https://dx.doi.org/10.4314/dujopas.v10i2a.29
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 2a June 2024*

Exploring Microbial Analysis of Tap Water in University of Benin Halls of Residence: Implications for Health and Sustainability

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Abstract

This study aimed to analyze the bacterial and fungal composition of tap water found in the University of Benin's halls of residence, located in Benin City, Nigeria. Standard methods were employed to isolate, enumerate, characterize, and identify the bacterial and fungal isolates.
Clean and safe water access is crucial for human well-being and sustainable development. This study examines the bacterial and fungal contaminants in the tap water obtainable in the halls of residence at the University of Benin in Benin City, Nigeria. Standard methods were employed to isolate, enumerate, characterize, and identify the bacterial and fungal isolates. Microbial analysis revealed the presence of bacteria such as Klebsiella spp., Staphylococcus spp., Streptococcus spp., Bacillus spp., and Pseudomonas aeruginosa, along with potential pathogens like Escherichia coli in all samples. Fungi detected included Aspergillus niger, Fusarium spp., and Mucor spp. The detection of E. coli suggests waterborne disease risks, emphasising the need for water treatment and hygiene. Maintaining microbiological quality is crucial for a healthy learning environment. Variations across residential halls indicate the importance of infrastructure and maintenance. Compliance with regulations and standards, infrastructure upgrades, education on water safety, collaboration with health authorities, and continuous monitoring are recommended to ensure safe tap water for students in university residences.

Keywords: Microbial Analysis, Tap Water Quality, University Residences, Waterborne Diseases, Public Health

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INTRODUCTION

Clean and accessible water is essential for the well-being of all living organisms and plays a pivotal role in advancing sustainable development (UN World Water Development Report 2021; https://datatopics.worldbank.org/sdgatlas/archive/2020/goal-6-clean-water-and-sanitation/). However, despite their widespread availability, water sources can serve as reservoirs for pathogenic microorganisms, posing significant health risks to communities, especially in developing nations (Pandey *et al*., 2014). In Nigeria, water quality issues are prevalent, affecting both urban and rural areas. The University of Benin, located at Ugbowo road, Benin City, Edo state, Nigeria, faces similar challenges concerning water quality within its infrastructure.

One of the few available studies addressing water quality at the University of Benin was conducted by Agatemor and Okolo in 2007. Their assessment of the university's water supply system provided valuable insights into the microbiological and physicochemical aspects of water quality. However, the literature on water quality specifically within the university's halls of residence remains limited, warranting further investigation.

Globally, ensuring access to clean and safe water is a priority outlined by organizations such as the World Health Organization and UNICEF (2021). Despite efforts to improve water quality, challenges persist, particularly in regions with inadequate infrastructure and limited resources. Inadequate access to quality water contributes significantly to waterborne diseases, which disproportionately affect developing nations (World Health Organization, 2009; GBD, 2016; Prüss-Ustün *et al*., 2019; Charles *et al*., 2020). Shockingly, more than two billion people do not have access to clean and safe water, underscoring the need for immediate action to address water-related health (World Health Organization and UNICEF, 2021).

Contaminated water sources, often due to human or animal waste, introduce pathogenic microorganisms into the water supply, leading to disease transmission (Cabral *et al*., 2010; Pandey *et al*., 2014). Waterborne diseases can range from mild gastroenteritis to severe and life-threatening conditions such as dysentery, hepatitis, and typhoid fever. The primary source of pathogens that cause these diseases is faecal contamination, including bacteria, viruses, and protozoa, which perpetuate the transmission of disease (Centers for Disease Control and Prevention, 2006; Van Seventer *et al*., 2016).

This study aimed to address this gap in the literature by analyzing the bacterial and fungal composition of tap water in the University of Benin's halls of residence. By employing standard methods for microbial analysis, we seek to identify potential health risks associated with waterborne pathogens and assess the overall quality of tap water within the university community. The investigation aimed to contribute towards a better understanding of water quality issues in the university context and propose strategies to ensure safe water access for students and staff. This research has implications for public health and sustainability, highlighting the importance of addressing water quality challenges in educational institutions.

