Article

Bacteriological Evaluation of Raw Milk from Selected Dairy Farms in Akoko South West, Ondo State Nigeria

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Abstract: The study was carried out to evaluate the microbiological quality of raw milk produced in Supare-Akoko of Ondo State, Nigeria. Two study areas (Supare and Akungba Villages) both in the Akoko South-West L.G.A. of Ondo State, in Nigeria's South-West geopolitical zone, were selected for the study. The study area served as the raw milk collection points. Raw milk samples were collected with the aid sterile container into the milking bucket and then transported to the laboratory of the Adekunle Ajasin University's department of microbiology in Akungba-Akoko, Ondo state, Nigeria, for further analysis. Several tests such as test for serial dilution, PH measurement, gram staining, and biochemical analysis, to identify and evaluate the microbe species in the raw milk selected for the study. The findings showed that numerous microbe species, including Bacillus, Staphylococcus, Pseudomonas, Alcaligens, Actinomycetes, Enterobacteria, Licheniformis, Listeria, and Streptococcus species, were present in all of the raw milk samples. Therefore, the study recommended that there should be adequate awareness & education on handling of milk in the most hygienic way possible, as well as the establishment of bulk milk tanks and milk processing plants in key locations that could enable appropriate test and production of quality milk before the product is being made available to the general public for consumption.

Keywords: raw milk; bacteriological analysis; human consumption

INTRODUCTION

Milk is extremely perishable because to its complex composition, nutritional value, high water content, and pH that is close to neutral (Maldaner *et al.*, 2012). It is a substance that encourages the growth of microorganisms, particularly bacterial pathogens (Chye *et al.*, 2004). The quantity of bacteria in milk directly affects the quality and safety of dairy products because milk from a healthy udder contains very few bacteria (3x104 cfu/mL), but it can still become contaminated by microorganisms from the environment during milking and milk handling, from water, and from milk equipment (Arcuri *et al.*, 2006). Milk requires specific consideration in its production, processing, marketing, and consumption due to its unique properties. The microbiological quality of milk products may be impacted by a number of variables, including the health of the herd, the cleanliness of the tools and utensils used to obtain them, the conditions of the milking facility, the excretion from an infected animal's udder, and the quality of the water used on the farm (Amaral *et al.*, 2003). Although output is currently increasing with the help of new technologies, many milk producers continue to employ outdated practices that lead to subpar raw milk (Correa et al., 2009). High bacterial contamination in milk usually renders it unfit for further processing because it falls short of the nutritional value, safety, and satisfaction standards set by the consumers (Nanu *et al.*, 2007). Customers have become increasingly demanding regarding the quality and safety of the products offered as a result of the globalization of markets, which includes the accessibility of a variety of imported dairy products (Nada et al., 2012). In an effort to become more competitive, the dairy

 doi:10.20944/preprints202306.1315.v1

business has experienced significant changes that are advantageous to the producer in terms of quality (Gonzalez et al., 2004).

The industry has implemented physico-chemical, microbiological, hygienic, and sanitation techniques to test and confirm the quality of milk (Guerreiro et al., 2005). Thus, the production of high-quality milk should come first for the marketing of value-added products and good-quality end products with a long shelf life. Due to issues like poor hygiene and sanitation during milking and milk handling, dirty water, high ambient temperatures, a lack of cooling facilities, and inadequate infrastructures for milk transportation to the processing facilities, this is typically difficult to accomplish in developing countries (Bille *et al.,* 2000).To prevent or reduce the entry and subsequent growth of microorganisms in milk is a constant challenge for those involved in milk production (O'Connor, 1994). These are primarily a result of the significance of producing milk with good hygienic quality, which is required to milk product of superior quality and prolonged shelf-life thus to provide a safe and wholesome food for the consumers (O'Connor, 1994). In order to add value, ensure safety, extend shelf life, and manage dairy herds, this study will ascertain the microbiological quality of raw milk produced in Supare-Akoko, Ondo State, Nigeria.

