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Research Article

Isolation and Identification of Bacterial and Fungal Spoilage Organisms in Branded and Unbranded Milk; Consumer Perception of Safety Hazard for Milk

Favour Ogheneoruese Onoharigho[1], Praise Ahuose Ighede[2], Great Iruoghene Edo[3*], Patrick Othuke Akpoghelie[4], Emmanuel Oghenekome Akpoghelie[5]

[1]Faculty of Science, Department of Biochemistry, Elizade University, Ondo, Nigeria
[2]Faculty of Life Sciences, Department of Microbiology, Ambrose Ali University, Ekpoma, Nigeria
[3]Faculty of Science, Department of Petroleum Chemistry, Delta State University of Science & Technology, Ozoro, Nigeria
[4]Faculty of Science, Department of Food Science and Technology, Delta State University of Science and Technology, Ozoro, Nigeria
[5]Faculty of Social Science, Department of Economics, Delta State University, Abraka, Nigeria
E-mail: greatiruo@gmail.com

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Abstract: Background: This research aims to isolate and identify bacterial and fungal spoilage organisms in branded and unbranded milk, as well as to assess the parameters linked to milk safety risk. A total of 30 samples were collected for laboratory testing. For analysis, the samples were inoculated on several mediums. The bacterial and fungal isolates were identified using Gram staining and biochemical identification methods. **Results:** According to the findings, *Klebsiella spp*. and *Escherichia coli* are the most common bacteria found in isolated organisms from branded and unbranded milk (22.6%). Meanwhile, 8.8% of both *Enterococcus faecalis* and *Streptococcus faecalis* were found in the samples. 3.7% of Serratia marcescens and some Streptococcus species were all identified in the samples. At the milk outlets, regularly opening containers to sell milk and predisposing the milk to hand contamination increased the risk of contamination by environmental contaminants. Survey was done with 60 respondents, 37 (61.67%) said they were aware of the health risks associated with milk. Twenty-three people (38.33%) said they knew about diseases linked to drinking contaminated milk. It also discovered that there was no formal food hygiene training for any of the food handlers. However, 11.7% of the workers had advanced training in a variety of fields. **Conclusions:** The microorganisms associated with milk products' spoilage in this study are of economical and public health significance. Some strains of *A. flavus* have been reported to produce potent mycotoxins called ochratoxin that can be harmful to human beings and animals. Cares should be taken on the handling of milk and milk products. And the improved preservation methods should be suggested to enhance the quality of milk products. The findings of the study provide a foundation for developing better milk policies.

***Keywords*:** microbes, hazard, outlets, contaminants, hygiene

1. Introduction

Milk has been long recognized as the most nutrient-dense and nutritionally balanced food due to its abundance in

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vitamins, minerals, vital amino acids, proteins, lipids, and carbohydrates [1]. Nevertheless, milk might be an excellent environment for a variety of bacteria and fungus to grow and thrive, posing a risk to the public's health [2]. Colonization by some food-borne microorganisms improves the nutritional value and shelf life of agricultural products, which are known as food fermentation in some contexts [3]. Colonization changes nutritional quality of the consumables, such as flavor, structure, nutritional value, and at times produces toxic secondary metabolites, which could lead to food deterioration [4]. They are two sides of the same coin when it comes to food colonization. Food spoilage poses a severe threat to our food supply and causes enormous costs [5].

Powdered milk is a manufactured dairy product prepared by drying milk with heat that is milk is dehydrated to about 5% of moisture by evaporation [6]. One reason for drying milk is to preserve it. Also, due to its low moisture content, milk powder has a much longer shelf life than liquid milk, and does not require refrigeration. Meanwhile, because of their chemical composition and nutritional value, all dairy products are susceptible to microbial spoilage [7]. When the raw milk that is used to process milk products is contaminated or cross-contaminated, pathogenic microorganisms are introduced [6].

The tough plant cell wall components that might accumulate in global ecosystems are primarily broken down by fungi. Prior to spoiling, the fungi can be seen in small quantities on or inside the crop, or as survival structures. If the food has been pasteurized, rotting fungi can likewise be introduced to an uninhabited area. According to [1] an important yeast source, which is the teat surface. It should be mentioned that yeast growth during milk storage is rare [8]. Moreover, except for a few fungal species, yeast and molds are not heat-resistant and should be killed after pasteurization. Therefore, during manufacturing, fungal contamination generally occurs after milk heat-treatment [9].

Indeed, it has been widely documented that milk-borne pathogenic bacteria can cause a number of illness epidemics that are lethal [10]. Microorganisms can enter milk and milk products from a number of places, such as the milking equipment, the air quality outside, the soils, the water or moisture content, etc [11]. Numerous types of bacteria are known to live on the surface of the bovine teat [12]. Furthermore, the microbial contamination of milk may be partly attributed to the operators' unsanitary handling. Despite the fact that milk is a very good source of nutrients, it might be dangerous to the health because it contains antimicrobial drug residue and zoonotic viruses. So information are needed to enable the implementation of risk-based measures in milk and milk product outlets [12].

Although contamination of milk and milk products by pathogenic microbes is a problem for worldwide health, its deadly effects on human health in underdeveloped nations, particularly Nigeria, have not yet been fully resolved outside of a few research paper. Dairy goods urgently require a rigorous microbiological inspection for the purpose of quality assurance in order to ensure the safety of consumers, as the components of milk and milk-based products are sufficient to support microbial growth and replication. According to some of the earlier local studies conducted in the state of Edo, raw or unpasteurized milk and milk products could be a very effective means of exposing a large number of individuals to potential microbiological risks, which could ultimately lead to the development of a number of diseases.

Therefore, the safety of milk products in relation to the development of food borne diseases stands as a significant worldwide health issue, particularly in developing countries where production of milk and milk products typically takes place while following poor hygienic measures [13]. So there is needed to identify these micro-organisms especially those that are pathogenic to humans in order to reduce the risk of contamination and infection arising from handling and consumption of milk products. The goal of this research is to isolate and identify bacteria and fungi spoilage organisms in branded and unbranded milk, as well as to determine the factors linked to milk safety risk and the extent of contamination by estimating the viable bacterial count.

