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Microbial Diversity, Seasonal Dynamics and Food Safety Implications of *Wara* in Okada, Edo State, Nigeria

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Abstract: *Wara* is a traditional soft, white, unsalted, and unripened cheese made primarily by the Fulani people of West Africa from cow milk. Despite its widespread consumption, information on the microbiological quality and safety of street-vended *wara* remains limited. This study was conducted to assess the microbiological characteristics of *wara* samples sold in Okada over a four-month period. Eighty (80) *wara* samples were collected aseptically between January and April. They were bulked and homogenized and subjected to microbiological investigation using standard isolation, culturing, and identification procedures. Isolates were characterized through cultural, biochemical, and morphological examinations, and monthly variations in microbial counts were evaluated using one-way analysis of variance (ANOVA). Microbial examination results revealed the presence of bacterial and fungal contaminants. The bacterial isolates included *Bacillus subtilis and Klebsiella pneumoniae*, whereas fungal isolates comprised *Aspergillus niger*, *yeast species*, *Mucor sp*, *Neurospora sp*, and *Sclerotium spp.* Fungal counts increased markedly from 2.40×10^6 CFU/mL in January to 201.60×10^6 CFU/mL in April, with April having significantly higher counts ($p < 0.05$) than the preceding months. Bacterial loads varied between 6.20×10^6 and 117.00×10^6 CFU/mL, reaching a peak in March. The observed fluctuations suggest that environmental conditions, handling practices, and storage environments may influence microbial proliferation in *wara* across different periods of the year. In addition, the microbial loads recorded in this study exceeded recommended limits for ready-to-eat dairy products, indicating potential risks to consumer health. The findings demonstrate that *wara* marketed in Okada is susceptible to contamination and may serve as a vehicle for foodborne microorganisms if produced and handled under inadequate sanitary conditions. Strengthening hygienic practices during processing, transportation, and retailing, alongside routine microbiological surveillance and food safety training for producers and vendors, is recommended to improve product safety and protect public health.

Keywords: *Wara*; Traditional cheese; Microbial load; Food safety; Microbial contamination; Public health; Dairy microbiology.

INTRODUCTION

Cheese locally known as *wara* in Nigerian, is a soft, white, unsalted, and unripen cheese traditionally processed from cow milk by the Fulani tribes who are predominantly cattle rearers in Nigeria (Raheem, 2006; Ogunbanwo *et al*., 2011). *Wara* making started with the Fulani people, but as a result of their nomadic lifestyle, it spread to other parts of the country, such as Kwara, Oyo, Ogun, Ondo, and the Benin Republic (Raheem, 2006). It is obtained by coagulating the casein with rennet or similar enzymes in the presence of lactic acid produced by adding microorganisms (Adegoke *et al*., 1992). Production of lactic acid by the starter flora during manufacture of cheese results in a decrease in the pH of milk, and this, in combination with cooking and stirring, promotes syneresis of the curd and expulsion of whey (Walstra, 1993). *Wara* can also be curdled using the juice extracted from Sodom apple leaves (*Calotropis procera*) or unripe

pawpaw. Cheese is usually stored in its whey and consumed fresh, but this can only last for 3-5 days, after which spoilage occurs. It is sometimes fried and used as a meat substitute in stews and soups, or smoke-dried to enhance its keeping qualities. However, all these increase its shelf life by only a few extra days or a few weeks at best (Belewu *et al*., 2005). Cheese is made mostly from the milk of cows, buffaloes, sheep or goats, and it is an important food component in the healthy diet of human's highly nutritious, rich food and source of protein, peptides, amino acids, vitamins, salt, and essential minerals, including calcium (Augusti, 1996; Belewu and Aina, 2000). However, cheese has been shown to have antimicrobial properties that prevent disease; it has been used as a drug for certain infection when common antimicrobial agents failed. It had also been found that soft cheese has growth inhibitory activity against common bacteria that caused diarrhoea in South West Nigeria (Ibrahim and Falegan, 2013). Microorganisms such as *Streptococci, Lactobacilli, Coliform* bacteria, and some fungi are associated with milk and milk products (Baba *et al.,* 2004; Raheem and Saris, 2009). The consumption of local cheese is of utmost public health importance, and its high consumption rate in Nigeria calls for the periodical examination of cheese produced in different localities.