MATERIALS AND METHODOLOGY

Study area: The Supare and Akungba Villages in the Akoko South-West L.G.A. of Ondo State, in Nigeria's South-West geopolitical zone, were the sites of the study. The Fulani cattle settlement farms located in the LGAs served as the raw milk collection points. Using a sterile container with a cover, the raw milk samples were immediately collected into the milking bucket. The milk was collected early in the morning (during milking), and it was then transported to the laboratory of the Adekunle Ajasin University's department of microbiology in Akungba-Akoko, Ondo state, Nigeria, for analysis.

Materials used: Sterile cotton, Disposable latex gloves, Sterile agar plates (Petri plates filled with a bacterial food preparation (Nutrient agar), A permanent marker, tubes, autoclave, conical flask, measuring cylinder, Bunsen burner, metal inoculating loop, incubator, glass microscope slide, inoculating loop, distilled water, crystal violet dye, Gram's iodine solution, Alcohol or methlylated spirit, Safranin, Microscope and media Nutrient agar.

Sterilization: The culture media in the conical flasks was sterilized in an autoclave at 121^0C for 15 minutes. To lower the microbial load and prevent contamination, the work benches will be swabbed with 70% ethanol and the inoculating loops will be appropriately flamed using a Bunsen burner.

Measurement of pH: 20 mL of distilled water was used to dilute a 2 mL sample of "Milk" that was measured in a beaker. The measurement was performed using a glass electrode pH meter, and the outcome was recorded.

Serial dilution*:* Each sample of raw milk was measured into a sterile test tube at a rate of one (1) ml. 9 ml of sterile distilled water were combined with the raw milk in each test tube. There will be ten (10) fold serial dilutions performed. Following microbiological procedures, 1 ml of the sample from the first tube was taken and dumped into the second test tube, and 1 ml from the second test tube was collected and discarded into the third test tube. This was carried out up to the tenth dilution, and the final 1ml will be thrown away. Then, Nutrient Agar was evenly distributed with 0.1ml of each dilution from a suitable dilution over the surface of the media plates that had been made (NA). Duplicate plates were created and infected. They were hatched out after bacterial growth at 37°C for 24 h. After 24 h of the incubation period, the plates were checked and observed for bacterial growths.

Gram staining: After looking at the agar plates, the glass slide was flamed for 10 s, then allowed to cool, to classify the isolates into Gram positive and Gram negative bacteria. On a clean, grease-free glass slide, a tiny smear of a young bacterial culture (24 hours old) was applied. After allowing it to air dry, it was heat fixed by being passed through a Bunsen burner flames roughly three times. Crystal violet dye was then applied to the heat-fixed smear for 60 seconds. Clean water was used to swiftly remove the stain. The water was turned off, and Lugol's iodine was applied to the smear for 60 seconds. Using clean water, the iodine was cleaned. The smear was quickly decolored for 20 seconds with 95% ethanol. The slide was cleaned of the stain with clean water, and then left to air dry. First,

 doi:10.20944/preprints202306.1315.v1

an x40 objective lens was used to assess the staining and distribution of the gram-stained bacteria on the slide. Next, an x100 oil immersion objective lens was used to search for the bacteria. Gram-negative bacteria showed up as red or pink, while gram-positive bacteria showed up as purple.

BIOCHEMICAL CHARACTERISTICS

The following biochemical tests were carried out on each air isolate for further characterization and identification:

- **Catalase Production Test:** This was accomplished by applying a loopful of the isolate to the surface of a sterile, dry glass slide, adding a drop of 3% hydrogen peroxide, mixing the slide, and then watching for the formation of bubbles, which is a sign of a successful outcome.
- **Indole Production Test:** This was done using tubes of tryptophan broth. 0.5 mL of Kovac's reagent was added to each tube after the isolates had been inoculated into the medium-filled tubes and had been incubating at 370C for 24 hours in the presence of ambient air. Any time a pink ring appears in a tube, the test was successful.
- **Citrate Utilization Test:** Simmon's citrate agar slant was used in test tubes for this. The slant were lightly inoculated with the isolate and incubated for 24 hours at 370C. When the color turns blue, it means the test was successful..
- **Oxidase Production Test:** Oxidase test strips were used for this. The strips were inserted with a loopful of each isolate, and a color change to blue or purple after 2 minutes signifies a good reaction.
- **Hydrogen Sulphide Production Test:** SIM (Sugar, Indole, and Motility Medium), which contains 20.0 g of pancreatic digested casein, 6.1 g of peptic digested animal tissue, 3.5 g of agar, and 0.2 g of Fe(NH4)2(SO4)26H2O and 0.2 g of Na2S203.5H2O, was used for this. The air isolate was introduced using the stab inoculation method into labeled tubes containing SIM media. The tubes were then incubated at 370°C for 24 hours before being checked for the appearance of black precipitate, a sign of a successful outcome.
- **Starch Hydrolysis:** This was accomplished by incorporating 1% starch into the used medium. A loopful of each air isolate was inoculated into solidified agar medium in a labeled petriplate, incubated at 37^0C for 24 hours, and then each plate was flooded with iodine. A color change to violet was then noticed for each plate, which denotes a successful outcome for starch hydrolysis.
- **Voges-Proskauer Test:** This was transported in test tubes using MRVP broth. A positive result is indicated by the development of a red color, therefore each isolate was inoculated in the medium-containing tube and incubated for 24 hours at 350°C before a few drops of Barritt's reagent were added. The tubes were then gently shaken for 2 minutes and left undisturbed for 15 minutes.
- **Sugar fermentation test**: The ability of an organism to ferment a certain carbohydrate (sugar) added to a medium and produce acid or acid with gas is determined by this principle. The test organism was inoculated in a sugar medium using my method, and it was then incubated for 24 hours at 37°C. Sugar makes up 1% of the media. Andrade's indicator is the one that is used (a solution of acid fuchsin to which is added sodium hydroxide). Yellow is a good color. *Negative:* rosy-pink. In Durham's tube, gas generation may be visualized as bubbles. Lactose, Maltose, Fructose, Galactose, Glucose, Sucrose, and Mannitol are among the sugars used.

RESULTS

Bacterial Count

Total viable counts of bacterial isolates from raw milk are presented in Table 1. The viable count of bacteria present ranged between 3.5×10^5 in Supare-Akoko to 7.5×10^6 in Akungba-Akoko.

 doi:10.20944/preprints202306.1315.v1

Table 1. Total viable counts of bacteria isolates from raw milk.

SAMPLE	BACTERIA (10^4)
SUPARE (SA1)	$4{\cdot}5\times10^4$
SUPARE (SA2)	$3{\cdot}5\times10^5$
SUPARE (SA3)	$6{\cdot}0\times10^6$
AKUNGBA (SB1)	$7{\cdot}0\times10^4$
AKUNGBA (SB2)	$3{\cdot}0\times10^5$
AKUNGBA (SB3)	$7{\cdot}5\times10^6$

pH MEASUREMENT

The pH ranged between 6·5 in Supare-Akoko to 6.9 in Akungba-Akoko (Table 2).

Table 2. pH measurement.

SAMPLE	Ph
SUPARE (SA1)	6·5
SUPARE (SA2)	6·7
SUPARE (SA3)	6·9
AKUNGBA (SB1)	6·7
AKUNGBA (SB2)	6·8
AKUNGBA (SB3)	6·9

Morphological Characterization

Table 3. The morphological characteristics of bacterial isolates from raw milk samples.

Isolate Code	Surface	Colour	Elevation	Edge	Mode of Spread
SA3D	Smooth	Milky	Raised	Irregular	Moderate
SBB	Rough	Creamy	Flat	Undulate	Swarm
SA2B	Irregular	Milky	Raised	Entire	Moderate
SB3A	Rough	Milky	Convex	Entire	Moderate
SBC	Smooth	Milky	Raised	Cremated	Moderate
SB2B	Irregular	Milky	Raised	Cremated	Moderate