2. Materials and methods

2.1 *Geographical description of the study area*

Warri is one of the major industrial cities in Delta State, Nigeria. It is an oil hub in Southern Nigeria and houses a refinery, petrochemical plants, a steel company and an annex of the Delta State Government House. It served as the colonial capital of the then Warri Province. It shares boundaries with Ughelli/Agbarho, Sapele, Okpe, Udu and Uvwie although most of these places, notably Udu, Okpe and Uvwie, have been integrated to the larger cosmopolitan Warri. Warri lies on the Latitude: 5° 33' 15.8364" N and Longitude: 5° 47' 35.5236" E. The area is characterized by a tropical

monsoon climate with a mean annual temperature of 32.8 °C (91.0 °F) and an annual rainfall amount of 2,770 mm (109 in). There are high temperatures of 28 °C (82 °F) and 32 °C (90 °F). The surrounding region is predominantly rainforest, tending to swamplands in some areas.

2.2 *Experimental design and data collection*

The study involved a lab-based investigation to determine the safety of powder milk samples by analyzing the microbial profile and load of branded and unbranded milk. On the 8th of February 2021, thirty samples of milk (fifteen branded and fifteen unbranded) were purchased from six Warri locations (Iruekpen market, creamy milk store, austrock market, market square, davtina supermarket, royal market) aseptically, maintaining the standard procedure of sampling. Samples were transported immediately (approximately within 1 hour) to the laboratory for microbiological analysis (to Department of Petroleum Chemistry, Delta State University of Science and Technology, Ozoro in Nigeria). Prior to the estimation of bacterial and fungal load, samples were subjected serial dilutions up to 10^{-2}. The branded milk which are foreign made, were packaged nylon, plastic and aluminum.

Petri dishes, test tubes, conical flasks, beakers, pipettes and spatulas where sterilized in a hot air oven at 180 °C for two hours, followed by storage at 40 °C.

A pre-tested and pre-structured questionnaire was used to collect information on risk assessment for a specific milk food safety hazard. Information on socio-demographic characteristics were collected (age, residence, marital status, and educational level). Some of the questions included in the questionnaire are as follows:

- What is their perceptions of health risks and awareness of diseases associated with milk consumption?
- How often do they consume milk?
- Which type of containers used in packaging milk do they normally buy?
- Have they ever come across trained workers serving in milk shops?
- What are the Methods and factors they used in checking for the quality of milk? (Question for owners of milk outlets) etc.

The primary investigator instructed all data collectors prior to the start of data collection to guide the participant efficiently, so that the question can be answered accurately. Every day, the acquired data was reviewed for accuracy and reliability. In this survey, the total of people that received the questionnaire was 71, with a response rate of 60 people (84.51%).

2.2.1 *Serial dilution*

Test tubes containing 9 mL of physiological saline (0.9 percent NaCl) were autoclaved before to use. Tenfold serial dilutions of the powdered milk sample were prepared. To make a tenfold dilution, Pipetting was used to repeatedly combine 1 ml of powdered milk samples with 9 ml of saline water in a test tube. Repeated pipetting was used to transfer 1 ml from the 10^{-1} test tube to the 10^{-2} labeled test tube and mix it with 9 ml saline solution. This procedure was repeated for the 10^{-3} and 10^{-4} test tubes [14].

2.2.2 *Culture media preparation*

24.8 g of Sabouraud dextrose agar (Biolab, Nigeria) was weighed and dissolved in 200 ml of distilled water, the mixture was then autoclaved for 15 minutes at 121 °C. The prepared medium was poured into 20 sterile petri dishes, and allowed to cool for a short while, The surface of the table was sterilized with 70% ethanol and cotton wool.

2.2.3 *Preparing spread plates for fungus culture*

Thirty prepared sterile plates were labeled using five plates per samples. Using a micro pipette, 0.1ml of each labeled dilution was pipetted onto each plate as indicated by the diluting factor, which was 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. To sterilize the spreader, a glass spreader was dipped in ethanol and flamed for a few seconds. To avoid contamination, the plate was quickly opened. The inoculum was spread around the surface of the agar with the spreader until no traces of liquid remained. The spreader was re-flamed, and the process was repeated in the next plate, working quickly to avoid airborne organisms contaminating the agar. For growth observation, the plates [20] were placed inversely at room

temperature for 24-72 hours [15].

2.2.4 *Spread plate method for bacterial*

Five Nutrient agar plates (Total viable count) were labeled as raw, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} after serial dilution was completed. 2 ml from the test tubes labeled raw, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were added to the plates, and the drops were spread using a spreader or Pasteur pipettes that dispense 0.02 ml, placing a drop on the corresponding agar strip. The plates were then incubated for 24-48 hours at 37 °C. Finally, the colonies on the plates were counted and noted for future research [16].

2.2.5 *Isolating pure culture*

Individual fungal colonies were removed using a sterile wire loop from any of the plates that represented each colony. A high diluted plate was chosen since it frequently contains pure, well-separated colonies. The chosen colony was carefully picked, the chosen agar plate was rapidly opened next to the Bunsen burner, and the corked bottle's slant was extracted as the bottle's tip was being flamed. A sterile wire loop was used to spread the colony across the sterile agar slant's surface. For several macroscopically detected growths, this process was repeated. The slant tip was re-flamed, covered, and maintained at room temperature for a period of 3 to 7 days. To obtain a pure colony, this was done.

2.2.6 *Slide culture method*

Using sterile petri dishes, one (1) milliliter of sterile water was added to each dish. A U-shaped glass rod was inserted into each petri dish. Sterile slides were positioned on each of the U-shaped glass rods in the petri dishes. Using a scalpel blade, the SDA that had already been prepared was cut into cubes. A sterile wire loop was used to collect and spread four to seven days' worth of fungus growth across the four sides of the SDA. A sterile cover slip was gathered and applied to the inoculum in the petri dish using well-flamed forceps. The plate was covered and left at room temperature for 4 to 7 days before testing in order to observe development.

3. Microscopic and bacteriological examination

3.1 *Morphology characteristics*

The growth pattern, colour, and colony size of each microorganism were noted in order to help identify them. This was done according to [17].

