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PRESENTS

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**A SYNERGY BETWEEN AGRICULTURAL PRODUCTION,  
TECHNOLOGICAL INNOVATION & MANAGEMENT EFFICIENCY:  
A PANACEA TO ECONOMIC STABILITY  
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## MICROBIOLOGICAL QUALITY OF MILK PRODUCED FROM SOME SETTLEMENTS IN IBARAPA.

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### Abstract

Milk is a food that inherently favours microbial growth and due to its characteristics, several precautions must be taken to prevent contamination during production, processing and consumption. This study was carried out to evaluate microbial quality of raw milk taken from different locations of Jeke, Opeji and Sekere, all in Ibarapa Central Local Government Area, Oyo State. The samples were serially diluted and inoculated using the streaking technique. The heterotrophic, coliform, yeast and mould count of milk produced were  $7.5 \times 10^7$ - $7.2 \times 10^7$  cfu/ml;  $4.3 \times 10^7$ - $2.8 \times 10^7$  cfu/ml and  $1.6 \times 10^7$ - $1.2 \times 10^7$  cfu/ml respectively. Amongst the bacteria isolated were *Bacillus* spp, *Pseudomonas* and for fungi; *Aspergillus* spp and *Fusarium* spp were isolated. Raw milk collected from dairy farm at Jeke had the highest number of fungal isolates. High heterotrophic, coliform, yeast and mould counts obtained indicated poor sanitary condition. Hence, there is need for scrupulous hygienic measures during the handling of milk and product.

**Keywords:** Microbiological, Quality, Milk, Settlement.

### Introduction

Milk is a complex mixture, nutritious with a high level of water, pH close to neutral and highly perishable (Maldaner *et al.*, 2012). Milk production in commercial dairy herds is an economically viable and profitable enterprise. It generated around 973mm days of gainful employment per year in an average dairy herd, though, these dairy herds have helped in increasing the supply of milk (khem chand *et al.*, 2020)

Milk from a healthy udder contains very few numbers of bacteria ( $<3 \times 10^4$  cfu/ml) but may become contaminated by microorganisms from the surrounding environment during milking and milk handling, from water and milk equipments. Thus, the number of bacteria in milk directly influences the quality and safety of dairy products (Arcuri *et al.*, 2006).

Despite the fact that production is growing using modern technology, many milk producers still use non-specialized methods, resulting in raw milk of poor quality. The milk contaminated by high levels of bacteria usually become unsuitable for further processing since it does not meet the consumers' expectations in terms of healthy (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes) (Nanu *et al.*, 2007). The parameters such as physicochemical, microbiological, hygienic and sanitation measures have been deployed by the industry to test and verified the quality of milk (Guerreiro *et al.*, 2005). Pathogens commonly found in milk includes; *Salmonella* species, *E. coli*; *Listeria* and monocytogens. These bacteria are found naturally in the environment where cows could be easily contaminated during milking and also allow high amount of pathogenic microbes to enter the milk. During large-scale pasteurized milk production, unprocessed milk stored in the tank can be contaminated. After the processed milk are distributed, failure to keep milk at refrigeration temperatures can also allow pathogenic microbes to multiply, greatly increasing the risk of illness from consuming the milk. (Gould *et al.*, 2014). Therefore, there is a need to evaluate the micro flora of raw milk produced by subjecting it to microbial quality test.

## **Materials and methods**

### **Study Area and Sample Collection**

The study was conducted at Ibarapa Central Local Government Area, Oyo State, Nigeria. The collection points of raw milk were Sekere, Jeke and Opeji Fulani cow settlement farms, via Igboora. The raw milk sample was directly collected from the cows using sterile Ziploc. The time of collection was between 7.00 -8.30am (during milking), and after, the milk were conveyed to the laboratory for analysis.

### **Media and Instruments**

Medial used are McConkey Agar for enumeration of Coliforms, Nutrient Agar (NA) for purification of cultures and storage in slants and Potato Dextrose Agar (PDA) for isolation of fungi: the media were prepared according to the manufacturers specifications. Material used includes; glass petri dishes, conical flask and wire loops which were all sterilized in hot air oven at 160°C. The benches were sterilized with 95% ethanol to reduce the microbial load on the work bench as described by Cheesebrough, (2003).