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SAA	Smooth	Milky	Raised	Entire	Moderate
SA3A	Smooth	Milky	Flat	Entire	Swarm
SB2C	Rough	Milky	Flat	Entire	Swarm
SB3B	Rough	Creamy	Convex	Entire	Swarm
SA3B	Dry	Creamy	Convex	Entire	Swarm
SB3C	Dry	Creamy	Convex	Undulate	Moderate
SB3D	Irregular	Creamy	Flat	Undulate	Moderate
SB3E	Rough	Creamy	Flat	Undulate	Moderate
SB3F	Smooth	Creamy	Raised	Undulate	Moderate
SB2D	Smooth	Creamy	Raised	Cremated	Moderate
SB2E	Rough	Creamy	Convex	Cremated	Swarm
SA3C	Rough	Creamy	Flat	Cremated	Swarm
SB3G	Rough	Creamy	Flat	Cremated	Swarm
SAB	Dry	Creamy	Convex	Cremated	Swarm
SB3H	Dry	Creamy	Convex	Irregular	Swarm
SB3I	Smooth	Creamy	Convex	Irregular	Swarm
SA3E	Smooth	Creamy	Convex	Irregular	Moderate
SAC	Smooth	Milky	Convex	Irregular	Moderate
SBD	Rough	Milky	Convex	Irregular	Moderate

ANTIBIOTICS SENSITIVITY FOR GRAM POSITIVE

A total of 19 bacterial isolates from milk culture samples have been confirmed to be responsive to Levofloxacin but resistant to streptomycin and norfloxacin. Norfloxacin, Chloramphenicol, Streptomycin, Ciprofloxacin, and others could not kill Staphylococcus aureus. (Table 4).

Table 4. Antibiotics sensitivity for Gram positive bacteria.

PROBABLE ORGANISM	**S**	**NB**	**CH**	**CPX**	**E**	**LEV**	**CN**	**APX**	**RD**	**AMX**
Bacillus cereus	0	0	0	0	0	20	0	0	16	0
Bacillus licheniformis	0	0	0	0	0	16	0	0	20	0

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Bacillus subtilis	0	0	0	0	0	20	0	0	20	0
Actinomyces bovis	0	0	0	0	0	20	0	0	18	0
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0
Streptococci pneumonia	0	0	15	23	20	30	0	0	0	0
Listera grayi	0	0	0	0	0	22	0	0	0	0
Streptococci pyogenes	0	0	0	0	0	23	20	0	24	0
Brevibacterium linens	0	0	0	23	0	0	18	0	14	0
Cellulomonas flavigena	0	0	16	25	0	20	0	0	0	0
Bacillus coagulans	0	0	0	15	0	15	0	0	22	0
Streptococcus epidermis	0	0	0	0	0	18	0	0	20	0
Microbacterium lacticum	0	0	16	24	21	24	19	0	0	0
Bacillus subtilis	0	0	0	0	0	15	0	0	20	0
Streptococcus pneumonia	0	0	0	0	0	20	0	0	20	0
Bacillus cereus	0	0	0	18	0	0	0	0	0	0
Streptococcus pneumonia	0	0	0	0	0	20	0	0	20	0
Bacillus subtilis	0	0	24	25	0	20	0	0	0	0

KEY: Resistant = 0, NB= Norfloxacin (10 mcg), CH= Chloramphenicol (30 mcg), S=Streptomycin (30 mcg), CPX= Ciprofloxacin (10 mcg), E= Erythromycin (30 mcg), LEV= Levofloxacin (20 mcg), CN= Gentamycin (10 mcg), APX=Ampiclox (20 mcg), RD= Rifampicin (20 mcg), AMX= Amoxil (20 mcg).

3.5. ANTIBIOTICS SENSITIVITY FOR GRAM NEGATIVE

All the 6 bacterial isolates (Flavobacterium aquatile,Enterobacter agglomerans,Alcaligens paradoxus,Pseudomonas fluorescens, Enterobacter agglomerans Pseudomonas fluorescens,) were

 doi:10.20944/preprints202306.1315.v1

resistant to Amplicin, Nalidixic acid, and only Enterobacter agglomerans was susceptible to Reflacine (Table 5).

Table 5. Antibiotics sensitivity for Gram negative bacteria.

PROBABLE ORGANISM	AU	CPX	STX	S	PN	CEP	OFX	NA	PEF	CN
Flavobacterium aquatile	0	16	0	0	0	0	0	0	0	22
Enterobacter agglomerans	18	24	0	0	0	20	0	0	0	22
Alcaligenes paradoxus	0	20	15	10	0	0	0	0	0	15
Pseudomonas fluorescens	0	21	25	0	0	0	20	0	0	22
Pseudomonas fluorescens	0	21	25	0	0	0	20	0	0	22
Enterobacter agglomerans	0	0	0	0	0	20	0	0	25	0

KEY: Resistant = 0, CEP= Ceporex (10 mcg), OFX= Tarivid (10 mcg), NA= Nalidixic acid (30 mcg), PEF=Reflacine (10 mcg), CN= Gentamycin (10 mcg), AU= Augmentin (30 mcg), CPX= Ciproflox (10 mcg), STX= Septrin (30 mcg), S= Streptomycin (30 mcg), PN= Amplicin (30 mcg).