3.2 *Gram staining*

Gram staining was performed using standard procedures on culture that produced growth. The growth on the culture plate was carefully placed on a sterile, grease-free microscope slide and allowed to air dry, fixed by passing it three times over the Bunsen burner's pilot flame. Crystal violet was flooded for 30 seconds on the fixed smear before being washed off with tap water. Lugol's iodine was added and washed away after about 30 seconds, and then acetone was used to quickly decolorize it and wash it away. After that, neutral red (Counter stain) was added and washed away after about 60 seconds. After that, the slides were placed in a draining rack to air dry the smear. After drying, a drop of immersion oil was applied to the smear, which was then examined under the microscope with oil immersion objectives [18].

3.3 *Staining with lactophenol blue*

Using a sterile forceps, the culture's cover slip was carefully taken off, and a drop of lactophenol (lp) was put a clean microscopic slide. The media culture was slowly withdrawn from it. Sterilized cover slips were put on the slide that had a drop of lactophenol blue for microscopical examination. After checking that the lens was properly focused at ×10, it was then seen at ×40 for a better vision [19].

3.4 *Catalase test*

The purpose of the catalase test was to see if the bacteria could degrade hydrogen peroxide by producing the enzyme catalase. A petri dish was filled with a microscopic slide. A small number of bacteria from a 24-hour pure culture were inoculated onto the microscopic slide using a sterile inoculating loop. A drop of 3 percent H_2O_2 was dropped onto the organism on the microscopic slide with a dropper, and the bubble formation was observed immediately [20].

3.5 *Coagulase test*

Staphylococcus aureus is distinguished from other *staphylococci species* using this test. The sterile plasma was diluted in normal saline by a factor of ten. Two separate drops of saline were placed close to both edges of the slide using a sterile wire loop. A colony of the organism was picked with a sterile wire loop and emulsified in both drops of normal saline to make a thick suspension. One of the bacterial suspensions received a drop of sterile plasma, while the other suspension received no plasma (negative control). Within 10 seconds, coarse clumping was observed [21].

3.6 *Indole production test*

The ability of the bacteria to degrade the amino acid tryptophan by the enzyme tryptophanase was determined through an indole production test. 5 mL tryptophan broth was autoclaved at 15 pounds per square inch (psi) at 121 °C in each test tube. A small amount of the experimental bacteria from a 24-hour-old pure culture was inoculated into the tubes using a loop inoculation method with an inoculating loop, and the tubes were incubated for 48 hours at 37 °C using sterile technique. 5 drops of Kovac's reagent were directly added to the tubes to test for indole production [22].

3.7 *Urease test*

This is a crucial test for distinguishing between enterobacteria. Inoculated and incubated at 37 °C in a water bath, the entire surface of Christensen's urea slope. Four hours later, it was examined. A color shift was noticed [23].

3.8 *Blood agar test*

The bacteria's hemolytic capability was determined by using blood agar to produce hemolysins and lyse red blood cells. In a conical flask, blood agar base was prepared and autoclaved at 121 °C, 15 pounds per square inch (psi). The nutrient agar medium was allowed to cool to 45-50 degrees Celsius before adding 5% (vol/vol) sterile defibrinated sheep blood that had been warmed to room temperature and gently mixed to avoid air bubbles. The liquid media was then dispensed into sterile plates and allowed to solidify for a while. A blood agar plate was streaked using sterile technique by picking a loopful colony of 24-hour old pure culture with an inoculating loop using the streak plate method. The plates were then incubated for 24 hours at 37 °C. The plates were examined for gamma, beta, and alpha hemolysis after incubation [24].

3.9 *Citrate utilization test*

The enzyme citrate permease was used to perform a citrate utilization test to distinguish between enteric organisms based on their ability to ferment citrate as a sole source of carbon. Autoclaving at 15 pounds per square inch (psi) 121 °C yielded 2 ml of Simmons citrate agar slants in each vial. Small amounts of the experimental bacteria from a 24-hour-old pure culture were streak inoculated into the vials with an inoculating needle using sterile technique, and the vials were incubated for 48 hours at 37 °C [25].

3.10 *Oxidase test (cytochrome oxidase)*

Pseudomonas, *Neisseria*, *Vibrio*, and *Pasteurella* species, all of which produce oxidase enzymes, are identified using the oxidase test. In a clean petri dish, place a piece of filter paper and 2 or 3 drops of freshly prepared oxidase

reagent. Remove a colony of the test organism with a stick or glass rod (not an oxidized wire loop) and smear it on the filter paper. Within a few seconds, you should notice a blue-purple color developing [26].

3.11 *Hydrogen sulphide (H_2S) production*

The detection of hydrogen sulphide gas (H_2S) is primarily used to aid in the identification of *enterobacteria* and, on rare occasions, to distinguish between Bacteroides and *Bruceila* species. When sulphur-containing amino acids decompose, H_2S is produced [27].

3.12 *Lead acetate paper test to detect H_2S*

When a sensitive method for measuring H_2S generation is needed, the lead acetate paper test is advised. Introduce the test organism into a sterile peptone water or nutrient broth tube or bottle. A lead acetate paper strip used as a stopper is placed in the bottle or tube's neck above the medium. Keep the inoculation medium warm (35-37 °C) and monitor the strip's lower portion every day for blackening [28].

3.13 *Data analysis*

For the prevalence of bacteria in milk, descriptive statistics, frequencies, and percentages were applied to the data collected. The antimicrobial agents' zones of inhibition on bacterial growth were compared using Analysis of Variance (ANOVA).

4. Results

4.1 *Fungal isolates*

On the agar, different genera grew with varied cultural traits (Table 1). These fungi are from the *Penicillium*, *Aspergillus*, *Fusarium*, and *Mucor* genera. *Aspergillus* was the most commonly isolated genus (Table 2). *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* are some of the main species that were Isolated. These *Aspergillus* species were isolated from various samples investigated, along with additional taxa that were also discovered.

4.2 *Bacterial isolates*

Table 3 illustrates the frequency distribution of isolated organisms from branded and unbranded milk, with *Klebsiella spp*. and *Escherichia coli* accounting for 23.8% of the total, followed by *Enterococcus faecalis* and *Streptococcus faecalis* accounting for 9.5 percent each. *Serratia marcescens*, *Streptococcus pyrogen*, *Streptococcus viridias*, *Streptococcus agalatine*, *Streptococcus spirogen*, and *Streptococcus pneumococcus*, on the other hand, each accounted for 4.8 percent of the total. The isolated organism frequency from individual branded and unbranded milk was recorded in Table 4. The Bacteria count of individual branded and unbranded milk was recorded in Table 5. The bacteriological biochemical test was recorded in Table 6, Table 7.