MATERIALS AND METHODS

Study Area

The study was conducted in 2023 at the University of Benin located at Ugbowo road, Benin City, Edo state, Nigeria. The study included Halls 1-4.

Sample Collection
Sterile bottles were used to collect water samples while maintaining strict aseptic measures during the collection process. The collected samples were placed immediately in a cooler with a controlled low temperature to ensure sample integrity was maintained. The samples were used within two hours of collection.

Culture Media
For bacteriological analysis, Nutrient Agar was used. For mycological analysis, Potato Dextrose Agar was employed. The culture media were prepared following the manufacturer's instructions.

Isolation of Microorganisms
To isolate microorganisms, 10 mL of tap water samples from each hall of residence were measured into sterile beakers. Subsequently, 90 ml of sterilized distilled water was added to create a 10-1 suspension. Serial dilution was then carried out through tenfold serial dilutions, resulting in dilutions up to 10-10. Aliquots of 0.1 mL from appropriate dilutions were plated on nutrient agar for bacterial isolation and on potato dextrose agar for fungal isolation. This process was replicated four times for each set of samples. In control experiments, sterile water replaced the 0.1 mL from the serial dilution. Incubation conditions for nutrient agar plates were set at 37 °C for 24–48 hours, while potato dextrose agar plates were incubated at room temperature (28 °C) for 72 hours. Following incubation, the discrete colonies were counted (Kumar et al., 2022).

Enumeration of Microorganisms
The method outlined by Holt *et al*. (2000) for estimating bacterial and fungal counts was adopted for enumerating the total viable counts of the isolates. The mean colony counts on nutrient agar and potato dextrose plates for each specific dilution were utilized to determine the total viable count for the samples, presented as colony-forming units per millilitre (CFU/mL).

Characterization and Identification of Bacterial Isolates
Bacterial isolates were subcultured from single isolated colonies on nutrient agar and incubated at 37 °C for 24 hours to obtain pure cultures. The bacterial strains that had been purified were stored in a refrigerated environment at a temperature of 4 °C. Cultural characteristics on nutrient agar plates were visually assessed for size, shape, surface, opacity, texture, elevation, and pigmentation using light microscopy. The Gram staining technique, as outlined by Tripathi and Sapra (2023), was employed to differentiate between Gram-positive and Gram-negative bacterial cells.

Additional Tests for Bacterial Identification: Further characterization and identification of Bacterial isolates was conducted through various tests:

The Spore Staining Test uses a malachite green solution and microscopic examination to detect the presence of spores; the Acid-fast Stain, which applies the Ziehl-Neelsen staining method to identify acid-fast organisms (Faith, 2022); the Motility Test, which involves incubating isolates on motility agar at 28 ± 2 °C for 24 hours to observe spreading growth outside the stab line; the Coagulase Test, which assesses clotting by mixing the test organism with human plasma to observe clotting (Cheesbrough, 2000); the Catalase Production Test, which uses hydrogen peroxide to determine the presence of catalase in bacterial isolates; the Citrate Utilization Test, which observes colour changes in Simmon citrate broth; and the Methyl Red

and Voges-Proskauer Tests (MR-VP), which determine the presence of methyl red and Voges-Proskauer reactions in glucose phosphate medium. Additionally, the Indole Test is used to identify indole-producing bacteria by adding Kovac's reagent to peptone water (Cheesbrough, 2000), while the Oxidase Test uses filter paper soaked in an oxidase reagent to detect oxidase production in bacterial isolates (Cheesbrough, 2000). The Urease Production Test observes colour changes in urea slopes to identify urease production (Cheesbrough, 2000), while the Voges-Proskauer Test aims to demonstrate bacterial fermentation of carbohydrates and the production of acetyl methyl carbinol.

Identification of Fungal Isolates

A lactophenol blue stain was applied to mycelium on a glass slide to identify fungal isolates. The slide was observed under a microscope for characteristics including spore-bearing structures, septated hyphae, and the presence of spores (Mokobi, 2022).

Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 27. Data on Microbial Counts in Sample Sources were subjected to analysis of variance (ANOVA) to assess treatment effects. Tukey's Honestly Significant Difference (HSD) test was employed for posthoc comparisons to identify significant differences among treatment means (García Cañedo, *et al*., 2017).

RESULTS

Six bacterial species were isolated from the bacteriological experiment (B1, B2, B3, B4, B5, and B6), while three fungal species were isolated from the mycological experiment (F1, F2, and F3). The results of the cultural characteristics, morphological characteristics, and biochemical tests for the organisms isolated from the water samples collected from different halls of residence (B1, B2, B3, B4, B5, and B6) are presented in Table 1.

The cultural characteristics of the isolated organisms varied across the different halls of residence. The shapes of the colonies were predominantly round or circular. The colours of the colonies ranged from milky white to creamy and light green. In terms of size, the colonies were observed to be either large or small. Elevation characteristics ranged from flat to raised, and the transparency of the colonies varied from transparent to opaque.

Gram staining results revealed a mix of positive and negative outcomes among the isolated bacteria. Cell types ranged from rod-shaped to cocci, with distinct arrangements such as single cells, chains, clusters, and short chains. Notably, the cell arrangement of one isolate indicated a bipolar, thick rod morphology.

A series of biochemical tests were conducted to characterize the isolated organisms further. The urease test yielded varying results, with some bacterial isolates showing positive (+) reactions and others negative (-). Similarly, the indole test exhibited a mix of positive and negative outcomes among the bacterial isolates. Acid-fast staining tests yielded negative results for all the bacterial isolates. The citrate utilization test revealed positive reactions for most of the bacterial isolates. The catalase production test exhibited positive reactions in the majority of the isolated bacteria. In terms of motility, a few isolates displayed positive reactions, while others were non-motile. Results for the methyl red, Voges-Proskauer, coagulase, starch hydrolysis, and oxidase tests varied among the different isolates.

Fermentation tests were conducted to assess the utilization of various sugars by the isolated organisms. The results demonstrated that the ability to ferment specific sugars varied among the isolates. Galactose, maltose, mannitol, fructose, glucose, and lactose were tested, with differing fermentation outcomes.

Based on the results of the various tests conducted, the probable identities of the isolated organisms were determined. The isolates were identified as *Klebsiella* spp., *Staphylococcus* spp., *Escherichia coli*, *Streptococcus* spp., *Bacillus* spp., and *Pseudomonas aeruginosa*.

Table 1: The cultural characteristics, morphological characteristics, and biochemical tests of the organisms isolated.

Cultural characteristics	B1	B2	B3	B4	B5	B6
Shape	Round	Circular	Round	Round	Circular	Round
Colour	Milky	White	Milky	Milky	Creamy	light green
Size	Large	Large	Large	Small	Large	Large
Elevation	Flat	Flat	Flat	Raised	Raised	Raised
Transparency	Transparent	Opaque	Opaque	Translucent	Opaque	Opaque
Morphological characteristics						
Gram stain	Negative	Positive	Negative	Negative	Positive	Negative
Cell type	Rod	Cocci	Rod	Rod	Bipolar Rod	Thick rod
Cell arrangement	Single	Chains	Cluster	Cluster	Short Chains	Single
Biochemical test						
Urease	+	-	+	-	+	+
Indole	+	-	+	+	-	+
Acid-fast	-	-	-	-	-	-
Citrate utilization	+	-	+	+	+	+
Catalase production	+	+	+	+	+	+
Motility	-	-	+	+	+	+
Methyl red	-	-	+	-	+	+
Vogues prauskeur	+	-	+	+	+	+
Coagulase test	-	+	-	-	-	-
Starch hydrolysis	+	+	+	-	+	+
Oxidase test	-	-	-	-	-	-
Fermentation test						
Galactose	+	+	+	+	+	+
Maltose	+	+	-	+	-	-
Manitol	-	+	+	+	+	+
Fructose	+	+	+	+	+	-
Glucose	+	-	+	-	-	+
Lactose	+	-	+	-	-	+
Probable Identity	*Klebsiella* spp.	*Staphyloccocus* spp.	*Escherichia coli*	*Streptococcus* spp.	*Bacillus* spp.	*Pseudomonas aeruginosa*

