. The isolates will then be inoculated into the cooled medium and incubated at 370C for 48 hours, after which three drops of 0.02% methyl red reagent were added to 5ml of the culture and observed. A red colour reaction signified a positive result and a yellow colour indicates a negative result (Omemu et al., 2018)

**Voges Proskaeur Test**

This test was carried out to determine the ability of some isolates to ferment carbohydrates and produce acetyl methyl carbinol or reduced product 2, 3 butylenes glycol (CH3 CHOH.CH3). Also, 5 ml culture from methyl red test would be used and 3 drops of 40% KOH and 6 drops of alpha naphthol added. The colour was observed to change from amber to pink or red indicating a positive reaction (Sah et al., 2010)

**Indole Test**

This test was carried out to test the ability of the isolates to break down tryptophan for nutritional needs using the enzyme tryptophase and producing indolent using Kovac's reagent. The test was carried out as described by (Harley, 2005). The isolate was incubated in a test tube containing peptone water for 48 hours at 37°C. After incubation, 0.5ml (about 5 drops) of Kovac's reagent was poured into the test tube and observed after agitation for a minute, a positive indole test was indicated by the formation of a pink to red color, ('cherry-red ring ') in the reagent's layer on top of the medium within seconds of adding the reagent. In contrast, the formation of yellow or slightly cloudy colour indicated a negative result (McFaddin, 2006).

**Citrate Test**

This is based on the ability of the organism to use citrate as its sole source of carbon and energy, and ammonium salt as a sole source of nitrogen. The medium used was Simmon’s citrate agar. The medium was boiled by heating and dispensed into test tubes which are then autoclaved for 15 minutes at 1210C to achieve sterilization. After sterilization, the medium would be allowed to set in a slanting position before inoculating the organism. The culture was incubated at 370C for 24-48 hours. Positive results show a colour change from green to blue while for a negative result, there is no colour change (Hemalatha et al., 2011).

**Sugar Fermentation Test**

This test was carried out to determine the utilization of some sugars by the isolates. The sugars tested are glucose, lactose, maltose, and sucrose. Exactly 1g of peptone water powder was weighed and transferred into a conical flask. One gram of each sugar was weighed and added to the flask, and 1 mL of Bromo-thymol blue indicator was added to the flask and was dispensed in 100 mL of water. The broth was mixed and dispensed into bijou bottles or test tubes containing inverted Durham tubes. The peptone water broth in the bijou bottles or test tubes was sterilized in the autoclave at 1150C for 15 minutes. The procedure was repeated for other sugars; the peptone water broth was allowed to cool before inoculating the culture and was then incubated at room temperature for 24 hours. The ability of the isolates to utilize sugar will show a colour change to light blue and in some cases, the production of gas in the Durham tubes (Bhardwaj et al., 2012)

**2.8 Determination of Biofilm Formation**

Brain heart infusion was prepared, glucose was added and supplemented with 0.8% Congo red indicator and autoclaved at 121oC for 15 minutes at 15psi. A pure culture of bacteria isolates was then streaked Congo red agar plates incubated for 24 hours and inspected for black pigmentation (Azzawi et al.,2015)

**2.9 Antibiotics Susceptibility Testing**

Antibiotic sensitivity patterns of all the confirmed E. coli isolates were performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by CLSI (2012). Twelve commonly used antibiotics (µg/disc) viz. Gentamycin (GN) 10ug; Ofloxacin (OFX) 5ug; Imipenem (IMP) 10ug; (LEV) Levefotaxim (CTX) 25ug; Ampiclox (ACX) 10ug; Cefuroxim (CXM), 30ug; Levofloxacin (LEV) 5ug; Nalixidic acid (NA) 30ug; Cefixime (ZEM) 5ug; Augmentine (AUG); 30ug; Ceftriaxone sulbactam (CRO) 45ug; and Nitrofurantoin (NIT) 300ug. Abtek, (UK) was tested. From an overnight culture E. coli, (0.5 MacFarland turbidity standards) bacterial culture was prepared in sterile saline, from which 0.1mL was inoculated onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37oC for 24h. The zone of inhibition was measured in millimeters (Naqid et al., 2020)

**Haemaglutination testing**

Tripticase soy agar was prepared according to the manufacturer’s instructions and autoclaved at 121oC for 15 minutes at 15psi. The media was allowed to cool up to 37oC and then supplemented with sheep blood immediately before pouring the plates. Pure culture of bacterial isolates was then streaked on blood agar plates and incubated for 24 hours for haemolysis to be visualized (Puglia et al., 2000)

**3. RESULT**

**3.1 Total Heterotrophic Bacteria Count of Borehole Water Obtained from Off-Campus Hostels**

The total heterotrophic bacteria count (THBC) obtained from the different water samples studied showed that FESTAC hostel had the highest THBC of 1.77x104 CFU/ml followed by CAM hostel with 1.86x104CFU/ml. LAN, CHI, ONU, and TROY hostels had THB counts of 1.87x103, 1.64x103, 4.75x103, and 2.0x103 CFU/ml respectively. See Table 1 below.

**3.2 Staphylococcus Counts of Borehole Water Obtained from Off-Campus Hostels**

The Staphylococcus count obtained from the different water samples studied showed that CAM, hostel has the highest Staphylococcus count of 1.45x102 CFU/ml followed by TROY hostel with 1.15x102 CFU/ml. ONU and FESTAC hostels had the same Staphylococcus counts of 2.5x101, while LAN and CHI hostels had no staphylococcus count at all. See table 2 below.

