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MICROBIAL EVALUATION OF BOREHOLE WATER USED BY SELECTED BAKERIES IN IJEBU-NORTH, OGUN STATE, NIGERIA

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ABSTRACT

Water is one of the most important components of dough systems used to produce bread and other bakery products. Water could serve as a direct or indirect source of microbial contamination during baking, handling, or packaging, thereby affecting the quality and safety of bakery products. This study investigated the presence of faecal coliforms in water sources of selected bakeries in Ijebu North Local Government Area. Water samples were collected from the main sources of three bakeries located in the Ijebu North Area. Physical and microbiological parameters were then investigated and compared to the WHO guidelines. The average temperature and pH ranged between 24.30 and 25.27^{o}C and between 6.40 and 6.73, respectively. The average total bacterial and coliform counts of the water samples collected from the three bakeries were in the ranges of 1.80-3.60 × 10^{4} CFU ml^{-1} and 1.60-2.70 × 10^{4} CFU ml^{-1}, respectively ($p < 0.0001$). Twenty-nine isolates comprising four species (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella spp.) were observed in this study. The study showed that all water samples under analysis were tainted with faecal coliform, which makes the water unsafe for use in bakeries.

Key words: borehole, water, bakeries, faecal coliform

INTRODUCTION

The bakery industry plays a significant role in Nigeria's economy as it provides employment opportunities, contributes to food security, and generates income for the government through taxes (Omiyale, 2021). Baked goods such as bread, cakes, and pastries are staples in the Nigerian diet and are consumed daily by millions of Nigerians (Abideen, 2022). The industry also supports the growth of other sectors such as agriculture and transportation, as it relies on the supply of raw materials such as flour, sugar, and eggs, and requires reliable transportation to deliver products to consumers. In recent years, there has been an increase in the number of small and medium-scale bakeries, which has further contributed to the growth and development of the industry (Nwewi *et al*., 2017). However, ensuring the safety and quality of bakery products is crucial, and this requires strict adherence to good manufacturing practices and the use of clean and safe water in production.

Borehole water is an important source of water for many communities in Nigeria (Raimi *et al*., 2019). However, concerns have been raised about the safety of borehole water due to the potential for contamination with harmful microorganisms (Adesakin *et al*., 2020). In the bakery industry, water is a critical ingredient and is used for various purposes such as mixing dough, washing equipment, and cleaning surfaces (Sinani *et al*., 2014). Therefore, the quality of water used in bakery production is of utmost importance to ensure that the final product is safe for human consumption. Any contamination of the water source could potentially lead to the contamination of the final product and pose a risk to public health (Fida *et al*., 2022). As a result, it is crucial to evaluate the microbial quality of borehole water used by bakeries to ensure that it meets the required standards for safe food production.

Bakery product contamination via water can occur during baking, packaging, and handling (Pacher *et al*., 2022). The consumption of baked goods made with contaminated water can pose significant health risks to the consumers. The presence of pathogenic microorganisms in water used during the production of baked goods can lead to the contamination of the finished product, which can cause foodborne illnesses such as diarrhoea, cholera, and typhoid fever (Fida *et al*., 2022). Additionally, the use of contaminated water can also lead to the growth of spoilage microorganisms in baked goods, which can impact their shelf life and quality (Qian *et al*., 2021). The risk of contamination is even higher in areas with poor sanitation and hygiene practices, where water sources may be contaminated with faecal matter or other contaminants (Fida *et al*., 2022).

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This study investigated the presence of faecal coliforms in water sources of selected bakeries in Ijebu North Local Government Area (LGA). The findings of this study will provide valuable information that can be used to improve water quality and ensure the safety of bakery products.

MATERIALS AND METHODS

Sampling Sites

Three bakeries were selected for this study. The bakeries are located in different areas in Ijebu North LGA. Ijebu North LGA of Ogun State geographically lies between latitudes 6.056′ north and 7.000′ N and longitudes 3.054′ E and 4.000′ east, comprising of towns such as Ago, Awa, and Ijebu Igbo.

Collection of Samples

Borehole water samples were collected from the bakeries in Ago Iwoye, Oru and Awa thrice into sterile sample bottles and immediately transported to the microbiology laboratory of Olabisi Onabanjo University, Ago Iwoye, Ogun State for microbiological analysis. Labels were used to prevent sample misidentification.

Materials Used

The following were the specific materials used for this study: Eosin Methylene Blue agar (EMB), MacConkey agar, *Salmonella shigella* agar, Cetrimide agar, nutrient agar, petri dish, gram staining reagents, biochemical test reagents, inoculating loop, ethanol, industrial bottle, syringes, and aluminum foils.