The general microbial counts in the water samples collected from different halls of residence are presented in Table 2. The total heterotrophic bacterial count (THBC), total coliform count (TCC), and the total heterotrophic fungus count (THFC) respectively showed significant differences among the difference halls of residences. For Hall 1, the THBC was recorded at 6.4 × 10^5 colony-forming units per millilitre (CFU/mL). The TCC was measured as 56 most probable numbers per 100 millilitres (MPN/100 mL). Additionally, the THFC was determined to be 1.1 × 10^5 CFU/mL. In Hall 2, the THBC was observed at 7.1 × 10^5 cfu/ml. The TCC exhibited a count of 72 MPN/100 mL, while the THFC was measured to be 3.5 × 10^5 CFU/mL. For Hall 3, the THBC was found to be 3.8 × 10^5 CFU/mL. The TCC was recorded at 81 MPN/100 mL, and the THFC was measured at 1.4 × 10^5 CFU/mL. Lastly, in Hall 4, the THBC was determined to be 5.1 × 10^5 CFU/mL. The TCC yielded a count of 33 MPN/100 mL, and the THFC was measured to be 2.5 × 10^5 CFU/mL.

Table 2: Microbial Counts in Sample Sources

Sample source	THBC (10^5 CFU/mL)	TCC (nMPN/100 mL)	THFC (10^5 CFU/mL)
Hall 1	6.4	56	1.1
Hall 2	7.1	72	3.5
Hall 3	3.8	81	1.4
Hall 4	4.51	33	2.5
Lsd	0.6857	5.999	0.2804

Keys: THBC = Total Heterotrophic Bacterial Count; TCC = Total Coliform Count; THFC = Total Heterotrophic Fungal Count; MPN = Most Probable Number. $\alpha = 0.05$.

The distribution of microbial isolates in the water samples collected from different halls of residence is summarized in Table 3. In Hall 1, *Staphylococcus* spp., *Escherichia coli, Pseudomonas aeruginosa, Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. were present, while *Klebsiella* spp., *Streptococcus* spp., and *Bacillus* spp. were absent. *Klebsiella* spp., *Escherichia coli,* Streptococcus spp., and *Pseudomonas aeruginosa* were Hall 2, while *Staphylococcus* spp., *Bacillus* spp., *Aspergillus* spp., Penicillium spp., and *Fusarium* spp. were absent. *Klebsiella* spp., *Escherichia coli, Pseudomonas aeruginosa, Aspergillus* spp., Penicillium spp., and *Fusarium* spp. were detected in Hall 3, while *Staphylococcus* spp. and *Streptococcus* spp. were absent. *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Pseudomonas aeruginosa*, and *Aspergillus* spp. were found in Hall 4, while *Escherichia coli*, *Klebsiella* spp., *Penicillium* spp., and Fusarium spp. were absent.

Table 3: Distribution of microbial isolates in the sampling points

Sample source	B1	B2	B3	B4	B5	B6	F1	F2	F3
Hall 1	-	+	+	-	-	+	+	+	+
Hall2	+	-	+	+	-	+	-	-	-
Hall 3	+	-	+	-	+	+	+	+	+
Hall 4	-	+	-	+	+	+	+	-	-

Keys: + = present, - = absent

The result in Table 4 summarizes the cultural and microscopic characteristics of the fungal isolates obtained from the water samples. Isolate 1 had black, fluffy colonies with a yellow reverse side on the culture medium. In the microscopic examination, it was observed that the isolate had simple septate and branched conidia organized in chains. These characteristics were consistent with those of *Aspergillus niger*, a commonly known fungal species.

Isolate 2 had white and cottony mycelium on the culture medium. In the microscopic examination, multi-segmented canoe-like spores accompanied by branched and segmented conidiophores were observed. These features aligned with the characteristics of *Fusarium* spp., which is a notable fungal genus.

