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## Microbial Assessments of Bulk Milk Before and After Pasteurization in Two Different Dairy Farms in Zaria, Nigeria

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### ABSTRACT

Milk has high nutritional values and an important source of protein, minerals, vitamins and fat in human diet. It provides excellent medium for growth of both pathogenic and spoilage microorganism. This study was carried out with the aim of assessing the microbial quality of bulk milk before and after pasteurization, in two different dairy farms in Zaria. Bulk milk samples were collected on daily basis for a period of 30 days during which 30 samples each, of pasteurized and raw milk were collected from each of the farms making a total of 120 milk samples. The both samples of raw milk before pasteurization and post-pasteurization of farm A and B were analyze for total aerobic, coliform plates counts and *E. coli* isolation rates. Mean results of aerobic plate counts of raw milk for farm A and B before pasteurization were; 5.70 and 6.04 log<sub>10</sub> CFU mL<sup>-1</sup>, respectively. These counts decrease to 3.76 and 4.20 log<sub>10</sub> CFU mL<sup>-1</sup> after pasteurization of the milk. Similarly, the *E. coli* isolation rate for farm A and B were; 20 and 53.3%, respectively. These also decrease to 6.7 and 13.3% after pasteurization. In addition, coliform counts also follow similarly trend. The coliform count in farm A and B were; 5.32 and 6.49 log<sub>10</sub> CFU mL<sup>-1</sup>, respectively. The counts decrease to 3.16 and 3.74 log<sub>10</sub> CFU mL<sup>-1</sup> after pasteurization of the milk. Total coliform and aerobic plates counts before bulk pasteurization in both farms were significantly different (p<0.05), with post-pasteurization values when subjected to paired t-test. The coliform and aerobic plates demonstrated poor hygiene practices and inefficient pasteurization methods in both farms. High isolation rate of *E. coli* in post pasteurization is an indicative of fecal contamination of the bulk milk indicative of serious public health concern. In conclusion, the present study has demonstrated poor method of pasteurization couple with lack of good hygienic practices such as: proper solid waste management, potable water, cleanliness of milking area and absent of milking installation. These are all factors that contributed to production of poor quality milk in both farms with high coliform, aerobic plates and high isolation rate of *Escherichia coli* post pasteurization.

**Key words:** Aerobic count, bulk milk, coliform count, *Escherichia coli*, Nigeria, pasteurization

### INTRODUCTION

Milk is defined as a fresh, clean, whole undigested and normal mammary secretion obtained by draining of the udder of healthy cows that are properly fed, kept and contains no appreciable colostrums (Frank and Mahony, 1988; Ajogi *et al.*, 2005). Milk is an important source of protein,

minerals, vitamins and fat in human diet (Pirestani and Eghbalsaeed, 2011) which approximately comprises of 87% water, 3.7% protein, 4.9% lactose and 0.7% ash 3.6% fat (Ramesh *et al.*, 2008). With these constituents, milk is described as the most nearly perfect food (Barrett, 1986). This complex biochemical composition, nutritional values and high water content render milk an excellent growth medium for both pathogenic and spoilage microorganism (Bryne, 2004; Parekh and Subhash, 2008; Okonkwo, 2011).

Dairy products are consumed by millions on daily basis worldwide and as such the potential for food-borne illness is a major concern to producers, regulators and consumers (Bryne, 2004). Fresh milk may be contaminated with different microorganisms depending on methods used in cleaning and handling of milk during processing and may originate from udder, the exterior of the udder, milking equipment used and milker's hand (Bramley and McKinnon, 1990; Douglas *et al.*, 2002; Oliver *et al.*, 2005; Bashir and Usman, 2008; Shojaei and Yadollahi, 2008). Bacteria in raw milk can affect the quality, safety and consumer acceptability of dairy products (Elmoslemany *et al.*, 2009). Such microorganisms include *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus* and *Campylobacter jejuni* (Navratilova *et al.*, 2004; Bashir and Usman, 2008; Elmoslemany *et al.*, 2009). Most of the food-borne illnesses associated with milk consumption are linked to post-pasteurization contamination (Olsen *et al.*, 2004) as proper pasteurization supposed to destroy most of the pathogenic bacteria in milk. Post-pasteurization contamination of milk is mostly by contaminated hands of dairy workers, unsanitary utensils and polluted water supply (Pantoja *et al.*, 2009). Detection of specific pathogens (bacterial, coliform, yeast and mould) and their toxins are used as index of contamination of milk and its products with possibility of presence of pathogens which may constitute health hazards to consumers (Parekh and Subhash, 2008).