4.3 *Risk assessment on specific milk food safety hazard*

In this study, six communities in the Warri region sold three varieties of branded milk. The branded milk were the most popular brands offered at Iruekpen market, creamy milk shop, austrock market, market square, davtina supermarket, and royal market area (Figure 1). The sort of milk sold in the six settlements didn't differ much.

According to the result, Aluminum containers were found to be the most common packaging containers. Plastic containers were utilized in fewer quantities than aluminum containers. Meanwhile, some stores had milk in nylon (sachet) packaging (Figure 2). The type of container utilized in each location, on the other hand, did not differ significantly.

Overall, milk is primarily transported by motorcycle in the six settlements 47 (78.0 percent). Milk is carried by pick-up trucks and other vehicles 13 (22.0 percent) in addition to motorcycles (Figure 3). The consumer perceptions of

health risks and awareness of diseases associated with milk consumption was recorded in Figure 4. In all of the locations studied, it was discovered that all of the outlets preferred to verify the quality of milk using a casual technique (Table 8).

Table 1. Morphology appearance of the fungal isolates

Samples	Colour	Shape	Elevation	Size
Branded milk (1)	White	Irregular	Elevated	4-8 um
	Yellow-green	Irregular	Elevated	25 um
	Black	Irregular	Elevated	54-64 mm
	White-pink	Oval	Elevated	1-7 cm in diameter
Branded milk (2)	Yellow-green	Oval	Elevated	5-9 um
	Grey-green	Irregular	Elevated	3-5 um
	Gray-green	Irregular	Not elevated	45-65 mm
Branded milk (3)	Black	Irregular	Elevated	1-7 cm in diameter
	Grey-yellow	Oval	Elevated	45-65 mm
	White	Irregular	Elevated	35 um
	Pink	Oval	Elevated	54-64 mmd
	Green	Irregular	Flat	3-7 um
Unbranded milk (1)	Gray-*pink*	Flat	Elevated	55-75 mm
Unbranded milk (2)	Yellow-green	Oval	Elevated	5-8 um
	Grey-yellow	Flat	Elevated	2-4 um
Unbranded milk (3)	Yellow-green	Flat	Elevated	4-9 um

Table 2. Identified fungal

Genus	Specie	A	B	C	D	E	F
Fusarium	*F. Oxysporum*	-	+	-	-	-	+
Penecillium	*P. digitatum*	-	-	+	+	+	-
Mucor	M. hiemalis	-	-	+	+	+	-
Aspergilus bn	*A. flavus*	+	+	+	+	+	+
	A. fumigatus	-	+	-	-	-	+

A = Branded (1), B = Branded (2), C = Branded (3), D = Unbranded Milk (1), E = Unbranded Milk (2), F = Unbranded Milk (3)

Table 3. Frequency distribution of isolated organism from branded and unbranded milk

Organism	Frequency	Percentage
Streptococcus faecalis	3	8.8%
Klebsiella spp	6	22.6%
Streptococcus viridias	1	3.7%
Serratia marcescens	2	3.7%
Streptococcus pyrogen	2	3.7%
Enterococcus faecalis	2	8.8%
Escherichia coli	4	22.6%
Streptococcus pneumcoccus	2	3.7%
Streptococcus agalatine	2	3.7%
Streptococcus spirogen	2	3.7%
Total	28	85%

Table 4. Frequency distribution of isolated organism from individual branded and unbranded milk

Organisms	Branded milk (1)	Branded milk (2)	Branded milk (3)	Unbranded milk (1)	Unbranded milk (2)	Unbranded milk (3)
Streptococcus viridias	1 (10%)	-	1 (10%)	-	2 (20%)	-
Streptococcus pyrogen	1 (10%)	2 (20%)	2 (20%)	1 (10%)	1 (10%)	2 (20%)
Streptococcus faecalis	1 (10%)	1 (10%)	1 (10%)	-	-	2 (20%)
Klebsiella spp	2 (20%)	-	-	1 (10%)	1 (10%)	-
Enterococcus faecalis	1 (10%)	1 (10%)	2 (20%)	2 (20%)	2 (20%)	2 (20%)
Escherichia coli	-	-	-	2 (10%)	1 (10%)	1 (10%)
Serratia marcescens	2 (20%)	1 (10%)	-	-	1 (10%)	-
Streptococcus agalatine	-	-	-	1 (10%)	-	-
Streptococcus pyrogen	1 (10%)	2 (20%)	1 (10%)	2 (20%)	1 (10%)	1 (10%)

This research included a total of 71 people, with a response rate of 60 people (84.51 percent). The majority of milk handlers (70.0%) wore clean garments, according to hygiene observations. Most establishments had restrooms. Only a few outlets had sanitary hand dryers, and there were no basins with flowing hot water. To obtain milk, outlets lacked sanitized equipment (Table 9). There was no hygiene training for all of the staff. However, 11.7% of the workers

obtained further training in areas such as computer science (Table 10). In general, 61.67 percent of local residents were aware of the health concerns linked with milk. Only 38.33 percent of those polled were aware of the ailment linked to milk drinking (Figure 4).