### **Samples Inoculation**

Ten (10)mls of the raw milk was measured into a sterile beaker containing 100mls of sterile distilled water. The raw milk in the beaker was mixed and stirred thoroughly with sterile glass rod. One (1)ml of each of the raw milk samples were measured into a sterile test tube containing 9mls of sterile distilled water. Ten (10) fold serial dilutions were carried out (Cheesebrough, 2003). Then from this suitable dilution, 0.1ml of each dilution was uniformly spread over the surface of prepared media plates of NA and PDA. The plates were prepared and inoculated in duplicates. They were incubated for bacteria growth at 37°C for 24hours while PDA was kept at 32°C for 5-7 days for fungal growth. After the expiry of the incubation period, the plates were checked and observed for bacterial fungal growths. The numbers of colonies in each plate were recorded. The culture plates with colonies less than 300, and it's duplicate for each sample were selected. The count obtained was multiplied by the dilution factor and expressed as colony. The total viable, total coliform and total fungal counts were all calculated using the formula;  $TVC (cfu/ml) = \frac{1}{v} \times N \times D$

V =Volume of inoculum

N =Number of colonies counted

D =Dilution factor

### **Identification of Bacteria**

Identification and characterization of bacteria isolates was carried out using standard methods as described by Cheesebrough, (2003). The shape and arrangement of the cells were observed by the use of compound microscope. They were further identified following biochemical analysis which includes; Gram's Staining, Catalase test, oxidase test and sugar fermentation.

### **Identification of Fungi**

Fungal isolates were identified macroscopically and microscopically using lactophenol cotton blue (LCB). A portion of the isolated fungi was collected using sterile needle to tease the fungal cells so that the LCB will penetrate into it, then covered with slips and viewed under the microscope.

## Results

**Table 1: Biochemical Characteristics of Bacteria Isolates From Raw Milk Samples**

| Sample code | Gram reaction | Shape | Catalase | Oxidase | Urease | Citrate | Glucose | Fructose | Sucrose | Galactose | Probable Organism Identified                          |
|-------------|---------------|-------|----------|---------|--------|---------|---------|----------|---------|-----------|-------------------------------------------------------|
| M1          | +             | Rod   | +        | +       | -      | -       | +       | -        | +       | +         | <i>Bacillus pulmilus</i>                              |
| M2          | -             | Rod   | +        | +       | -      | +       | +       | -        | +       | D         | <i>Pseudomonas fluorescence</i>                       |
| M3          | +             | Rod   | +        | +       | -      | -       | +G      | -        | +       | (+)       | <i>Bacillus polymyxa</i> / <i>Pseudomonas</i> species |

Key: +=Positive; +G= Positive with Gas; (+)= Weakly positive, M1= Raw milk from jeke via Igboora, M2= Raw milk from opeji via Igboora, M3= Raw milk from sekere via Igboora

**Table 2: Morphological Characterization of Fungi Isolates From Raw Milk Samples**

| Sample code | Macroscopic Morphology                                                                | Microscopic Morphology<br>Hyphae structure | Conidiophore                                                                      | Probable fungi identification |
|-------------|---------------------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------|
| M1          | Rapid growth rate within three days colonies appear velvety and pale green in colour. | Septate                                    | Conidiophores are long and rough I appearance with pale brownish colour.          | <i>Aspergillus flavus</i>     |
| M2          | Fast growing colonies which appear within four days of growth while aerial mycelium   | Septate                                    | Conidiophores are short single with lateral monophialides in the aerial mycelium. | <i>Fusarium oxyporum</i>      |
| M3          | Mycelia are black with granulate appearance                                           | Septate                                    | Conidiophores are long colourless and smooth.                                     | <i>Aspergillus niger</i>      |

**Keys:** M1= Raw milk from jeke via Igboora, M2= Raw milk from opeji via Igboora, M3= Raw milk from sekere via Igboora

**Table 3: The microbial population in the raw milk samples**

| Colony counts<br>cfu/ml | Total coliform<br>counts | Total<br>counts   | Heterotrophic | Total yeast and mould<br>count |
|-------------------------|--------------------------|-------------------|---------------|--------------------------------|
| M1                      | $4.3 \times 10^7$        | $7.5 \times 10^7$ |               | $1.6 \times 10^7$              |
| M2                      | $4.8 \times 10^7$        | $5.2 \times 10^7$ |               | $1.8 \times 10^7$              |
| M3                      | $2.8 \times 10^7$        | $7.2 \times 10^7$ |               | $1.2 \times 10^7$              |

**Key:** M1= Raw milk from jeke via Igboora, M2= Raw milk from opeji via Igboora, M3= Raw milk from sekere via Igboora

Table 1: The result showed the biochemical characteristics of bacterial isolates from the raw milk samples of which the probable organisms were identified. In sample M1, (from Jeke), *Bacillus pulmilus* was identified which had positive gram reactions, rods in shape, catalase positive and urease negative. In the sugar fermentation test, glucose positive, fructose negative and sucrose positive, had no colour change which showed the absence of acid and air space.