Physiochemical Analysis of the Water Sample

The water samples were analyzed for major physiochemical parameters which included temperature, pH, colour, taste, and odour. Chemical properties of water involve parameters such as pH and temperature.

Sterilization of Glassware

All glassware were thoroughly washed with detergent, rinsed with clean water and sterilized in an oven at 160°C for at least 2 h. Bench and work tables were sterilized with 95% ethanol before embarking on any experiment and all laboratory work was carried out under aseptic condition.

Media Preparation

All media were prepared according to manufacturer's instruction. The media were sterilized by autoclaving at 121°C for 15 min. then allowed to cool before pouring into sterile petri dishes.

Microbiological Analysis

Serial dilution

After inverting sample bottles several times, the water samples were serially diluted as described below. A 1 ml aliquot from an inch below the surface was added to 9 ml of distilled water in a test tube using an automatic pipette and sterile 1 ml pipette tip, resulting in a 10^{-1} dilution. To obtain a 10^{-2} dilution, a sterile pipette was used to mix the 10^{-1} dilution by drawing 1 ml of the 10^{-1} and placed into another tube containing 9 ml of distilled water. The process was continued for the rest of the diluents.

Total viable count

Adding 1 ml of the sample water into sterile petri dish using a fresh sterile pipette tip for each dilution, the water was distributed evenly in the plates and cooled nutrient agar (37°C) was poured into the plates. The plates were left to cool down and solidify before being placed in the incubator at 37°C for 24 to 48 h. To avoid bubble formation, the plate was gently rotated between the palms after mixing the sample and agar. After dividing the plates into four sections, the clearest zone in each section was visually counted for microbial count. Finally, the total microbial count of each water sample was calculated by multiplying the number of dilution factor with the counted microbial count.

Total coliforms

Adding 1 ml of every dilution of the water sample was done using a fresh sterile pipette tip for each aseptically. Eosin methylene blue and MacConkey agar were used for the process, and 1 ml of every water sample from 10^{-3} and 10^{-4} was mixed with EMB and MacConkey agar. Growth was observed on both EMB and MacConkey agar plates after allowing them to grow for 24 h.

Identification and Characterization

Using the pour plate method, bacteria were obtained from the water sample. Sterile petri dishes were filled with different agar types, each containing 1 ml of the water sample, and were then incubated at 37°C aerobically for 24 h. Once distinct colonies were visible, they were transferred to fresh agar plates and stored on slants for further examination. To identify the microorganisms present, cultural and morphological characteristics were used to differentiate between them, followed by biochemical testing. The isolates were heat-fixed and smeared on a clean, grease-free slide for the gram staining procedure. After staining for 30 sec, the smear was decolorized with acetone, flooded with safranin, and left for 30 sec. The slide was then rinsed with tap water and allowed to dry, and finally examined using oil immersion under an objective lens of ×100 magnification (Allen, 2001).

Biochemical Test

The biochemical tests that were conducted included the indole test, catalase test, oxidase test, and citrate utilization test. For the indole test, the test organism was inoculated into sterile tryptone water and incubated at 37°C for 48 h. After that, a drop of

Kovac's reagent was added, and the solution was observed for colour change. A positive result was indicated by the formation of a pink to red color in the reagent layer. Meanwhile, for the catalase test, a colony of the isolate was picked using an applicator stick on a clean microscope slide and emulsified with a drop of 3% hydrogen peroxide. The presence of gas bubbles indicated a positive catalase reaction. In the oxidase test, filter papers were moistened with drops of oxidase reagent, and each test organism was picked with a sterile loop and smeared on a filter paper. A positive result was indicated by the appearance of a purple color within 5 to 10 sec. Lastly, for the citrate utilization test, sterile citrate media were inoculated with isolates of the test organism using a sterile needle and incubated at 35°C for 24 h. A change in colour of the media from green to blue indicated a positive reaction, showing that the organisms were able to utilize citrate as a sole carbon source. A negative reaction was indicated by trace or no growth, and the medium remained the deep forest green colour of the uninoculated agar (Allen, 2001).

RESULTS AND DISCUSSION

Faecal coliforms are frequent contaminants of undertreated water sources (González-Fernández *et al*., 2021). In bakeries, water has been regarded as one of the main sources of microbial contamination in dough and this contamination can occur at different stages of the baking process including high baking temperatures, handling, and packaging as a result of cross contamination, poor storage conditions or other factors (Minervini *et al*., 2019; Sami *et al*., 2020). This study observed the presence of faecal coliforms in bakery water sources and shows a prevailing relationship between the water sources of the sampled bakeries and possible contamination of the dough pre-baking and contamination of the finished product post baking. These findings supports previous studies that have investigated the presence of faecal coliforms in doughs and bakery products and shows the water used in these bakeries could be a source of enteropathogenic bacteria to consumers (Gómez-Aldapa *et al*., 2013; Khanom *et al*., 2017).