**3.3 Pseudomonas Counts of Borehole Water Obtained from Off-Campus Hostels**

The Pseudomonas count obtained from the different water samples studied showed that CAM, hostel has a Staphylococcus count of 2.2x102 CFU/ml, and there was no Staphylococcus count for samples from LAN, CHI, ONU, TROY, and FESTAC hostels. See table 3 below.

**3.4 Vibrio Counts of Borehole Water Obtained from Off-Campus Hostels**

The Vibrio count obtained from the different water samples studied showed that TROY hostel has the highest Vibrio count of 2.15x102 CFU/ml and ONU hostel has 1.7x102 CFU/ml. CHI, FESTAC, LAN, and CAM hostels had no Vibrio count at all. See table 4 below.

**3.5 Salmonella Counts of Borehole Water Obtained from Off-Campus Hostels**

The Salmonella count obtained from the different water samples studied showed that CAM hostel has the highest Salmonella count of 2.1x102 CFU/ml and LAN hostel has 1.3x102 CFU/ml. CHI, FESTAC, ONU, and CAM hostels had no Salmonella count at all. See table 5 below.

**Table 1: Total Count of Colonies on Various Media Plates**

**LogCFU/ml**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample location | THBC | Vibriod count | Faecal coliform count | Pseudomonad count | Staphylococcal count | Salmonella Shigella count |
| CAM HOSTEL | 4.27±0.01 | 0 | 0 | 2.15±0.21 | 2 | 0 |
| CHI HOSTEL | 2.77±0.1 | 0 | 0 | 0 | 0 | 0 |
| FESTAC HOSTEL | 4.25±0.02 | 0 | 0 | 0 | 0 | 0 |
| LAN HOSTEL | 4.27±0.02 | 0 | 0 | 0 | 0 | 3.11±0.05 |
| ONU HOSTEL | 4.7±0.01 | 3.23±0.07 | 0 | 0 | 2.39±0.12 | 0 |
| TROY HOSTEL | 4.3±0.03 | 3.33±0.1 | 0 | 0 | 3.04±0.19 | 0 |
| P value | <0.0001 |  |  |  |  |  |  |

**Table 2: Total Heterotrophic Bacteria Counts of Borehole water Obtained from Off Campus Hostels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hostel ID** | **Dilution Plated** | **Av. Count** | **CFU/ml** | **Log CFU/ml** |
| **CAM** | 10-1 | 186 | 1.86x104 | 4.270 |
| **LAN** | 100 | 187 | 1.87x103 | 3.272 |
| **CHI** | 100 | 164 | 1.64x103 | 3.215 |
| **ONU** | 10-1 | 47.5 | 4.75x103 | 3.677 |
| **TROY** | 100 | 200 | 2.0x103 | 3.301 |
| **FESTAC** | 10-1 | 177 | 1.77x104 | 4.278 |

**Table 3 Staphylococcus Counts of Borehole water Obtained from Off Campus Hostels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hostel ID** | **Dilution Plated** | **Av. Count** | **CFU/ml** | **Log CFU/ml** |
| **CAM** | 10-0 | 14.5 | 1.45x102 | 2.161 |
| **LAN** | - | - | - | - |
| **CHI** | - | - | - | - |
| **ONU** | 10-0 | 2.5 | 2.5x101 | 1.398 |
| **TROY** | 100 | 11.5 | 1.15x102 | 2.061 |
| **FESTAC** | 10-0 | 2.5 | 2.5x101 | 1.398 |

**Table 4 Pseudomonas Counts of Borehole water Obtained from Off Campus Hostels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hostel ID** | **Dilution Plated** | **Av. Count** | **CFU/ml** | **Log CFU/ml** |
| **CAM** | 10-0 | 22 | 2.2x102 | 2.342 |
| **LAN** | - | - | - | - |
| **CHI** | - | - | - | - |
| **ONU** | - | - | - | - |
| **TROY** |  | - | - | - |
| **FESTAC** | - | - | - | - |

**Table 5 Vibrio Counts of Borehole water Obtained from Off Campus Hostels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hostel ID** | **Dilution Plated** | **Av. Count** | **CFU/ml** | **Log CFU/ml** |
| **CAM** | - | - | - | - |
| **LAN** | - | - | - | - |
| **CHI** | - | - | - | - |
| **ONU** | 10-0 | 17 | 1.7x102 | 2.230 |
| **TROY** | 100 | 21.5 | 2.15x102 | 2.332 |
| **FESTAC** | - | - | - | - |

**Table 6 Salmonella Counts of Borehole water Obtained from Off Campus Hostels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hostel ID** | **Dilution Plated** | **Av. Count** | **CFU/ml** | **Log CFU/ml** |
| **CAM** | 10-0 | 21 | 2.1x102 | 2.322 |
| **LAN** | 100 | 13 | 1.3x102 | 2.114 |
| **CHI** | - | - | - | - |
| **ONU** | - | - | - | - |
| **TROY** | - | - | - | - |
| **FESTAC** | - | - | - | - |