In Nigeria, most dairy farms that produce fresh milk for human consumption are not subjected to quality control to ascertain the safety of the milk for public consumption (Bertu *et al.*, 2010). However, during this study it was commonly observed that the pasteurization methods include heating of milk in large pots using kerosene, gas or in some instances fire woods. In all of these methods, automated temperature regulator is absent. Rather, visual observation of the milk being heated is often carried out to assess pasteurization parameters. The visual method is ineffective in ascertaining whether the milk pasteurization temperature is up to 63-76°C. It is also practically impossible to apply such pasteurization techniques as High Temperature Short Time (HTST) at 72-76°C for 15 sec which use the function of time temperature designed to kill pathogenic microorganism (ICMSF, 1998). In addition, there is high risk of post pasteurization contamination of milk with food-borne pathogens due to hygiene problems during preparation and handling (Obi and Ikenebomeh, 2007). The present study was therefore, designed to assess microbial quality of bulk milk before and after pasteurization and to isolate *Escherichia coli* as an index of milk contamination, from two major dairy farms in Zaria, Nigeria.

## **MATERIALS AND METHODS**

**Study area:** The study was carried out between November and December, 2010. In two major dairy farms (designated A and B) with different management systems located in Zaria, North-western Nigeria were used for the study. Farm A belongs to Friesian-white Fulani (Indigenous cattle breed X Northern Nigeria) maintained in semi-intensive manner, where animals were allowed to graze in a pasture field within the farm during the daytime and supplemented with concentrate in the evenings. The farm also had a parlour, where milking was done. Milk

pasteurization was carried out by heating milk in large pot using cooking gas, once the milk boils it allowed to cool before packaging into containers or further processes into yoghurt. Farm B consisted of White Fulani breed of cattle extensively managed, in which animals move for long distance in search of green fields or crop residues in farms after harvest. Farmers in both farms used bare hands for milking their cows. Milking pasteurization in farm B was done by heating of milk in large pot using fire wood and once the milk boils it allowed to cool and packaged into plastics container in both farms no thermometer used in measuring temperature.

**Milk sampling:** About 5 mL of milk was collected into clean sterile sample bottle from bulk milk in both farms before and after milk pasteurization and transported immediately to the bacteriology laboratory in ice packs. Sample collection was carried out daily over a period of 30 days during which 30 samples each of pasteurized and raw milk were collected from each of the farms making a total of 120 milk samples.

**Laboratory procedures:** The samples were diluted using a 10 fold serial dilution. By pour plate method, 0.1 mL of  $10^8$  dilutions were inoculated in both MacConkey and nutrient agar (Oxoid, UK) and incubated for 24 h at 37°C for enumeration of total coliform and aerobic plate counts, respectively. Another 0.1 mL was inoculated into Eosin methylene blue agar for isolation of *Escherichia coli*. *Escherichia coli* identification was carried out based on conventional methods of morphological features, Gram staining and biochemical characterization as described by Singh and Prakash (2008). Interview and observations were conducted in the two farms to know how the farmers carry out milking practice and the method of milk pasteurization.

**Data analyses:** Results expressed as CFU mL<sup>-1</sup> were converted to log<sub>10</sub> values using the methods of Lawan *et al.* (2011). Data generated were subjected to paired t-tests to determine significant differences in total coliform and aerobic plate counts between raw and pasteurized milk. Percentage isolation rate of *E. coli* was also calculated.

## RESULTS

The aerobic plates count of raw milk for farm A and B before pasteurization were; 5.70 and 6.04 log<sub>10</sub> CFU mL<sup>-1</sup>, respectively. These counts decrease to 3.76 and 4.20 log<sub>10</sub> CFU mL<sup>-1</sup> after pasteurization of the milk. Coliform counts also follow similar trend. The counts in farm A and B were; 5.32 and 6.49 log<sub>10</sub> CFU mL<sup>-1</sup>, respectively. The counts decrease to 3.16 and 3.74 log<sub>10</sub> CFU mL<sup>-1</sup> after pasteurization of the milk. The total coliform and aerobic plates counts before bulk milk pasteurization in both farms were significantly different with post pasteurization values (p<0.05) when subjected to paired t-test (Table 1).