Table 5. Bacteria count of individual branded and unbranded milk

	No of samples	Sample 1 cfu/mL mean count	Sample 2 cfu/mL mean count	Sample 3 cfu/mL mean count	Sample 4 cfu/mL mean count	Sample 5 Cfu/ml mean count
Branded milk(1)	5	6.6×10^9	5.7×10^9	6.6×10^9	6.8×10^9	5.5×10^9
Branded milk (2)	5	5.8×10^9	5.9×10^9	5.4×10^9	5.8×10^9	7.4×10^9
Branded milk (3)	5	1.64×10^9	1.75×10^9	1.54×10^9	1.69×10^9	9.24×10^9
Unbranded milk (1)	5	8.1×10^9	7.7×10^9	6.5×10^9	5.7×10^9	4.6×10^9
Unbranded milk (2)	5	7.8×10^9	6.8×10^9	7.7×10^9	6.9×10^9	8.8×10^9
Unbranded milk (3)	5	1.13×10^9	1.17×10^9	3.7×10^9	2.9×10^9	2.8×10^9

Table 6. Bacteriological biochemical test

Colony character	Morphology by gram stain	Motility	Catalase	Coagulase	Blood agar test	CVBA test	CAMP test	Bile Solubility	Nacl 6.5%	Identified organism
Mucoid pink on Mac Conkey	+ COCCI	NM	-	-	G		-	-	+	*Klebsiella spp.*
Beta on Blood agar	+ COCCI	NM	-	NA	B	+	-	-	NA	*Escherichia. Coli*
Beta on Blood agar	+ COCCI	M	NA	NA	NA	NA	NA	NA	NA	*Pseudomonas spp*
Alpha heam on bld agar	+ COCCI	M	NA	NA	NA	NA	NA	NA	NA	*Enterobacter spp*
Pink colonies on MacConkey	+ COCCI	NM	-	NA	B	NA	+	-	NA	*Strep. viridians*
Mucoid pink on MacConkey	- BACILI	NM	-	NA	A	NA	-	+	NA	*Enterococcus spp*
Yellow colony on MSA	- BACILI	M	NA	NA	NA	NA	NA	NA	NA	*Pseudomonas spp*
Beta on Blood agar	- BACILI	NM	+	NA	NA	NA	NA	NA	NA	*Strep. pneumonia*
Beta on Blood agar	- BACILI	M	+	NA	NA	NA	NA	NA	NA	*Strep. pyrogen*

Key: NA-Not Applicable, A/G-Acid /Gas

Table 7. Bacteriological biochemical test

Colony character	Sugar fermentation										
	Indole	Urease	Citrate	Oxidase	Hydrogen sulphide	Glucose	Lactose	Sucrose	Manitol	Starch Hydrolysis	Identified organism
Mucoid pink on MacConkey	NA	NA	NA	NA	NA	NA	NA	NA	A/G	NA	*Klebsiella spp.*
Beta on Blood agar	NA	NA	-	NA	NA	NA	NA	-	NA	NA	*Escherichia. Coli*
Beta on Blood agar	NA	NA	NA	NA	-	NA	NA	-	NA	NA	*Pseudomonas spp*
Alpha heam on bld agar	NA	-	NA	NA	NA	NA	-	NA	NA	NA	*Enterobacter spp*
Pink colonies on MacConkey	NA	NA	-	NA	NA	NA	-	NA	NA	NA	*Strep. viridians*
Mucoid pink on MacConkey	+	-	A/G	-	-	-	A/G	d	A/G		*Enterococcus spp*
Yellow colony on MSA	-	-	+	A/G	-	A/G	-	-	A/G	A/G	*Pseudomonas spp*
Beta on Blood agar	-	+	NA	-	-	A/G	-	-	A/G	-	*Strep. pneumonia*
Beta on Blood agar	-	-	NA	+	+	-	-	+	-		*Strep. pyrogen*

Key: NA-Not Applicable, A/G-Acid/Gas

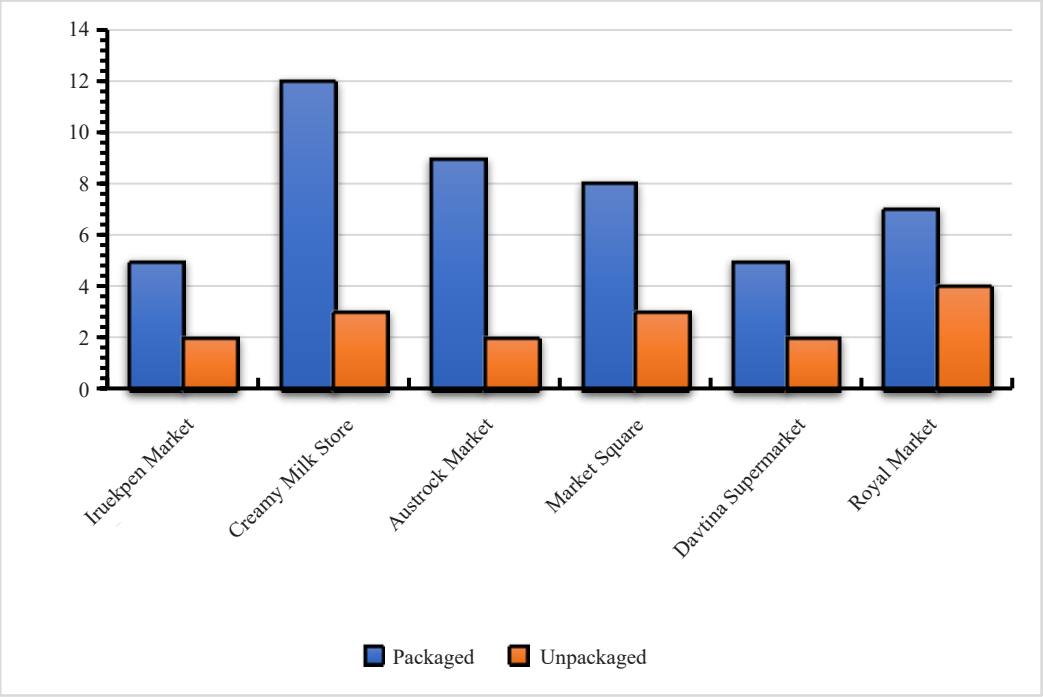

Figure 1. Number of outlets selling milk within the study area

Table 8. Methods and factors used in checking for the quality of milk

Region evaluated	N	Lactometer/Quality checking equipment	Color, smell & viscosity
Iruekpen market	15	0 (0.0%)	15 (100%)
Creamy milk store	15	0 (0.0%)	15 (100%)
Austrock market	15	0 (0.0%)	15 (100%)
Market square	15	0 (0.0%)	15 (100%)
Davtina supermarket	15	0 (0.0%)	15 (100%)
Royal market	15	0 (0.0%)	15 (100%)