Isolate 3 exhibited thick and abundant cottony mycelium and a white reverse side on the culture medium. Microscopic analysis highlighted non-septate hyphae with sporangia containing black sporangiospores. The presence of a columella separated by a septum was also observed. These traits were indicative of *Mucor* spp., a genus of fungi.
Each isolate exhibited unique cultural and microscopic features, allowing for their identification to the genus or species level.

Table 4: Fungi cultural and microscopic characteristics

Isolate	Cultural	Microscopic Examination	Fungal isolates
1	Black fluffy colonies with yellow reverse side	Simple septate and branched conidia observed in chains	*Aspergillus niger*
2	White and cottony Mycelium	Multi-segmented canoe-like spores with branched and segmented conidiophores	*Fusarium* spp
3	Thick abundant cottony mycelium with white reverse side	Non-septate hyphae with sporangium containing black sporangiospores, columella separated by a septum	*Mucor* spp

Table 5 provides information on microbiological limits and their associated health impacts. It presents permissible levels of various microbial parameters and their potential health implications.

When the Total Coliform Count exceeds the permitted level of 10 CFU/mL, it can cause health risks due to the presence of potentially pathogenic microorganisms associated with faecal contamination.

For Thermo Tolerant Coliform or Escherichia coli, the acceptable level is 0 CFU/100 mL. If this level is exceeded in water samples, it indicates the presence of thermotolerant coliforms, which can lead to serious health issues such as urinary tract infections, bacteremia, meningitis, diarrhoea (a major cause of morbidity and mortality among children), acute renal failure, and hemolytic anaemia.

The maximum allowable level for faecal Streptococcus is 0 CFU/100 mL. The presence of faecal streptococcus indicates recent faecal contamination, which can pose risks to water quality and human health.

For Clostridium perfringens spores in water samples, a 0 CFU/100 mL limit is established. The presence of these spores indicates intermittent faecal contamination, indicating potential health risks associated with waterborne pathogens.

Table 5: Maximum permitted levels of microbiological limits and health impacts

Parameter	Unit	Maximum Permitted Levels	Health Impact
Total Coliform Count	CFU/mL	10	Indication of faecal contamination
Thermo tolerant Coliform or *E. coli*	CFU/100 mL	0	Urinary tract infections, bacteremia, meningitis, diarrhoea, (one of the main causes of morbidity and mortality among children), acute renal failure and hemolytic anemia
Faecal streptococcus	CFU/100 mL	0	Indication of recent faecal contamination
Clostridium Perfringensspore	CFU/100 mL	0	Index of intermittent faecal contamination

DISCUSSION

The findings of our study provide valuable insights into the microbial composition of tap water in the University of Benin's halls of residence, contributing to the existing literature on

water quality in the region. Our analysis builds upon the work of Agatemor and Okolo (2007), who conducted a comprehensive assessment of the university's water supply system, focusing on microbiological and physicochemical aspects. While their study provided important baseline data on water quality within the university, our research examined specifically into the microbial contaminants present in tap water within the halls of residence.

Our study identified a range of bacterial and fungal species in the tap water samples collected from different halls of residence. The presence of *E. coli*, *Klebsiella* spp., S*taphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Pseudomonas aeruginosa*, *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. highlights the potential health risks associated with waterborne pathogens and sustainability concerns associated with water quality in shared water sources. These findings align with previous research highlighting the prevalence of microbial contaminants in water sources, with inadequate sanitation infrastructure (Agatemor & Okolo, 2007; Pandey *et al*., 2014; Bengoechea *et al*., 2019; Chang *et al*., 2021; Guerra *et al*., 2022).

Our findings are also in line with global concerns regarding water quality and public health. The high total coliform counts (TCC) found in all halls and the presence of E. coli in all samples indicate faecal contamination, which is consistent with studies highlighting the prevalence of waterborne pathogens in inadequately treated water sources (Centers for Disease Control and Prevention, 2006; GBD, 2016; Van Seventer et al., 2016; Prüss-Ustün *et al*., 2019; Charles *et al*., 2020). Previous research has shown that contaminated water sources often contain potential pathogens such as *Klebsiella* spp., *Staphylococcus* spp., and Pseudomonas aeruginosa, which were also detected in our samples (Pandey *et al*., 2014; Cabral *et al*., 2010). These waterborne diseases associated with faecal contamination can result in a range of health issues, from mild gastroenteritis to severe and potentially life-threatening conditions (Centers for Disease Control and Prevention, 2006; Van Seventer *et al*., 2016).