**3.7 Morphological/Biochemical Characterization of Isolates from Water Samples Studied**

The morphological and biochemical characteristics revealed the predominance of eight bacterial genera including Salmonella, Pseudomonas, Proteus, Staphylococcus, Corynebacterium, Bacillus, Yersinia, VIbrio, Micrococcus species. (Table 7)

**3.8 Antibiotic Susceptibility Profile of Gram-Positive Isolates That Tested Positive to Biofilm, Alpha or Beta Hemolysis**

The antibiotic susceptibility profile of gram-positive isolates showed that LAN4 was more resistant to antibiotics than other gram-positive isolates (It was resistant to eight antibiotics; Cefalexin, Erythromycin, Ampicillin, Sulfamethoxazole, streptomycin, Rifampicin, Azithromycin, Ampiclox) while CAM 7 and CHI1 was susceptible to all antibiotics (Cefalexin, Erythromycin, Ampicillin, Sulfamethoxazole, streptomycin, Rifampicin, Azithromycin, Pefloxacin, Ciprofloxacin, Ampiclox - (Table 8)

**3.9 Antibiotic Susceptibility Profile of Gram-Negative Isolates That Tested Positive to Biofilm, Alpha or Beta Hemolysis**

The antibiotic susceptibility profile of gram-negative isolates showed that TRO7 was more resistant to antibiotics than other gram-negative isolates (it was resistant to 7 antibiotics on the antibiotics sensitivity disc; Pefloxacin, Ofloxacin, Azithromycin, Levofloxacin, Cefalotin, Ampicillin and Aureomycin) while CHI2 was susceptible to all antibiotics (Cefalexin, Spectinomycin, Ciprofloxacin, Pefloxacin, Ofloxacin, Azithromycin, Levofloxacin, Cefalotin, Ampicillin and Aureomycin (Table 9)

**3.10 Biofilm Formation and Haemolytic Characteristics of Bacteria Isolates from Water Samples Studied**

The study showed that six isolates tested positive for biofilm formation, eleven tested positive for alpha haemolysis, eleven also tested positive for beta haemolysis and fourteen tested positive for gamma haemolysis. (Table 10 to 12)