Table 9. Hygiene, milk handling practices and training for workers in stores

Hygiene practice	f (n = 60)	%
Workers clothes		
Clean	42	70.0
Dirty	18	30.0
Toilet available		
Available	34	56.7
Not available	26	43.3
Hand basin with running hot water		
Available	0	0.0
Not available	60	100.0
Hygiene hand drier		
Available	13	21.7
Not available	47	78.3
Soap for washing hands		
Available	52	86.7
Not available	8	13.3
Equipment sterilized		
Sterilized	0	5.0
Not sterilized	60	100.0
Cold storage (Freezer)		
Available	39	56.7
Not available	21	48.3

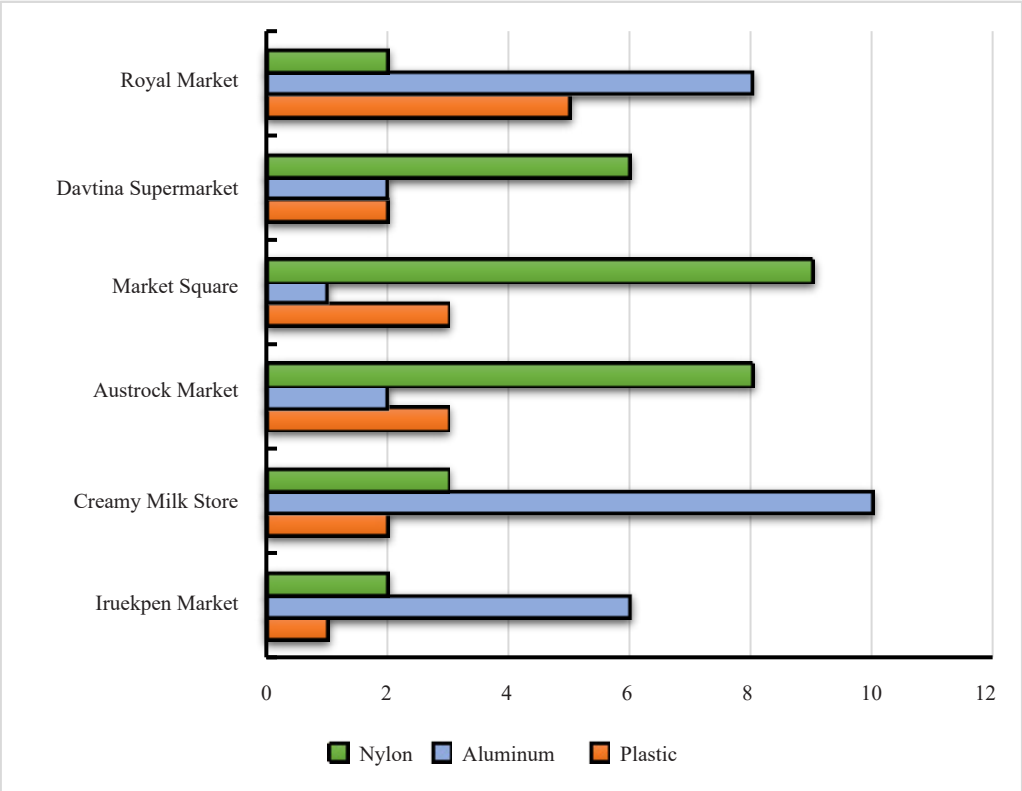

Figure 2. Types of container used in packaging milk

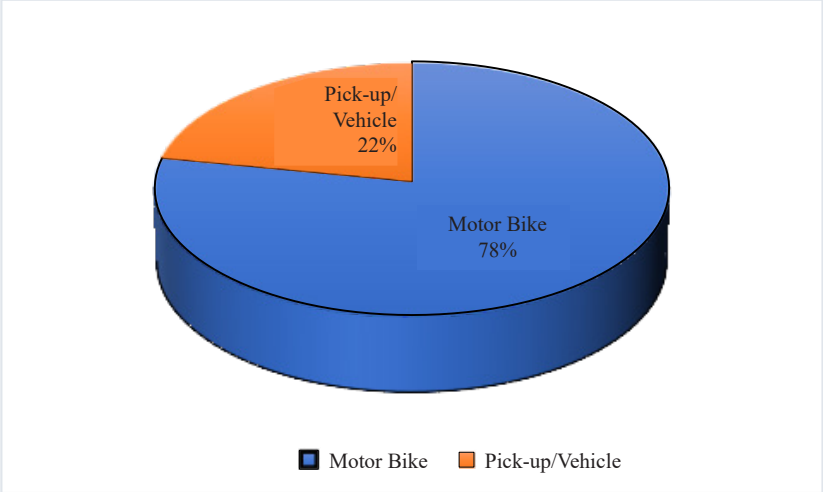

Figure 3. Means of transport used in carrying milk to the outlets

5. Discussion

Many genera and species of fungus were isolated and identified from powder milk [both branded and unbranded] studies, including *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor* specie. These food products may have been contaminated by biotic or abiotic factors [16], which appear to be one of the most important elements that promote fungus development in food [29]. Also, it was recorded that various species of fungus were extracted from each sample

[30]. The Branded [1] stored in open containers was more contaminated than other cultured samples. Also, air-tight storage facilities like sacks, polythene bags, and natural fiber, which are employed by market traders for storage of all types (Personal observation), may have aided fungal development. This is because they can produce a constant rise in the milk's humidity and warmth, favoring fungal development, as described by [29]. The most prevalent fungus that causes food spoiling is *Aspergillus flavus*, which was found from the samples gathered in this study as having the highest prevalence (Table 2). Furthermore, external effects such as insect infestation, wounds, and the presence of foreign matter such as sand, dust, and debris can cause fungal infestation in milk. And any of these sources might have provided some of the discovered fungal species. Similarly, unbranded milk's frequent contact to the outside environment at the time of sale may have contributed in the deposition of fungus spores on them [31]. As a result, when temperature and humidity activate the development processes, spores can germinate on food goods. Insect damage has also been shown to create entrance sites for fungi and help in their fast spread [32]. As a result, the presence of insects may be necessary for infection establishment under some settings. While various fungal species cause food spoiling across the world, it's worth noting that the presence of recognized organisms isolated from these samples has been linked to the production of mycotoxins such as ochratoxin, neurotoxic, and aflatoxin. If formed in certain dietary items, this mycotoxin can cause significant mycotoxicosis in humans and animals. The ochratoxin is a naturally occurring foodborne mycotoxin that may be found in a wide range of agricultural goods and is generated by a number of fungal species and genera, including *Penicillium* and *Aspergillus* [33]. *Aspergillus* species create aflatoxin, which is the most prevalent and dangerous mycotoxin.