Wara, a staple cheese-like food in West Africa made from curdled milk, lacks in-depth scientific study despite its widespread consumption. This research aimed to fill this gap by analyzing its nutritional content and microbial safety, offering valuable insights for several reasons (Ajala, 2018)**.** Currently, data on *wara*'s nutritional content is limited and potentially inconsistent due to variations in production methods and ingredients used. *Wara*'s short shelf life and traditional production methods raise concerns about potential microbial contamination, which can pose health risks if not properly addressed. A thorough microbial evaluation would identify the presence and types of microorganisms present in *wara*, informing strategies to improve hygiene practices during production and storage. Thus, improving product quality extends shelf life (reducing waste), benefiting local producers through increased marketability and potential for new *wara*-based products and boosting the West African economy (FAO, 2009).

MATERIALS AND METHODS

Study Location

This study was conducted in Okada town in Ovia North East Local Government Area of Edo state, with an area of 2,301 km^2, which lies on Longitude 5°23'24"E and Latitude 6°43'58"N. It is located in the rainforest region of Southern Nigeria; average annual rainfall of about 2000mm; temperature range 25-35°C; and relative humidity 75-95%. The State is bordered in the north by Kogi State, in the south and east by Delta State, and in the west by Ondo State. The experiment was carried out in the Department of Animal Science laboratory, University of Benin, Benin City, Edo State, Nigeria. (Google Map accessed January, 2025)

Eighty (80) *wara* samples were collected from sellers in Okada in Ovia North East Local Government Area. Ten (10) samples were collected monthly, for four months (January, February, March, and April), and taken immediately to the laboratory for microbial evaluation.

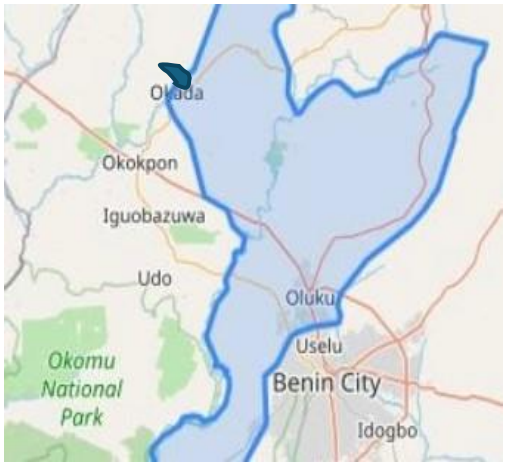

Figure 1: Map of Study Area

Microbial Analysis

Sterilization of Materials

Pipette, conical flask, round bottom flask, and bottles were washed, drained, dried, wrapped in aluminum foil, and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before use. An aseptic working environment was ensured.

Preparation and Sterilization of media

Nutrient Agar (NA)

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 mL of distilled water in a conical flask, corked with cotton wool and foil paper. The medium was placed in an autoclave to sterilize it for 15 mins at 121°C and thereafter allowed to cool.