Physiochemical Properties of Water Samples

All bakery water samples collected in this study were clear, odourless, and tasteless (Table 1); however, the pH and temperature varied with sample collection. The observed pH of the Ago bakery samples ranged between 6.20 and 6.80 (6.50±0.30) while the Oru bakery samples ranged between 6.00 and 7.00 (6.40±0.51) and the Awa bakery samples were between 6.4-7.2 (6.73±0.41) (Table 1). Although a few of the water samples observed fell below the WHO guidelines for the pH of drinking water, the water samples were generally within the acceptable limit. The findings of this study are similar to those of Duressa *et al*. (2019), who investigated the drinking water quality from source-to-household tap connections in Ethiopia. Although most of his samples were generally acceptable, it is believed that the geological conditions of the sample areas could have influenced the pH of the water samples. The temperature observed among the water samples also varied with the sample area, but were all within the WHO guidelines for drinking water. Observed temperature of Ago bakery samples ranged from 23.40 to 25°C (24.30°C±0.82), while Oru bakery water samples ranged from 22.40 to 26°C (24.70°C±2.00) and Awa bakery water samples were between 23.70 and 27.2°C (25.27°C±1.80) (Table 1). These findings also agree with those of Daghara *et al*. (2019), who observed normal temperatures among the water samples collected in their study.

Total Bacteria and Coliform Count

The average total bacterial count of the water samples discovered from the three bakeries ranged from 1.80-3.60 × 10^4 CFU ml^{-1}. The highest average total bacterial count of 3.60 × 10^4 was obtained from the Ago bakery, and the lowest bacterial count of 1.80 × 10^4 was got from the Awa bakery water samples (Table 2). The average total coliform count obtained from the water samples ranged from 1.60-2.70 × 10^4 CFU ml^{-1}. The highest coliform count was obtained from the Awa bakery (2.70 × 10^4), while the lowest was obtained from the Oru bakery water samples (1.60 × 10^4) (Table 3). These findings

Table 1: Physicochemical properties of water samples

Sample area	Appearance	Taste	Colour	pH	Temperature (°C)
Ago A	Clear	None	Colourless	6.20	25.00
Ago B	Clear	None	Colourless	6.80	24.50
Ago C	Clear	None	Colourless	6.50	23.40
Oru A	Clear	None	Colourless	6.30	26.00
Oru B	Clear	None	Colourless	7.00	25.70
Oru C	Clear	None	Colourless	6.00	22.40
Awa A	Clear	None	Colourless	6.60	23.70
Awa B	Clear	None	Colourless	7.20	27.20
Awa C	Clear	None	Colourless	6.40	24.90
WHO	Clear	None	Colourless	6.50-8.50	22.00-28.00

Table 2: Total bacteria and coliform count of water samples analyzed from the selected bakeries

Sample area	Average total bacterial count (CFU ml^{-1})	Average total coliform count (CFU ml^{-1})	Significance (*p*-value)
Ago	3.60×10^4	1.90×10^4	< 0.0001
Oru	2.90×10^4	1.60×10^4	
Awa	1.80×10^4	2.70×10^4	
WHO standard	1.00×10^2	1.00×10^2	

agree with those of Akrong *et al*. (2019) in a Ghanaian study investigating the bacteriological quality of drinking water sources, as well as fecal coliforms and other bacteria in water samples used for drinking and other domestic purposes in these communities. Nevertheless, another study by Shams *et al*. (2019) found that bottled drinking water samples were free of pathogenic bacteria or fecal coliforms in Gonabad city of Iran. Sterilization techniques and processing methods utilized in the study areas prior to water usage can have an impact on the existence or non-existence of pathogenic bacteria and fecal coliforms.

Identification of Bacteria Isolates

A total of 29 isolates were characterized and identified from the three analyzed water samples. Four genera of microorganisms were identified in 29 isolates. These genera included *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella* spp. (Table 3). Of the four genera, *Escherichia coli* had the highest percentage of occurrence (31.03%), followed by *Pseudomonas aeruginosa* (27.58%) *and Salmonella* spp. *(*17.24%), as shown in Figure 1. While the findings of this study disagrees with that of Mahmood *et al.* (2019) who recorded negative growth in a study assessing potable water quality in Iraq, but a study carried out by Luvhimbi *et al.* (2022) recorded the *Escherichia coli* to have the highest percentage from samples collected for street taps and household storage in the Limpopo province study.