Table 10. Training of the workers serving in milk shops on food hygiene training

Training	f	%
Food hygiene training		
Trained	0	0.0
Not trained	60	100
Training for personnel		
Trained	7	11.7
Not trained	53	88.3

In recent years, *Aspergillus* infections have become more prevalent. However, the majority of research has concentrated on *Aspergillus fumigatus*, the genus' most common species. For unknown causes, *Aspergillus flavus* is more abundant in the air than *Aspergillus fumigatus* in some areas and hospitals [34]. *A. flavus* is the second most prevalent cause of invasive *Aspergillosis* after *A. fumigatus*, and it is the most common cause of superficial and systemic infection. In contrast to *A. fumigatus*, outbreaks linked with *A. flavus* tend to be connected with a single or closely similar strain. Furthermore, *A. flavus* creates aflatoxins and aflatoxin is known to be heat stable; heating food products infected with aflatoxin will not eradicate it. This recognized mycotoxin, which may be produced by this fungal species, enters the body by spore inhalation, ingestion, or skin wounds, and this mycosis is more severe in immunocompromised individuals. The spores of this fungus exist in the air, making it very simple for them to penetrate exposed food products (R).

By cultural and staining characteristic and biochemical tests, the bacterial isolates were confirmed as *Streptococcus faecalis*, *Streptococcus pyrogen*, *Klebsiella spp*, *Serratia marcescens*, *Streptococcus viridias*, *Enterococcus faecalis*, *Escherichia coli*, *Streptococcus agalatine* and *Streptococcus spirogen*.

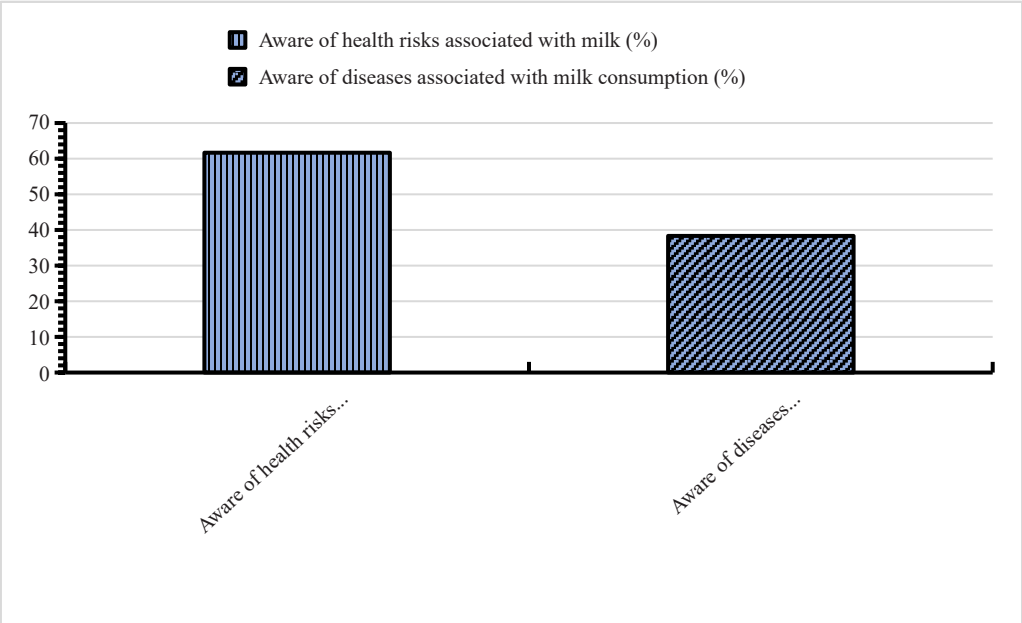

Figure 4. Consumer perceptions of health risks and awareness of diseases associated with milk consumption

The most common bacteria were *Klebsiella spp*. and *Escherichia coli*, followed by *Enterococcus faecalis* and *Streptococcus faecalis*, while the least common were *Serratia marcescens*, *Streptococcus pyrogen*, *Streptococcus viridias*, *Streptococcus agalatine*, *Streptococcus spirogen*, and *Streptococcus*. Previous research has shown that *Escherichia coli* is a common bacteria found in milk. Milk is produced, transported, handled, and sold in completely unsanitary conditions [1].

Klebsiella species are commonly found as normal flora in the human nose, mouth, and gastrointestinal system, but they may also serve as opportunistic human infections. The presence of *Klebsiella spp*. on teat skin during and before milking is linked to fecal contamination of the udder, which can be induced by cow movement through filthy alleyways and holding pens [35]. Meanwhile, hygiene in holding pens and alleyways should be permitted as a key component in preventing *Klebsiella spp. mastitis*.

Also, the study showed the frequency of distribution of isolated organism from individual branded and unbranded milk sold in Warri and those which were associated with *Streptococcus faecalis*, *Streptococcus pyrogen*, *Klebsiella spp*, *Serratia marcescens*, *Streptococcus viridias*, *Enterococcus faecalis*, *Escherichia coli*, *Streptococcus agalatine*, *Streptpcoccus spirogen* and *Streptococcus pneumococcus*. The result is relevant to the fact that *Streptococcus pyrogen* and *Enterococcus faecalis* are the most frequent pathogen isolated across the samples. The first unbranded milk sample showed the presence of *Streptococcus pyrogen*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus agalatine*, *Streptpcoccus spirogen* and *Streptococcus pneumococcus*. The second sample of unbranded milk showed the presence of *Streptococcus pyrogen*, *Escherichia coli*, *Enterococcus faecalis*, *Streptpcoccus spirogen*, and *Klebsiella spp*. The third sample of unbranded milk showed the presence of *Streptococcus faecalis*, *Streptococcus pyrogen*, *Escherichia coli* and *Enterococcus faecalis*. These organisms may survive for a long time in milk before resuming growth when the powder is reconstituted and kept at a suitable temperature [36]. Many infectious illnesses with various etiologies can be transmitted through milk [2]. Milk can be contaminated by TB, brucellosis, and mastitis-infected cows, as well as human carriers of typhoid fever, diphtheria, dysentery, and scarlet fever [37].