Potato Dextrose Agar (PDA)

Similarly, 39 g PDA was dissolved in 1000 mL of distilled water in a conical flask with a cork stopper, allowed to completely dissolve, and then sterilized by autoclaving at 121°C for 15 minutes. NA and PDA were allowed to cool then dispensed aseptically into sterile Petri dishes. The petri dishes were covered and allowed to solidify (Cheesebrough, 2006). Finally, the smear was counterstained with safranin for 1 min and washed off. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnification to view the slides. The number of colony-forming units per milliliter (CFU/mL) was calculated (Cappuccino, 2006).
Colony forming units were calculated using a colony counter to determine the microbial load from distinct colonies in Pure

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cultures obtained from plates incubated with samples for one day at 28°C. Microorganisms were identified culturally (shape, elevation, margin, colour and size); morphologically (gram staining, cell type and cell arrangement), and biochemically (catalase, oxidase, urease, citrate utilization and indole tests)

Phenotypic identification of fungi from samples

Isolation and culture were made from a single colony and characterized using cultural features, which include shape, size, colour of spores, reverse colour of fungus, texture of fungus (Samson et al., 2010)

Morphological identification of fungi

A drop of mounting fluid, lactophenol cotton blue solution, was placed on a grease-free slide. Scrapings of the pure isolates were taken from the PDA and transferred onto the fluid using a sterilized, cooled wire needle. It was then pressed gently to enable it mix properly with the stain. Sterile forceps were used to place a cover slip over the slides and blotting paper was also used to wipe excess stain and then examined under low magnification (x10) and high magnification (x40) objectives (Cheesebrough, 2006).

Biochemical identification

Biochemical test was carried out to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

Oxidase test

This is mainly used to differentiate between *Pseudomonas* and other gram-negative rod bacteria. The oxidase test was carried out to identify bacterial species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli*, which are gram positive and gram negative, respectively, were employed as the control. A piece of filter paper using sterilized wire loop; 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-p-phenylenediamine dihydrochloride solution) were added. A positive oxidase test was indicated by purple colouration within 10 seconds.

Urease test

A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37°C for 24 hours. Colour changes from yellow to red-pink confirmed the presence of urease.

Indole production test

Five grams of commercially available peptone broth was dissolved in 1 L of distilled water. The medium was then sterilized by autoclaving at 121°C for 15 minutes. Four millilitres of the medium were dispensed into a sterile test tube, and each of the bacterial isolates was inoculated into the peptone broth. The inoculated media was incubated at 37°C for 24 hours, after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150 mL of amyl alcohol, 10 g of dimethylamino benzaldehyde, and 150 mL of concentrated hydrochloric acid. A positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

Citrate utilization test

Twenty-two grams of commercially prepared Simmon's citrate agar was dissolved in one litre of distilled water and sterilized by autoclaving at 121°C for 15 minutes. The medium was dispensed into test tubes, and the test organism was inoculated by stabbing the medium in the tubes using a sterile straight inoculation wire containing culture. The tubes were incubated at 37°C for approximately 24 hours. A colour change from green to bright blue indicated positive results.

Catalase test

A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. Gas bubble production indicated a positive catalase enzyme.

Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following the manufacturer's instructions, and the prepared media were placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium were inoculated with the test bacterium using a sterile loop, and it was incubated for 18-24 hours. The results were read based on acid or alkaline production in the slant or butt region of the tube, and gas production was confirmed by the presence of cracks or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chart was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

Statistical Analysis

Data collected were subjected to one-way ANOVA using the GENSTAT (12th edition) statistical package, and means were separated using Duncan Multiple Range Test, where significant differences existed and were tested at 5% level of significance.

RESULTS AND DISCUSSION

Microbial load of *wara* samples collected from Okada

The fungal count for *wara* collected in January, February, March, and April were 2.40, 5.00, 11.40, and 201.60 CFU/mL, respectively. The fungal count for April was significantly ($P<0.05$) different from those obtained in January, February, and March. In addition, the bacterial count for the *wara* collected from Okada in January,

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February, March, and April were 79.20, 6.20, 117.00, and 27.20 CFU/mL, respectively, and the values were significantly affected by the months of collection, with March *wara* samples having the significantly highest count/load.