Table 3: Morphological characteristics of bacteria isolates

Sample area	Gram reaction	Cellular morphology	Colonial morphology	Biochemical test				Probable organism
				Oxidase	Catalase	Indole	Citrate	
Ago	-	Rod	Pink and slightly mucoid	-	+	+	-	*Escherichia coli*
	-	Rod	Translucent, colourless with black center	-	+	-	+	*Salmonella* spp.
	-	Rod	Pink or red, and mucoid	-	+	-	+	*Klebsiella* spp.
	-	Rod	Irregular greenish-blue	+	+	-	+	*Pseudomonas* spp.
Oru	-	Rod	Translucent, colourless with black centre		+	-	+	*Salmonella* spp.
	-	Rod	Pink and slightly mucoid		+	+	-	*Escherichia coli*
	-	Rod	Pink or red, and mucoid		+	-	+	*Klebsiella* spp.
Awa	-	Rod	Irregular greenish-blue		+	-	+	*Pseudomonas* spp.
	-	Rod	Pink and slightly mucoid		+	+	-	*Escherichia coli*
	-	Rod	Pink or red, and mucoid		+	-	+	*Klebsiella* spp.

All probable organisms were oxidase negative except *Pseudomonas* spp.

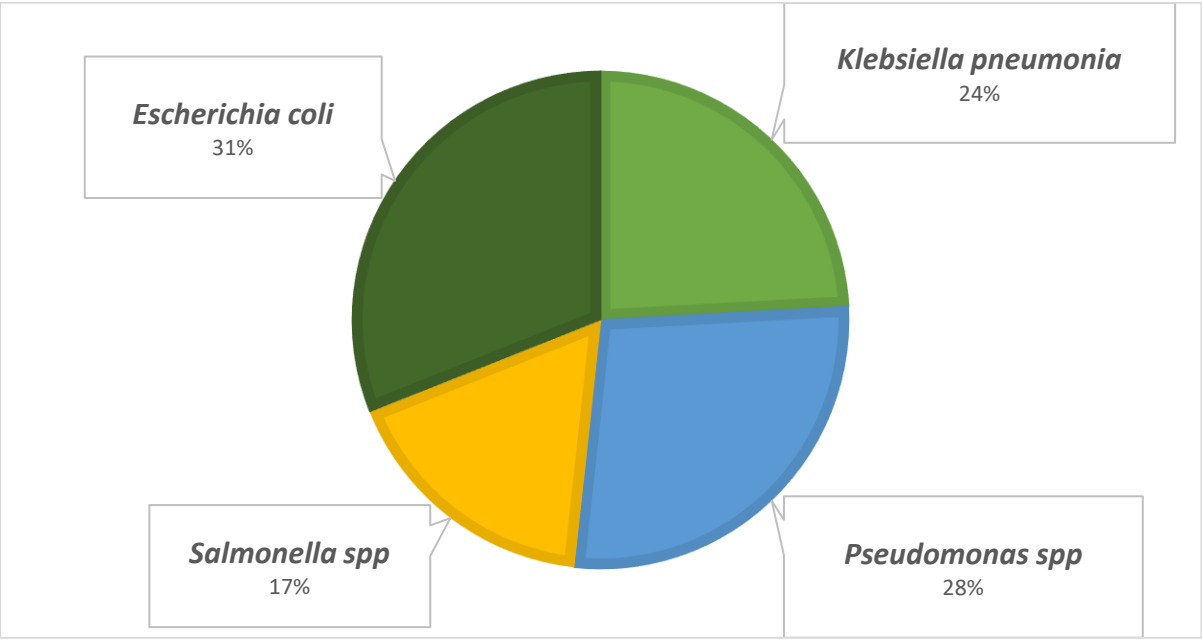

Figure 1: Percentage occurrence of bacterial isolate from the bakery water sample

CONCLUSION

This study has identified the presence of enteric bacteria in the water sources of bakeries. These bacteria have the potential to cause foodborne illnesses in consumers, and therefore, it is imperative to ensure that the water used in bakery processes is free of contamination. Bakery operators should be aware of the importance of regular monitoring of their water sources, and should take necessary measures to prevent contamination. This could include regular testing of the water sources, treatment of water before use, and proper sanitation of equipment and surfaces in contact with water. It is our hope that this study will contribute to improved food safety practices in bakeries and ultimately lead to better health outcomes for consumers.

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