**Table 7 Cultural Morphology of Bacterial Isolates from Water Samples studied.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SN** | **ISOLATE****CODE** | **SHAPE** | **SIZE** | **COLOR** | **MARGIN** | **ELEVATION** | **OPACITY** | **SURFACE** | **TEXTURE** |
| 1 | CAM1 | Round | 4mm | Pink | Entire | Convex | Opaque | Shiny | Moist |
| 2 | CAM2 | Round | 2mm | White | Entire | Flat | Opaque | Shiny | Moist |
| 3 | CAM3 | Round | 4mm | Milky | Entire | Flat | Opaque | Shiny | Moist |
| 4 | CAM4 | Round | 4mm | Pink | Entire | Convex | Opaque | Shiny | Moist |
| 5 | CAM5 | Round | 4mm | White | Entire | Flat | Opaque | Shiny | Moist |
| 6 | CAM6 | Round | 2mm | Yellow | Entire | Convex | Opaque | Shiny | Moist |
| 7 | CAM7 | Round | 4mm | White | Entire | Flat | Opaque | Shiny | Moist |
| 8 | CHI1 | Round | 6mm | White | Lobate | Flat | Opaque | Dull | Dry |
| 9 | CHI2 | Round | 3mm | Yellow | Entire | Flat | Opaque | Shiny | Dry |
| 10 | CHI3 | Round | 2mm | Orange | Entire | Flat | Opaque | Shiny | Dry |
| 11 | CHI4 | Round | 2mm | Milky | Entire | Flat | Opaque | Shiny | Dry |
| 12 | LAN1 | Round | 4mm | Milky | Entire | Flat | Opaque | Shiny | Moist |
| 13 | LAN2 | Round | 4mm | Milky | Entire | Flat | Opaque | Shiny | Moist |
| 14 | LAN3 | Round | 3mm | Yellow | Entire | Flat | Opaque | Shiny | Moist |
| 15 | LAN4 | Round | 4mm | White | Entire | Flat | Opaque | Shiny | Moist |
| 16 | LAN5 | Round | 7mm | White | Wavy | Flat | Opaque | Dull | Moist |
| 17 | LAN6 | Round | 2mm | Creamy | Entire | Flat | Opaque | Shiny | Moist |
| 18 | LAN7 | Round | 2mm | Milky | Entire | Flat | Opaque | Shiny | Moist |
| 19 | TRO1 | Round | 3mm | Peach | Entire | Flat | Opaque | Shiny | Moist |
| 20 | TRO2 | Irregular | 5mm | White | Lobate | Flat | Opaque | Dull | Moist |
| 21 | TRO3 | Round | 2mm | Milky | Undulate | Convex | Opaque | Dull | Dry |
| 22 | TRO4 | Round | 1mm | White | Entire | Convex | Opaque | Shiny | Dry |
| 23 | TRO5 | Spindle | 5mm | White | Entire | Flat | Opaque | Dull | Moist |
| 24 | TRO6 | Round | 3mm | Yellow | Entire | Flat | Opaque | Shiny | Moist |
| 25 | TRO7 | Round | 4mm | Milky | Entire | Flat | Opaque | Shiny | Moist |
| 26 | TRO8 | Rhizoid | 10mm | White | Entire | Flat | Opaque | Dull | Dry |
| 27 | TRO9 | Round | 1mm | Yellow | Entire | Convex | Opaque | Shiny | Moist |
| 28 | TRO10 | Round | 5mm | Creamy | Entire | Flat | Opaque | Shiny | Moist |
| 29 | TRO11 | Irregular | 5mm | White | Lobate | Flat | Opaque | Dull | Moist |
| 30 | ONU1 | Round | 1mm | White | Entire | Flat | Opaque | Shiny | Moist |
| 31 | ONU2 | Irregular | 6mm | Creamy | Entire | Flat | Opaque | Shiny | Moist |
| 32 | ONU3 | Round | 1mm | Milky | Entire | Flat | Opaque | Dull | Dry |
| 33 | FES1 | Irregular | 5mm | White | Lobate | Flat | Opaque | Dull | Moist |
| 34 | FES2 | Round | 1mm | Creamy | Entire | Convex | Transparent | Shiny | Moist |
| 35 | FES3 | Irregular | 5mm | White | Lobate | Flat | Opaque | Dull | Moist |
| 36 | FES4 | Round | 1mm | White | Entire | Umbonate | Opaque | Dull | Dry |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ISO** | **MO** | **OXI** | **CAT** | **LAC** | **GLU** | **SUC** | **MR** | **VP** | **IND** | **CIT** | **BUT** | **SLT** |  **GAS** | **S** | **H2S** | **GRS**  | **SHAPE** | **NAME** |
| CAM3 | + | + | + | **-** | **+** | **-** | **-** | **-** | - | + | A | A | **-** | **+** | **-** | **-**ve |  Rod | *Pseudomonas* sp  |
| LAN4 | + | **+** | **+** | **-** |  **+** | **+** | **-** | **+** | - | **+** | **A** | B | **-** | **+** | **-** | +ve  |  Rod | *Bacillus* sp |
| ONU1 | - | + | + | - | **+** | - | + | **-** | - | - | B | B | - | **-** | **-** | -ve |  Rod | *Yersinia* sp |
| ONU2 | + | - | - | + | **+** | + | - | **+** | + | + | A | A | **-** | **+** | **-** | **-**ve  |  Rod | *Vibrio* sp |
|  ONU3 TRO1 TRO2 TRO3TRO4TRO5TRO6TRO8TRO10TRO11FES1FES4 | +----++-+--- | -+--**-****+****-** **+****-****+****+****-** | ++-+-++ ++++ + | -++++-- +++++ | +++++++ +-+ **A****+** | -++-**+****+****-** **+****+****+****A****+** | ++--+-- +-+- + | **-****-****+****+****-****+****-** **+****-****-****+** **-** | ----+-- -+-- + | +++++++ ++--+ | AABBBAB ABA**B****A** | AABBABB ABBBB | **+****-****-****-****-****-****-** **-****-****-****+****-** | **+****-****+****+****-****+****-****-****+****-****+****-** | **+****-****-****-****-****-****-** **-****-****-****-** **-** | -ve+ve+ve+ve+ve+ve+ve +ve+ve-ve+ve+ve |  Rod Cocci Cocci Rod Cocci Rod Cocci CocciRodRodRodRod | *Proteus* sp*Staphyloccocus* sp*Micrococcus* sp*Bacillus* sp*Micrococcus* sp*Bacillus* sp*Microccocus* sp*Staphyloccocus* sp*Corynebacterium* *Vibrio* sp*Corynebacterium**Bacillus* sp |

**Table 8 BIOCHEMICALCHARACTERISTICS OF MULTIDRUG RESISTANT ISOLATES FROM WATER SAMPLES STUDIED.**

**Table 9 ANTIBIOTIC SUSCEPTIBILITY PROFILE (mm) OF GRAM-POSITIVE ISOLATES THAT TESTED POSITIVE TO BIOFILM, ALPHA OR BETA HEMOLYSIS**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SN** | **ISOLATE****CODE** | **PEF** | **CN** | **APX** | **Z** | **AM** | **R** | **CPX** |  **S** | **SXT** | **E** |
| 1 | CAM1 | 22 | 14 | 12 | 11 | 8 | 12 | 24 | 10 | 14 | 8 |
| 2 | CAM5 | 21 | 10 | 9 | 0 | 0 | 8 | 22 | 20 | 18 | 22 |
| 3 | CAM7 | 28 | 18 | 16 | 16 | 14 | 20 | 30 | 20 | 22 | 18 |
| 4 | CHI1 | 26 | 18 | 12 | 14 | 6 | 16 | 24 | 20 | 24  | 20 |
| 5 | LAN4 | 12 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 |
| 6 | TRO1 | 20 | 0 | 0 | 0 | 0 | 0 | 25 | 8 | 16 | 16 |
| 7 | TRO2 | 16 | 0 | 0 | 0 | 0 | 0 | 20 | 5 | 0 | 0 |
| 8 | TRO3 | 15 | 14 | 14 | 12 | 10 | 0 | 25 | 0 | 0 | 0 |
| 9 | TRO4 | 8 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 12 |
| 10 | TRO5 | 24 | 9 | 0 | 0 | 0 | 10 | 20 | 0 | 12 | 14 |
| 11 | TRO6 | 12 | 8 | 10 | 0 | 0 | 6 | 22 | 0 | 10 | 9 |
| 12 | TRO9 | 20 | 0 | 0 | 0 | 0 | 6 | 24 | 0 | 10 | 9 |
| 13 | TRO10 | 18 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 12 | 9 |
| 14 | ONU2 | 20 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 5 | 16 |
| 15 | FES1 | 24 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 10 | 16 |
| 16 | FES3 | 15 | 12 | 10 | 9 | 10 | 8 | 20 | 8 | 0 | 10 |
| 17 | FES4 | 24 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 |