Cultural, morphological and biochemical characteristics of fungi isolates

Some fungi species were isolated and sub-cultured from *wara* samples collected in January/February ($PDA4_1$, $PDA2_2$), February/March (PDA 6_2, $PDA7_2$), January ($PDA9_1$), and January/March ($PDA5_1$) and analyzed. Results showed that $PDA4_1$ and $PDA2_2$ isolates from *wara* belonged to the class *Ascomycete.* It had a dark colour of mycelium on agar plates and dark colour behind; conidia were present but there was the absence of rhizoids and spore colour, as shown in Plates 1 and 5. Its possible identity was the *Aspergillus niger* species. Fungal species isolated from *wara* cultured from petri dishes $PDA6_2$ and $PDA7_2$ were identified to belong to the class *Saccharomyces* and were of the yeast species. They showed a cream front color on the agar plate and a dark cream color behind. They possess a septate nature of hyphae with the absence of conidia, rhizoids, and spore colour.

Mucor sp, belonging to the *Mucoromycetes* class was also identified to belong to the family and it is of the mucor species. It showed a pycnidia black front and a darkish color behind. It possesses a non-septate hypha with a *sporangiospore* kind of spore. There was the absence of conidia, rhizoids, and spore color.

$PDA9_1$ was identified to belong to the class *Ascomycota* and the specie *Neurospora.* It showed a pink wooly frontal colour and a yellowish colour behind. It showed a septate nature of hyphae and an *ascospore* type of spore with the absence of conidia, rhizoid but presence of a black spore colour. $PDA5_1$ was identified to belong to the *Ascomycota* family and a specie of *Sclerotinia*. It showed a white front colour and a cream colour behind plate. It posed a septate nature of hyphae and a sporangiospore type of spore with the presence of conidia and the absence of rhizoids and spore colour.

Cultural, Morphological and Biochemical Characteristics of Bacteria Isolates

Wara samples collected in January/February had bacteria as shown in Petri dish $NA10_2$ and were identified as *Klebsiella pneumonia.* Its cultural features include an irregular shape, cream colour, and small in size, while it morphological features include a rod shape with disperse cells and a negative gram staining property. Although petri-dish $NA8_2$ showed bacteria isolated from samples collected in February/April and was identified to be *Bacillus cereus*, it showed positive for catalase and citrate test but negative for oxidase and urease test. *Bacillus cereus* showed positive for lactase and negative for hydrogen sulphide gas test sub-cultured

Table 1: Microbial Load of *Wara* obtained from Okada in Edo State

Month	January	February	March	April	SEM
Fungi	$2.40 \times 10^{6\ a}$	$5.00 \times 10^{6\ a}$	$11.40 \times 10^{6\ a}$	$201.60 \times 10^{6\ b}$	19.21
Bacterial	$79.20 \times 10^{6\ bc}$	$6.20 \times 10^{6\ a}$	$117.00 \times 10^{6\ c}$	$27.20 \times 10^{6\ ab}$	20.4

Table 2: Cultural and Morphological Characteristics of Fungal Isolates

Parameters	**January/February**	**February/March**	**February/April**	**January**	**January/March**
Colour of mycelium on agar plate	Dark colored growth	Cream front color	Pycnidia black	Pink wooly front colour	White front colour
Colour of plate culture reverse	Dark	Dark cream	Darkish	Yellowish	Cream
Nature of hyphae	Septate	Septate	Non- septate	Septate	Septate
Type of Spore	Conidiospore	Haploid spore	Sporangiospore	Ascospore	Sporangiospore
Conidia	Present	Absent	Absent	Absent	Present
Rhizoids	Absent	Absent	Absent	Absent	Absent
Spore colour	Absent	Absent	Absent	Black	Absent
Appearance of special structure	Dark	Fruiting heads	White	Filamentous	White

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Class of fungi	Ascomycota	Saccharomycetes	Mucoromycetes	Ascomycota	Ascomycota
Possible Identity	*Aspergillus niger*	*Yeast*	*Mucor*	*Neurospora* sp	*Sclerotinia spp*