Table 1: Mean values of coliform and aerobic plate counts of pre and post pasteurized milk sample collected from two dairy farms in Zaria, Nigeria

| Farms | No. of samples | Coliform counts (log <sub>10</sub> CFU mL <sup>-1</sup> ) |                 | Aerobic counts (log <sub>10</sub> CFU mL <sup>-1</sup> ) |                 |
|-------|----------------|---|-----------------|--|-----------------|
|       |                | Pre-pasteurize  | Post-pasteurize | Pre-pasteurize   | Post-pasteurize |
| A     | 30             | 5.30±1.70   | 3.16±1.34       | 5.70±0.65  | 3.76±0.60       |
| B     | 30             | 6.49±0.82   | 3.74±0.98       | 6.48±0.84  | 4.20±1.82       |

Values are Mean±SD, p<0.05 was considered significant, p-value = 0.000812 for coliform count, p-value = 0.000326 for aerobic count

Table 2: Isolation rates of *Escherichia coli* from pre and post pasteurized milk from two dairy farms in Zaria, Nigeria

| Farms | No. of samples | Pre pasteurized |      | Post pasteurized |      |
|-------|----------------|-----------------|------|------------------|------|
|       |                | No.             | %    | No.              | %    |
| A     | 30             | 6               | 20.0 | 2                | 6.7  |
| B     | 30             | 16              | 53.3 | 4                | 13.3 |

The isolation rates of *E. coli* were 20 and 6.7% in farm A, 53.3 and 13.3% in farm B before and after milk pasteurization, respectively (Table 2). Result of the interview and personal observations showed that both farms carried out hand milking methods. However, milking in farm B was done in open field and hygiene practices were poor, water was sourced from well which was not wholesome for milking procedures. In farm A, milking was done in milking parlour, udder were washed with mild disinfectants before milking, but the hygiene and sanitation of milking parlour was poor so also was the lightening of the milking parlour inadequate.

## DISCUSSION

Results of the study revealed that there was a high level of contamination in the raw milk before pasteurization with mean count of 5.70 and 6.48  $\log_{10}$  CFU  $\text{mL}^{-1}$  in farm A and B, respectively. This may be related to the unhygienic milking practices, unclean environment and absence of potable water for cleaning procedures in both dairy farms studied. This finding concurred with the report of Bramley and McKinnon (1990) in which counts exceeding  $10^6$  mL for raw milk were indicative of poor and unhygienic milking practices.

By comparing the mean counts of both aerobic and coliform counts post pasteurization, Farm B was observe to have higher values than Farm A (Table 1). This might be due to the fact that milking in farm B was done in an open space in which the bulk milk was in direct contact with dust from road and fecal materials from manure generated in the farm. This may be suggestive of the possible roles played by environmental and management factors in milk contamination during milking and processing. Such features were reported to include farm construction and design, cleanliness of milking area and surrounding buildings and installations, solid waste management, practice and pollution from within or outside the premises (Abid *et al.*, 2009). The environmental factors such as building and milking installations were absent from Farm B and the solid waste management disposable was also observed to be very poor. This might have contributed to the level of contamination in milk samples from the farm.

The mean coliform counts of pre and post pasteurized bulk milk from both farms were high, exceeding the recommended acceptable level 100 cell  $\text{mL}^{-1}$  of milk (Shojaei and Yadollahi, 2008). This is suggestive of unsanitary conditions and poor hygiene practice in the dairy farms. However, these high coliform counts might also have been contributed by fecal contamination in the bulk milk and could have been indicative possible presence of other enteric pathogens like *Salmonella*, *Listeria* and *E. coli* O157:H7 which are of serious public health concern in consumption of such milk.

When both aerobic and coliform counts before and after pasteurization were subjected to t-test, there were significant differences in counts before and after pasteurization and even though the counts after pasteurization were slightly lower than before pasteurization of the bulk milk, the counts still exceeded the minimum acceptable level specified by World Health Organization of  $3 \times 10^2$  CFU  $\text{mL}^{-1}$  (Ajogi *et al.*, 2005). The high counts post pasteurization obtained could be due to

ineffective pasteurization, post pasteurization contamination from the containers, equipment, utensils and or hands of the handlers as reported by Harding (1999) that high bacteria counts an indicator of poor production hygiene or ineffective pasteurization of milk. Although 6 out of the 30 samples in farm A and 3 out of 30 samples in Farm B were positive for aerobic and coliform before pasteurization but there were no growths for both aerobic and coliform organism post pasteurization. The isolation rates of *E. coli* in both farms also decreased after pasteurization. However, the isolation rates post pasteurization were still high, indicative of possible post-pasteurization contaminations or fecal contamination of the bulk milk.

In conclusion, the present study has demonstrated poor method of milk pasteurization in both farms A and B. Lack of good hygienic practices such as: proper solid waste management, potable water, cleanliness of milking area and absent of milking installation. These are all factors that contributed to production of poor quality milk in both farms with high coliform, aerobic plates and high isolation rate of *Escherichia coli* post pasteurization.

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