In sample M2 (from Opeji) *Pseudomonas fluorescence* was probable organism identified which had a gram negative rods with a sugar fermentation test that showed the glucose as positive; fructose, maltose and urease appeared negative, citrate positive and galactose appeared delayed positive. There was no colour change and an air space which showed the absence of acid and gas in the sugar used.

In sample M3 (from sekere), the probable organisms identified are *Pseudomonas* species and *Bacillus* species. The catalase and oxidase were positive while urease and citrate were negative. In the sugar fermentation, sucrose was positive, fructose negative, glucose was positive with gas while galactose was weakly positive.

Table 2: The result showed the morphological characterization of fungi isolates from the raw milk samples.

Sample M1 (from Jeke location) have a gram positive reactions which are globose in shape, long and rough conidiophores and probable organisms identified was *Aspergillus* species (*A. flavus*). In Sample M2 (from Opeji location), the gram reaction was negative with a shape of septate hyphae and the probable organisms identifies was *Fusarium* species (*F. oxysporum*). In sample M3, the conidiophores was long, colourless and smooth with septate hyphae.

Table3: The result showed the total microbial population found in the different samples of the raw milk. All the milk samples had total coliform count of which sample M2 (Opeji) had the highest with  $4.8 \times 10^7$  cfu/ml. followed by sample M2 (Jeke) with  $4.3 \times 10^7$  cfu/ml. The highest mean total heterotrophic count was observed in sample M1 (Jeke) which had  $7.5 \times 10^7$  cfu/ml, followed by  $7.2 \times 10^7$  cfu/ml in sample 3 (Sekere) while sample M2 (Opeji) had the least count of  $5.2 \times 10^7$  cfu/ml. The highest mean total yeast and moulds count of  $1.8 \times 10^7$  cfu/ml was observed in sample M2(Opeji) followed by  $1.6 \times 10^7$  cfu/ml in sample M1 while sample M3 had the least total yeast and mould count of  $1.2 \times 10^7$  cfu/ml.

## Discussion

Temperature, chemical composition and total aerobic counts are usually used to determine the overall hygiene, sanitation, quality and storage conditions of raw milk. Sample M2 had the least total heterotrophic counts ( $5.2 \times 10^7$ ) while its total coliform counts and total yeast and mould counts were the highest because of the poor facilities and unhygienic farm settlement under which the cows had been reared. The result of the total viable bacterial count reported in the study is in agreement with Farhan and Salik, (2007).

The presence of high numbers of isolation of these organisms from milk could be attributed to the contamination of udder and teats from the feed, personnel or water *P.flourescence* was present in the milk samples analyzed but

in a consistence proportion with regard to microbiological criteria, this is in accordance to the report of Afif *et al.*, (2008) who reported that the presence of *P. fluorescence* in milk samples is related to environment conditions. The presence of *Bacillus polymyxa* in sample M3 does not necessarily indicate a direct contamination of milk, but considered to be an indicator of poor hygiene and sanitation during milking and post manipulation. Microbial load in *B. polymyxa* obtained in this study is comparable to that obtained by Farhan and Salik, (2007), this is likely to be as a result of the water or grass they are fed with the *Fusarium* species found in sample 2 could be as a result of the environment where cows are reared, this is because, *Fusarium* are widely distributed in the soil is associated with plants.

The total count of bacteria is one of the criteria for evaluating the classification and processing of dairy products (Mhone *et al.*, 2011). The production of high quality milk should therefore be a priority for good quality and products of long shelf life for marketing of value added products therefore, there is need to evaluate microbes associated with milk and determine the factors that necessitated the contamination of milk and its products.

### Conclusion and Recommendation

High bacterial counts and probable fungi isolated organisms are indications of poor sanitary condition and threat to human health. The quality of raw cow milk produced at the farm level in the study area was poor, hence, adequate sanitary measures should be taken at all stages from milking to consumption. Further investigation of the status of the animals health is required especially mastitis and the significance of the effect of containers to ascertain their contribution on microbial quantity. So, application of good veterinary practice, Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practice (GMP) to improve the quality of milk.

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