Plate 1: Ascomycota (*Aspergillus niger*)
Sub-cultured from plate in January/February

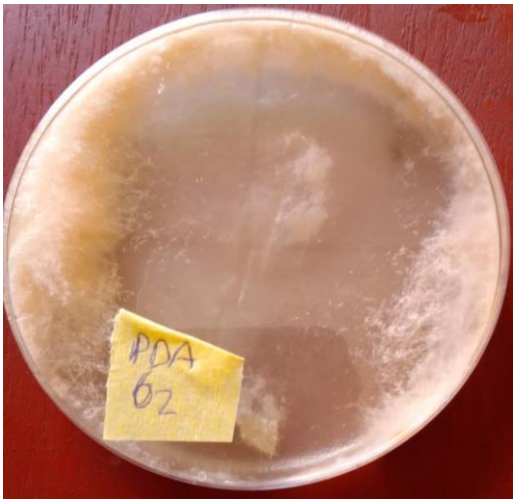

Plate 2: *Saccharomyces* (Yeast)
Sub-cultured from plate in February/March

Plate 4: Ascomycota (*Sclerotinia spp)*
Sub-cultured from plate in January

Plate **3:** Ascomycota *(Neurospora sp)*

Sub-cultured from plate in January/March

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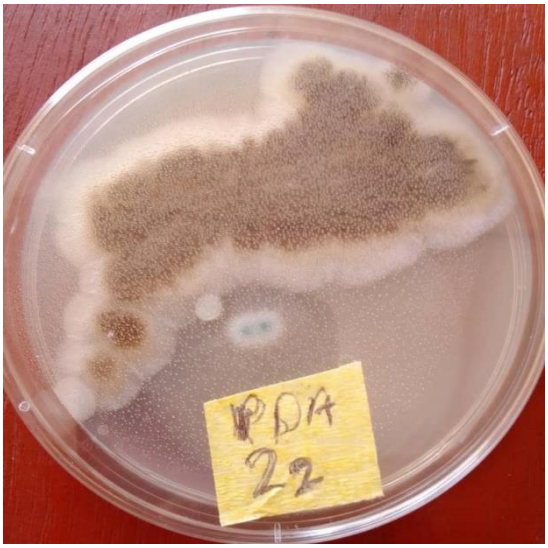

Plate 5: Ascomycota (*Aspergillus niger)* Sub-cultured from plate in from plate in January/February

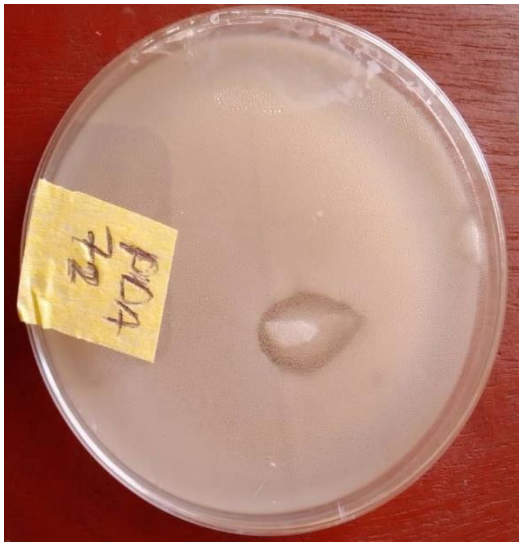

Plate 6: *Saccharomycetes* (Yeast) Sub-cultured February/March

Plate 7*: Klebsiella pneumonia* Sub-cultured from *wara* samples *wara* collected in collected in January/February

Plate 8: *Bacillus cereus* Sub-cultured from February/April

Table 3: Cultural, morphological, and biochemical characteristics of bacteria isolates

Parameters	January/February	February/ April
Cultural		
shape	Irregular	Circular
elevation	Flat	Flat
margin	Entire	Lobate
colour	Cream	Blue
size	Small	Big
Morphological		
gram staining	-	+
cell type	Rod	Rod