**Key:** Cefalexin (CN) 30µg, Erythromycin (E) 15µg, Ampicillin (AM )10µg, Sulfamethoxazole (SXT) 23.75 µg, streptomycin (S) 10µg, Rifampicin (R) 5µg, Azithromycin (Z) 15µg, Pefloxacin (PEF) 5µg, Ciprofloxacin (CPX) 5µg, Ampiclox (APX) 10µg.

**Table 10** **ANTIBIOTIC SUSCEPTIBILITY PROFILE (mm) OF GRAM-NEGATIVE ISOLATES THAT TESTED POSITIVE TO BIOFILM, ALPHA OR BETA HEMOLYSIS**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SN** | **ISOLATE****CODE** | CN | PEF | OFX | AZ | LEV | CF | SP | CPX | AM | AU |
| 1 | CAM3 | 0 | 20 | 21 | 0 | 20 | 18 | 0 | 26 | 0 | 0 |
| 2 | CAM4 | 21 | 10 | 9 | 0 | 19 | 8 | 0 | 20 | 18 | 22 |
| 3 | CHI2 | 28 | 18 | 16 | 16 | 14 | 20 | 0 | 20 | 16 | 18 |
| 4 | ONU1 | 12 | 14 | 12 | 0 | 6 | 0 | 0 | 18 | 0 | 8 |
| 5 | ONU2 | 12 | 14 | 0 | 0 | 0 | 0 | 10 | 23 | 0 | 0 |
| 6 | ONU3 | 10 | 18 | 0 | 0 | 0 | 0 | 0 | 18 | 10 | 10 |
| 7 | TRO7 | 16 | 0 | 0 | 0 | 0 | 0 | 8 | 23 | 0 | 0 |
| 8 | TRO8 | 12 | 14 | 14 | 0 | 10 | 0 | 0 | 21 | 0 | 0 |
| 9 | TRO11 | 10 | 18 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 12 |

Key: Cefalexin (CN) 30µg, Spectinomycin (SP) 100µg, Ciprofloxacin (CPX) 5µg, Pefloxacin (PEF) 5µg, Ofloxacin (OFX) 5µg, Azithromycin (AZ) 15µg, Levofloxacin (LEV) 5µg, Cefalotin (CF) 30µg, Ampicillin (AM )10µg, Aureomycin (AU) 30µg

|  |  |  |  |
| --- | --- | --- | --- |
| **SN** | **ISOLATE CODE** |  **HAEMOLYSIS** | **BIOFILM** |
| **ALPHA** | **BETA** | **GAMMA** |
| 1 | CAM1 | - | - | + | + |
| 2 | CAM2 | - | - | + | - |
| 3 | CAM3 | - | + | - | - |
| 4 | CAM4 | - | - | + | + |
| 5 | CAM5 | + | - | - | - |
| 6 | CAM6 | - | - | + | - |
| 7 | CAM7 | - | - | + | + |
| 8 | CHI1 | - | + | - | - |
| 9 | CHI2 | - | - | + | + |
| 10 | CHI3 | - | - | + | - |
| 11 | CHI4 | - | + | - | - |
| 12 | LAN1 | - | - | + | - |
| 13 | LAN2 | - | - | + | - |
| 14 | LAN3 | - | - | + | - |
| 15 | LAN4 | + | - | - | - |
| 16 | LAN5 | - | - | + | - |
| 17 | LAN6 | - | - | + | - |
| 18 | LAN7 | - | - | + | - |
| 19 | TRO1 | + | - | - | - |
| 20 | TRO2 | + | - | - | - |
| 21 | TRO3 | - | + | - | - |
| 22 | TRO4 | + | - | - | - |
| 23 | TRO5 | + | - | - | - |
| 24 | TRO6 | + | - | - | + |
| 25 | TRO7 | - | + | - | - |
| 26 | TRO8 | + | - | - | - |
| 27 | TRO9 | + | - | - | - |
| 28 | TRO10 | - | + | - | - |
| 29 | TRO11 | - | + | - | - |
| 30 | ONU1 | + | - | - | + |
| 31 | ONU2 | - | + | - | - |
| 32 | ONU3 | - | + | - | - |
| 33 | FES1 | - | + | - | - |
| 34 | FES2 | - | - | + | - |
| 35 | FES3 | + | - | - | - |
| 36 | FES4 | - | + | - | - |
|  | % Positive | 30.5% | 30.5% | 38.8% | 16.6% |
|  | % Negative | 69.5% | 30.5% | 61.2% | 83.4% |