5.1 *Risk factors associated with milk food safety hazard*

The majority of milk stores sell both branded and unbranded milk. Few people favored branded milk because they felt it was free of microbial contamination and hence wouldn't make them sick. Due to its lower cost, most customers preferred unbranded milk to packed milk. This discovery was similar to that of [38], who discovered that unbranded

milk is also offered in desired quantities, allowing low-income earners access since they may buy as little as they can afford. The impacts of packaging are numerous. Packaging can be for physical fortification. The milk enclosed in the package may require shield from many things like dust, dirt, microbes and high temperature, etc. Also, milk products are packaged for protection. Food products can be kept safe for a long time, unless Oxygen, water vapor, dust, etc. may not affect them. Some packages contain desiccants or Oxygen absorbers to help extend shelf life. Modified atmospheres or controlled atmospheres are also maintained in some food packages. Keeping the contents clean, fresh, disinfected and safe for the intended shelf life is a primary function of packaging milk. Lastly, milk products are packaged for safety measures [4]. Conversely, packaging of milk products can be bulky, expensive and environmentally damaging over the course of its life cycle. According to [36], there are more pros to buying packaged (branded) milk products than cons, so it is advisable to go for the branded milk products.

In the research region, the most common modes of milk transportation were motor bike (78%) and pick-up/ vehicles (22%). Motorcyclists claimed that due to space constraints, it was simpler to enter all of the planned locations, which were characterized by a bad road network and congestion. They further argued that as compared to automobiles, motorcycles consume extremely little petrol. Use of non-recommended plastic containers for handling milk is known to be fragile and is thought to contribute to high contamination in unbranded milk [39]. Plastic containers are particularly difficult to clean properly since they cannot be sterilized at high temperatures. Furthermore, if equipment is not properly cleaned and milk leftovers are left on damp surfaces, microbial growth will occur, potentially contaminating milk [40]. Plastic containers are known for scratching easily and providing bacteria hiding spots during cleaning. They are also poor heat conductors, preventing effective sterilization [40]. Consumers may be exposed to public health concerns as a result of milk handling issues and a lack of quality assurance for milk delivered to most merchants and households. The usage of plastic containers was linked to elevated coliform levels in raw milk, according to [40]. This is most likely owing to the difficulty of cleaning and sterilizing plastic containers. Meanwhile, the main reason for using aluminum cans is their excellent physical strength, durability, absolute barrier properties to moisture, O_2, and light, absence of flavor or odor, and rigidity [2]. To obtain appropriate closure (i.e., to maintain the integrity of the pack) an elastomeric compound is included in the end seam. Milk powder has a long shelf life when packed in aluminum cans due to their excellent barrier properties. The exchange of moisture and O_2 and the influx of light are not possible. According to [41] powders with a higher fat content are more susceptible to oxidation, and most powders are susceptible to deteriorative effects such as lumping and caking from moisture ingress. With adequately constructed cans, a shelf life in excess of 5 years is realistic, particularly when FMP products have been gas flushed with N_2 to minimize the amount of available O_2. However, national food safety authorities often adopt a conservative approach by reducing the nominated shelf life [42]. Furthermore, the use of sachets (Nylon) is well positioned to exploit the opportunities for convenience food markets. Flexible packages like reduce the volume of traditional packaging such as metal cans, reduce transport costs, reduce the cost of the packaging, and require less material, thus minimizing postconsumer waste [41]. To maintain the quality of the milk powder in such small sachets is a challenge given the very high surface area: volume ratio. A 2-year shelf life for milk powder in portion packs is normally required when distributing in the relatively complex environments of developing countries. In countries with more highly developed economies a maximum shelf life of up to 12 months is common [41]. Stated that the use of sachet (nylon) for the packaging of milk product is not highly advisable because it can be easily penetrated. When it is exposed, it can then be contaminated.

According to the findings, none of the shops studied had any suggested equipment for assessing milk quality, and they were 100 percent certain that color, smell, and viscosity were the only methods they used to decide if the milk was appropriate for sale (Table 8). According to [43], one of the most prominent sources of microbial contamination in milk is milk handling equipment. If equipment is not properly cleaned and milk leftovers are left on damp surfaces, microbial growth will occur, potentially contaminating the milk.

Poor personal hygiene or cross-contamination can make food handlers a source of food-borne illness dissemination. According to the findings, all milk handlers did not get any official food hygiene training and so did not have a high degree of general food hygiene. Lack of training in food hygiene, especially milk handling, may be a contributing factor to unsanitary milk handling by informal sector merchants and, as a result, an increase in milk contamination. These findings were mostly consistent with those of [44]. A total of 37 (61.67 percent) of the 60 people polled said they were aware of the potential health hazards linked with milk. The results of this study on milk-related health hazards correspond favorably to those reported by [37] from Tanzania, who found that 43 (71.67 percent) of the respondents

were informed. In this survey, 23 people (38.33 percent) said they were aware of problems linked to drinking tainted milk. Public awareness about the health and safety of milk in Warri, Delta State, should be regarded as a mandatory project by the competent authorities, because lack of understanding is a barrier to receiving excellent quality milk, as stated.

6. Conclusions

This study analyzed different spoilage organisms in branded and unbranded milk and at the end of the analysis *Aspergilus*, *Fusarium*, *Penicillium Mucor*, *Mucor Streptococcus*, *Enterococcus*, *Escherichia*, and *Klebsiella* species were isolated. It was recorded that the unbranded milk contained more spoilage organism than the branded milk. Also, some of the isolated fungus, such as *Aspergillus*, *Fusarium*, and *Penicillium*, are known to generate poisons. Some strains of *A. flavus* have been reported to produce potent mycotoxins called ochratoxin that can be harmful to human beings and animals. So care should be taken during handling of milk and milk products. Poor storage, the use of plastic containers, the lack of confirmation of milk quality, exposure of milk and the acquisition of raw milk from various sources have all been identified as risk factors for contamination of milk and milk products. To avoid re-infection, contaminated food products should be separated and discarded. This will aid in the reduction of mycoses.

Conflict of interest

The authors have no competing interests.

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