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cell arrangement	Disperse	Chains
Biochemical		
catalase	+	+
oxidase	-	+
urease	+	-
indole	-	-
citrate	+	+
Fermentation		
glucose	+	+
lactose	+	-
sucrose	+	+
Gas	+	-
H2S	-	-
possible identity	*Klebsiella pneumonia*	*Bacillus cereus*

DISCUSSION

The present study revealed substantial microbial contamination of street-vended *wara* obtained from Okada, Edo State, as indicated by the high bacterial and fungal loads recorded during this study. The fungal load increased progressively from January (2.40×10^6 CFU/g) to April (201.60×10^6 CFU/g), showing a strong trend of microbial proliferation over time due to contamination during production, handling, and marketing. This progressive increase may be linked to ambient storage conditions, high moisture content, and prolonged exposure to environmental contamination and poor storage practices. Saliu et al. (2014) and Malomo et al. (2020), have reported similar trends in studies on traditional Nigerian cheese (wara), where microbial population increased during storage under non-refrigerated conditions due to continuous exposure to air, dust, handling contamination of surfaces, and poor sanitary practices. The April peak in fungal contamination (201.60×10^6 CFU/mL) merits particular attention. This period coincides with the onset of the rainy season in Southern Nigeria, which is characterized by elevated humidity (75-95%), increased precipitation (approximately 2000 mm annually), and temperatures favourable for fungal development (25-35°C). The environmental conditions likely facilitate spore dissemination and subsequent colonization of *wara* surfaces. This is in line with the works of Akabanda et al. (2010) and Adegoke et al. (1992).

High fungal counts observed in this study are consistent with findings by Uzeh et al. (2006), who reported that *wara* naturally supports fungal growth due to its high moisture content and nutrient composition. Similarly, Laba and Arekemase (2016) observed that traditionally produced *wara* frequently contain fungal loads ranging from 10^6 to 10^8 cfu/g, especially when stored at ambient temperature. These findings collectively suggest that *wara* is highly susceptible to fungal spoilage under retail conditions in open markets.

The bacterial counts recorded in this study fluctuated significantly and exceeded the recommended total bacterial counts of 10^5 cfu/g for fresh cheese (ICMSF, International Commission on Microbiological Specifications for Foods, 2002). The highest value was recorded in March (117.00×10^6 CFU/g) and the lowest in February (6.20×10^6 CFU/g). These variations may be due to differences in raw milk quality, environmental contamination, and hygienic practices during production. Similar variations have been reported in locally produced dairy products, where absence of standardized processing methods leads to inconsistent microbial quality (Adebowale *et al*., 2008; Saliu et al., 2014; Adeleke et al., 2014).

The presence of *Aspergillus niger* as a dominant fungal isolate aligns with previous studies reporting *Aspergillus* species as common contaminants in food processing environments. Uzeh et al. (2006) and Saliu et al. (2014) reported the presence of *Aspergillus niger* in *wara* samples and attributed its occurrence to airborne spores, contaminated equipment, and poor sanitary conditions during processing and storage. Members of the genus *Aspergillus* are widely distributed in the environment and are known to colonize nutrient-rich dairy products under warm and humid conditions. Their presence is of concern because they contribute to spoilage and may adversely affect product quality during storage. These findings align with the observations of Ogunbanwo *et al*. (2004), who reported the presence of *Aspergillus species* and other potential contaminants in *wara* samples. Similarly, Akabanda *et al*. (2010) examined the microbiological characteristics of the Ghanaian traditional fermented milk product, *Nunu*, which shares similarities with *wara* cheese. The authors noted the presence of Aspergillus species, including *A. niger*, in some *Nunu* samples, highlighting the potential for cross-contamination during the production and handling of traditional dairy products.