**Table 11** **BIOFILM FORMATION AND HAEMOLYTIC CHARACTERISTICS OF BACTRERIA ISOLATES FROM WATER SAMPLES STUDIED**

**Table 12 ISOLATES POSITIVE TO ALPHA HAEMOLYSIS AND THEIR BIOFILM TEST RESULTS**

|  |  |  |  |
| --- | --- | --- | --- |
| SN | ISOLATE CODE | HAEMOLYSIS | BIOFILM |
| 1 | CAM5 | A | - |
| 2 | LAN4 | A | - |
| 3 | TRO1 | A | - |
| 4 | TRO2 | A | - |
| 5 | TRO4 | A | - |
| 6 | TRO5 | A | - |
| 7 | TRO6 | A | + |
| 8 | TRO8 | A | - |
| 9 | TRO9 | A | - |
| 10 | ONU1 | A | + |
| 11 | FES3 | A | - |

**Table 13 ISOLATES POSITIVE TO BETA HAEMOLYSIS AND THEIR BIOFILM TEST RESULTS**

|  |  |  |  |
| --- | --- | --- | --- |
| **SN** | **ISOLATE CODE** |  **HAEMOLYSIS** |  **BIOFILM** |
| 1 | CAM3 | B | - |
| 2 | CHI1 | B | - |
| 3 | CHI4 | B | - |
| 4 | TRO3 | B | - |
| 5 | TRO7 | B | - |
| 6 | TR010 | B | - |
| 7 | TR011 | B | - |
| 8 | ONU2 | B | - |
| 9 | ONU3 | B | - |
| 10 | FES1 | B | - |
| 11 | FES4 | B | - |

|  |  |  |
| --- | --- | --- |
|  | **Prevalence of Alpha haemolytic bacteria** | **Total** |
| Negative | positive |
| Isolate code | CAM HOSTEL | 6 | 1 | 7 |
| CHI HOSTEL | 4 | 0 | 4 |
| FESTAC HOS | 3 | 1 | 4 |
| LAN HOSTEL | 6 | 1 | 7 |
| ONU HOSTEL | 2 | 1 | 3 |
| TROY HOSTE | 4 | 7 | 11 |
| Total | 25 | 11 | 36 |

**Table 14 Prevalence of Alpha Haemolytic Bacteria**

|  |  |  |
| --- | --- | --- |
|  | **Prevalence of Beta haemolytic bacteria** | **Total** |
| Negative | positive |
| Isolate code | CAM HOSTEL | 6 | 1 | 7 |
| CHI HOSTEL | 2 | 2 | 4 |
| FESTAC HOS | 2 | 2 | 4 |
| LAN HOSTEL | 7 | 0 | 7 |
| ONU HOSTEL | 1 | 2 | 3 |
| TROY HOSTE | 7 | 4 | 11 |
| Total | 25 | 11 | 36 |

**Table 15 Prevalence of Beta Haemolytic Bacteria**

|  |  |  |
| --- | --- | --- |
|  | **Prevalence of Gamma haemolytic bacteria** | **Total** |
| Negative | positive |
| Isolate code | CAM HOSTEL | 2 | 5 | 7 |
| CHI HOSTEL | 2 | 2 | 4 |
| FESTAC HOS | 3 | 1 | 4 |
| LAN HOSTEL | 1 | 6 | 7 |
| ONU HOSTEL | 3 | 0 | 3 |
| TROY HOSTE | 11 | 0 | 11 |
| Total | 22 | 14 | 36 |

**Table 16 Prevalence of Gamma Haemolytic Bacteria**

**Table 17** **Prevalence of Biofilm Forming Bacteria**

|  |  |  |
| --- | --- | --- |
|  | **Prevalence of Biofilm forming bacteria** | **Total** |
| Negative | Positive |
| Isolate code | CAM HOSTEL | 4 | 3 | 7 |
| CHI HOSTEL | 3 | 1 | 4 |
| FESTAC HOS | 4 | 0 | 4 |
| LAN HOSTEL | 7 | 0 | 7 |
| ONU HOSTEL | 2 | 1 | 3 |
| TROY HOSTEL | 10 | 1 | 11 |
| Total | 30 | 6 | 36 |

**4. DISCUSSION**

The antibiogram profiles, biofilm formation capabilities, and hemolytic activities of bacteria isolated from domestic water sources in off-campus student hostels reveal significant microbial diversity and potential health risks. The total heterotrophic bacteria count (THBC) varied substantially among hostels, with FESTAC and CAM hostels exhibiting the highest counts (1.77x10^4 and 1.86x10^4 CFU/ml, respectively). These values exceed the World Health Organization's (WHO) recommended limit of 1.0x10^2 CFU/ml for drinking water (WHO, 2017). Similar findings were reported by Eze et al. (2020) in a study of borehole water quality in Enugu, Nigeria, where THBC ranged from 1.0x10^3 to 2.5x10^5 CFU/ml, indicating a widespread issue in water quality management in the region.

The predominance of eight bacterial genera (Salmonella, Pseudomonas, Proteus, Staphylococcus, Corynebacterium, Bacillus, Yersinia, Vibrio, and Micrococcus) aligns with previous studies on water quality in developing countries. Bain et al. (2014) conducted a systematic review of microbial drinking water quality in low and middle-income countries, identifying these genera as common contaminants. The presence of these potentially pathogenic bacteria in off-campus water sources raises concerns about the adequacy of water treatment and distribution systems serving student populations.