Comparing our results with those of Agatemor and Okolo (2007), we observe similar trends in microbial contamination within the university's water supply system. Both studies identified *E. coli* and other pathogenic bacteria, indicating persistent challenges in maintaining water quality standards. The presence of these contaminants underscores the importance of implementing effective water treatment and sanitation measures to ensure safe drinking water for the university community.

The variations in microbial counts across different halls of residence suggest the influence of factors such as water sources, infrastructure, and maintenance practices on water quality. Similar observations have been reported in other studies, highlighting the importance of local conditions and management practices in determining water quality within specific settings (Gupta et al., 2020; Kumar *et al*., 2020; Bilalova *et al*., 2023).

Despite the similarities between our finding and those of Agatemor and Okolo (2007), our study provides additional insights into water quality s specifically within the university's residential halls. By focusing on tap water samples from these locations, we highlight the importance of addressing water quality challenges at the point of consumption. Our results underscore the need for ongoing monitoring and intervention measures to mitigate the risks posed by microbial contaminants in tap water within university residences.

However, it is essential to acknowledge the limitations of our study. For instance, we only focused on microbial analysis without considering chemical contaminants or other water quality parameters, which could provide a more comprehensive understanding of tap water

safety. Additionally, the lack of comprehensive data on the sources of microbial contamination limits our ability to address the root causes of water quality issues. Addressing these limitations and building upon our findings through further research and collaboration will be critical for advancing our understanding of water quality issues within university settings and developing effective strategies for ensuring safe and sustainable water access for all.

Moving forward, interdisciplinary approaches that integrate microbial analysis with chemical monitoring and infrastructure assessment are crucial for promoting public health and sustainability in water management practices. The urgency of addressing the issues identified in this study cannot be overstated. Access to safe drinking water is a fundamental human right, and ensuring the safety of tap water in university residences is essential for the well-being and rights of students. Immediate action is necessary to safeguard the university community against potential health risks arising from microbial contamination.

CONCLUSION

The study conducted a comprehensive microbial analysis of tap water in the University of Benin's halls of residence, with a focus on bacteriological and mycological aspects. The findings of the study reveal alarming levels of contamination, including the presence of Escherichia coli and other potential pathogens, which pose significant health risks to students and staff who rely on this water source. The presence of high Total Coliform Counts (TCC) across all halls, along with the detection of E. coli in all samples, indicates faecal contamination. This highlights the pressing need for action to address waterborne disease risks. The variations in microbial counts across dormitories underscore the impact of water sources, infrastructure, and maintenance practices on water quality. Immediate measures are necessary to tackle these issues and safeguard the health of the university community.

However, the study acknowledges some limitations, such as the lack of comprehensive data on the sources of microbial contamination and the focus solely on microbial analysis without considering chemical contaminants or other water quality parameters. It is essential to address these limitations and take prompt action to ensure access to safe drinking water, a fundamental human right, and protect the well-being of students and staff in university residences.

To ensure that the University of Benin promotes water quality and sustainability, we recommend taking the following specific and actionable steps:
Implement immediate water treatment measures such as chlorination or filtration to reduce microbial contamination in tap water. Establish routine and systematic water quality testing to ensure compliance with microbial limits and prevent outbreaks of waterborne diseases. Initiate educational programs to raise awareness among students about the importance of water safety and proper water-handling practices to minimize health risks. Upgrade water infrastructure and maintenance practices to prevent microbial contamination at its source. Collaborate with local health authorities and water quality experts to gain valuable insights and resources for addressing water-related health concerns. Establish a robust and continuous monitoring system for water quality and microbial levels to track improvements and respond promptly to any emerging issues.

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