DISCUSSIONS

The findings showed that numerous microbe species, including Bacillus, Staphylococcus, Pseudomonas, Alcaligens, Actinomycetes, Enterobacteria, Licheniformis, Listeria, and Streptococcus species, were present in all of the raw milk samples. This observation supports Frazeir and Westhoof's (1998) findings that these microbes thrive in milk and hence compromise its quality. The milk could have been contaminated by the germs from a number of places, including the animal's skin, an infected, dirty udder, the milker's fingers, utensils, and feces. In his study, Olatunji (2009) emphasized the importance of handling milk and milk products hygienically to avoid the risk of microbial contamination.

Table 1 demonstrates that the average viable count across all samples was extremely high (7.5 x 106 cfu/ml), with Akungba Farm having the highest value. This exceeded Bergdoll's recommended upper limit of 1.0 × 105 cfu/ml (1998). According to the author, values exceeding these limits are a sign of major manufacturing hygiene issues. The low quantity of Streptococcus spp observed in these samples indicated that the raw milk examined contained little free fermentable sugar. These are the main bacteria in milk that produce lactic acid and are in charge of the fermentation of carbohydrates into lactic acid. As a result, these microorganisms cause milk to soured (O'Connor and Tripathi, 1992). In this endeavor, the majority of the microbes are either harmful or advantageous. Staphylococcus spp. were the most prevalent pathogenic bacteria discovered in the raw milk samples. Mastitis, a

devastating farm animal disease that frequently affects dairy industries, is linked to the Staphylococcus aureus. Mastitis is an infectious, highly contagious condition that affects the udder of cattle (Olatunji, 2009). In addition, Dalgeish (1995) noted that one cow out of every four has mastitis. Staphylococcus aureus strains that developed a strong exotoxin (Adesanya et al., 1995). Consuming a product that has strains that produce toxins may cause severe gastroenteritis. The majority of Staphylococcus strains belong to the coagulase positive category (Adams and Moss, 1995). Thus, only strains that are coagulase positive are thought to be enterotoxic. Moreover, the presence of Coliforms like Enterobacter is a sign of inadequate hygiene in the water used for milking and the surrounding area. This is consistent with Najib's 2003 report, which found Enterobacter in raw milk along with dirt, manure, sanitary supplies, and human waste. Pseudomonas spp. is a recognized cause of chronic mastitis in animals, and it has been suggested that the presence of these pathogens in developing nations may be related to substandard animal housing and poor milking hygiene practices by the majority of small-scale livestock keepers (Karimuribo et al., 2005; Mdegela et al., 2009). Because many milk vendors transport this product to the nearby town every day by trekking long distances along dusty footpaths in the bush, a higher percentage of milk producers are illiterates who are unaware of the possibility of contamination of milk from the type of water, utensils they use, and even from dung as well as from their own hands and transportation.

Most bacterial isolates tested positive for antibiotic susceptibility to streptomycin and norfloxacin. Only Enterobacter agglomerans was susceptible to Reflacine, while Staphylococcus aureus was resistant to norfloxacin, chloramphenicol, streptomycin, and ciprofloxacin.

CONCLUSION

According to the study, milk sold in Nigeria's Ondo state's Akungba and Supare Districts is severely contaminated, especially with more dangerous bacteria (Staphylococcus aureus and Enterobacter spp.) and less helpful bacteria (Streptococcus spp). The existence of these organisms indicates that the product (milk) has inadequate hygiene standards, and this suggests that locally processed cow milk from Akungba and Supare Akoko may be a cause of illness infection for those who consume these goods. Therefore, the study recommended that there should be adequate awareness & education on handling of milk in the most hygienic way possible, as well as the establishment of bulk milk tanks and milk processing plants in key locations that could enable appropriate test and production of quality milk before the product is being made available to the general public for consumption.

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