Antibiotic resistance patterns among the isolates varied significantly. The gram-positive isolate LAN4 exhibited resistance to eight antibiotics, including Cefalexin, Erythromycin, and Ampicillin. This multidrug resistance profile mirrors findings by Osunla and Okoh (2017) in their study of antibiotic-resistant bacteria in selected rivers in Osun State, Nigeria. They reported that 75% of Staphylococcus aureus isolates showed resistance to at least three antibiotics. The emergence of such resistant strains in water sources frequented by students could complicate treatment options for waterborne infections.

Conversely, the susceptibility of isolates CAM7 and CHI1 to all tested antibiotics suggests heterogeneity in resistance profiles within the bacterial population. This variability emphasizes the need for continuous monitoring and targeted interventions. A similar pattern of varying susceptibility was observed by Adesoji et al. (2015) in their analysis of antibiotic resistance in drinking water sources in Southwestern Nigeria, where they found both highly resistant and susceptible strains coexisting in the same water sources.

Among gram-negative isolates, TRO7 demonstrated resistance to seven antibiotics, including Pefloxacin, Ofloxacin, and Azithromycin. This extensive resistance profile aligns with global trends in antibiotic resistance among waterborne pathogens. In a study of urban water systems in Karachi, Pakistan, Imran et al. (2019) found that 68% of gram-negative isolates were resistant to multiple antibiotics, with particular concern for fluoroquinolone resistance. The presence of such resistant strains in off-campus water sources could contribute to the spread of antibiotic-resistant genes within student populations.

The identification of six isolates capable of biofilm formation underscores the potential for persistent contamination in water distribution systems. Biofilms serve as reservoirs for pathogens and facilitate the horizontal transfer of antibiotic resistance genes. Wingender and Flemming (2011) highlighted the role of biofilms in drinking water systems as reservoirs for opportunistic pathogens, emphasizing the challenges they pose to water safety. The co-occurrence of biofilm formation and antibiotic resistance observed in our study aligns with findings by Schwartz et al. (2003), who demonstrated the increased prevalence of antibiotic resistance genes in drinking water biofilms compared to planktonic bacteria.

Hemolysis patterns among the isolates (eleven each for alpha and beta hemolysis, fourteen for gamma hemolysis) provide insights into the potential virulence of the bacterial strains. Alpha and beta-hemolytic activities, in particular, are associated with increased pathogenicity. Ramteke et al. (2015) investigated hemolytic bacteria in drinking water sources in North India, finding that 42% of isolates exhibited hemolytic activity. They correlated this with increased virulence potential and the ability to cause extraintestinal infections. The presence of hemolytic bacteria in off-campus water sources thus represents a significant health risk for student populations.

The Staphylococcus counts in our study, particularly high in CAM (1.45x10^2 CFU/ml) and TROY (1.15x10^2 CFU/ml) hostels, exceed safety thresholds. Onanuga and Temedie (2011) reported similar findings in their study of waterholes in Amassoma, Nigeria, where Staphylococcus aureus counts ranged from 1.0x10^2 to 2.8x10^3 CFU/ml. The presence of Staphylococcus in drinking water sources is particularly concerning due to its ability to form biofilms and produce toxins, potentially leading to a range of infections in consumers.

Pseudomonas, detected only in CAM hostel (2.2x10^2 CFU/ml), presents a unique challenge due to its intrinsic antibiotic resistance and biofilm-forming capabilities. Vaz-Moreira et al. (2012) highlighted the ubiquity of Pseudomonas in water systems and its role as a reservoir of antibiotic-resistance genes. The localized presence of Pseudomonas in our study suggests potential contamination sources or favorable growth conditions in specific water systems, necessitating targeted investigation and remediation efforts.

The detection of Vibrio in TROY (2.15x10^2 CFU/ml) and ONU (1.7x10^2 CFU/ml) hostels is particularly alarming given the genus' association with severe gastrointestinal diseases. Mishra et al. (2020) reported the presence of potentially pathogenic Vibrio species in drinking water sources in coastal areas of Odisha, India, emphasizing the need for vigilant monitoring and treatment strategies. The occurrence of Vibrio in off-campus water sources underscores the potential for waterborne disease outbreaks among student populations.

Salmonella, detected in CAM (2.1x10^2 CFU/ml) and LAN (1.3x10^2 CFU/ml) hostels, poses a significant public health risk. Akinde et al. (2011) isolated Salmonella species from borehole water samples in Akure, Nigeria, highlighting the widespread nature of this pathogen in groundwater sources. The presence of Salmonella in off-campus water supplies necessitates immediate intervention to prevent potential outbreaks of typhoid fever and other salmonellosis among students.

The varying microbial profiles across different hostels suggest localized contamination sources or differences in water treatment efficacy. This heterogeneity aligns with findings by Okullo et al. (2017) in their study of drinking water quality in Greater Kampala, Uganda, where they observed significant variations in microbial contamination levels between different water sources and distribution points. Such variability emphasizes the need for comprehensive monitoring programs that account for spatial and temporal variations in water quality.

The co-occurrence of antibiotic resistance, biofilm formation, and hemolytic activity in several isolates presents a complex challenge for water safety management. This multifaceted virulence profile has been observed in other studies, such as that by Samie et al. (2012) in South Africa, where they found a correlation between biofilm formation, antibiotic resistance, and the presence of virulence genes in waterborne pathogens. The synergistic effect of these factors could enhance bacterial survival in water systems and increase the risk of infection among consumers.