Yeasts were also isolated from *wara* samples analyzed during this study, indicating active fungal colonization of wara. The presence of yeast in dairy products has been widely documented, where they may contribute to fermentation processes but also accelerate spoilage when uncontrolled. Malomo et al. (2020) reported yeast occurrence in Nigerian unripened cheese and noted that yeast proliferation is strongly influenced by storage temperature and hygiene conditions. Similarly, Uzeh et al. (2006) identified yeast species in *wara* and associated their presence to post-processing contamination and inadequate storage practices.

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The detection of *Mucor* species further reveals January/February environmental contamination of the product. *Mucor* spp. are common soil and air fungi that frequently contaminate food products exposed to unsanitary conditions. Their presence in dairy products has been associated with poor handling practices and exposure to dust and contaminated surfaces (Saliu et al., 2014). The isolation of *Neurospora species* and *Sclerotinia sp* in the present study also indicates environmental exposure, likely occurring during market display and transportation.

The bacterial isolates identified as *Klebsiella pneumoniae* and *Bacillus cereus* are of significant public health importance. The presence of *Klebsiella pneumoniae* suggests contamination from water sources, handlers, or equipment. Similar findings were reported by Uzeh and Imafidon (2022), who detected enteric bacteria including *Klebsiella* species in traditional Nigerian dairy products and linked their occurrence to poor hygienic conditions during production and retailing. Also, Saliu et al. (2014) reported the isolation of *Klebsiella sp* from traditionally processed wara and associated their presence with inadequate hygiene and contamination from environmental sources.

The isolation of *Bacillus cereus* is also consistent with earlier studies on *wara* and related dairy products in Nigeria. Adeleke et al. (2014) reported the presence of *Bacillus* species in wara and explained that contamination originates from raw milk, soil, and processing equipment. *Bacillus cereus* is particularly important because it is a spore-forming organism capable of surviving heat treatment and causing foodborne illness through toxin production under favorable conditions.

The overall microbial profile observed in this study is consistent with findings from multiple authors who have reported that traditional *wara* production systems in Nigeria are highly vulnerable to microbial contamination due to lack of pasteurization, inadequate sanitation, and exposure to environmental contaminants (Uzeh et al., 2006; Saliu et al., 2014; Owolabi et al., 2023). The high microbial loads recorded suggest that contamination occurs at multiple critical points, including milk collection, coagulation, handling, transportation, and retail display.

From a food safety perspective, these findings indicate potential health risks to consumers, particularly in environments where refrigeration is absent. The combination of high fungal loads and presence of opportunistic bacterial pathogens highlights the need for improved hygienic practices, including good manufacturing practices (GMP), use of potable water, proper equipment sanitation, and controlled storage conditions.

CONCLUSION

The present study demonstrated that *wara* marketed in Okada, Edo State, harbours a diverse range of bacterial and fungal microorganisms, including species with potential public health significance. Microbial counts varied across the sampling months, with fungal populations showing a progressive increase and bacterial populations reaching their highest levels during the study period. The observed microbial loads exceeded acceptable limits for ready-to-eat dairy products, indicating potential contamination during production, handling, storage, and marketing. The isolation of organisms such as *Klebsiella pneumoniae* and *Aspergillus niger* raises concerns regarding the microbiological safety of wara and underscores the need for improved sanitary practices throughout the production chain. The temporal variations observed in microbial populations further suggest that environmental conditions and handling practices may influence the microbiological quality of the product. To enhance consumer safety and product quality, strict adherence to good hygienic practices, routine microbiological monitoring, and the implementation of effective quality assurance measures are strongly recommended. Continuous education of producers and vendors on food safety principles is also necessary to minimize contamination risks. Further investigations using molecular identification techniques and broader geographical coverage are recommended to provide a more comprehensive understanding of the microbial ecology and safety status of traditionally produced wara.

Conflict of Interest

There are no conflicts of interest declared by the authors.

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