The presence of multidrug-resistant strains in off-campus water sources raises concerns about the potential for community-acquired infections resistant to conventional treatments. Machado and Bordalo (2014) reported similar findings in their study of antibiotic resistance in urban watersheds, highlighting the role of aquatic environments as reservoirs and conduits for the spread of antibiotic resistance. The proximity of student populations to these water sources could facilitate the rapid dissemination of resistant bacteria, potentially leading to challenging infection scenarios.

The detection of biofilm-forming bacteria in the water samples underscores the limitations of traditional water treatment methods. Cherchi and Gu (2011) demonstrated the increased resistance of biofilm-associated bacteria to chlorine disinfection, a common treatment method in many water systems. This resistance mechanism could explain the persistence of potentially pathogenic bacteria in treated water supplies, necessitating the exploration of alternative or complementary disinfection strategies for off-campus water sources.

The hemolytic activity observed in many isolates suggests an elevated virulence potential that could exacerbate the health risks associated with consuming contaminated water. Rajesh et al. (2012) correlated hemolytic activity with increased pathogenicity in environmental isolates from the Cochin estuary in India. The presence of hemolytic bacteria in off-campus water supplies could lead to more severe clinical outcomes in cases of waterborne infections among students.

The diversity of bacterial genera identified in this study reflects the complex microbial ecology of water distribution systems. Proctor and Hammes (2015) emphasized the importance of understanding microbial community dynamics in drinking water systems for effective management and risk assessment. The presence of opportunistic pathogens alongside commensal bacteria in off-campus water sources highlights the delicate balance between microbial populations and the potential for shifts towards more pathogenic communities under favorable conditions.

The antibiotic resistance profiles observed in this study mirror global trends in the emergence of antimicrobial resistance in environmental isolates. Wellington et al. (2013) reviewed the role of the environment in the dissemination of antibiotic resistance genes, highlighting water systems as critical vectors. The detection of multidrug-resistant strains in off-campus water sources suggests that these environments may serve as reservoirs for antibiotic resistance genes, potentially contributing to the broader issue of antimicrobial resistance in clinical settings.

**CONCLUSIONS**

In conclusion, the antibiogram profiles, biofilm formation capabilities, and hemolytic activities of bacteria isolated from off-campus water sources reveal a complex landscape of microbial contamination and potential health risks. The presence of antibiotic-resistant, biofilm-forming, and hemolytic bacteria in these water supplies necessitates urgent attention from public health authorities and water management agencies. Implementing comprehensive monitoring programs, exploring advanced water treatment technologies, and educating student populations about water safety are crucial steps in mitigating the risks associated with these microbial contaminants. Future research should focus on elucidating the mechanisms of antibiotic resistance transfer in these environments and developing targeted interventions to disrupt biofilm formation and neutralize virulence factors in water distribution systems serving off-campus student populations.

**RECOMMENDATION**

**Enhanced Water Quality Monitoring:** It is crucial to implement regular and comprehensive water quality testing for borehole sources. This should include screening for microbial contaminants, assessing bacterial resistance profiles, and identifying any potential sources of contamination. Routine monitoring will help in early detection of contamination and enable timely interventions to ensure safe drinking water.

**Improved Water Treatment Practices:** The study highlights the presence of biofilm-forming bacteria, which suggests that current water treatment practices may be insufficient. It is recommended to enhance water purification methods by incorporating advanced treatment technologies such as UV disinfection, ozonation, or filtration systems capable of removing biofilms and resistant bacteria. Regular maintenance and cleaning of borehole infrastructure should also be prioritized to prevent biofilm formation.

**Public Health Education:** Educating the student population and surrounding communities about water safety is essential. Awareness programs should focus on the importance of proper water handling, the risks associated with untreated water, and practical methods for household water treatment. Encouraging practices such as boiling water before consumption or using water purifiers can significantly reduce health risks.

**Strengthening Regulatory Frameworks:** There is a need for stricter enforcement of water safety regulations and standards. Regulatory bodies should ensure that water suppliers and operators adhere to established guidelines for water quality. This includes mandatory reporting of water test results and immediate actions to address any violations of water safety standards.

**Addressing Antibiotic Resistance:** Given the observed antibiotic resistance in waterborne bacteria, it is important to address the broader issue of antibiotic misuse and overuse. Efforts should be made to reduce the use of antibiotics in agriculture and aquaculture and to promote responsible antibiotic practices in healthcare settings. Additionally, incorporating antibiotic susceptibility testing into routine water quality assessments can help manage the risks associated with resistant bacteria.

**Infrastructure Improvement:** Investment in improving the infrastructure of borehole water systems is necessary. This includes ensuring proper construction, regular maintenance, and timely repairs to prevent contamination. Implementing measures to protect boreholes from environmental contaminants, such as ensuring proper sanitation and waste management practices around the water sources, can also help maintain water quality.

**Community Involvement:** Engaging local communities in water management efforts can enhance the effectiveness of interventions. Community-based programs that involve residents in monitoring, reporting, and maintaining water sources can foster a sense of ownership and responsibility. Collaborating with local stakeholders can also facilitate the development of tailored solutions that address specific needs and challenges.

**Research and Development:** Continued research into water contamination and treatment methods is essential for developing innovative solutions. Support for research into new water purification technologies, better understanding of microbial dynamics in water systems, and effective strategies for controlling biofilm formation can contribute to improved water safety and public health outcomes.

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