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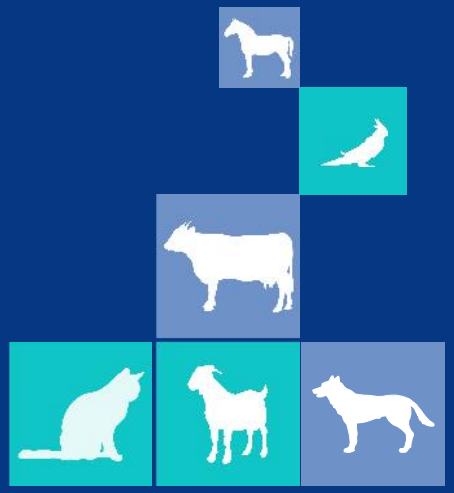

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Full length articles

Short Communications

ASSESSMENT OF THE BACTERIOLOGICAL QUALITY OF LOCALLY FERMENTED COW MILK (NONO) IN MAIDUGURI, NORTH EASTERN NIGERIA

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ABSTRACT

Contamination of locally fermented cow milk (Nono) by pathogenic bacteria constitute a major public health problem. This was because contaminated milk was a good medium that can support the growth and propagation of microbial pathogens. This study was designed to assess the bacteriological quality of locally fermented cow milk consumed in Maiduguri, northeastern Nigeria. Totally, forty (n = 40) samples of fresh, locally fermented cow milk (Nono) were collected and used in this study. Ten (10) milk samples each were collected from four randomly selected selling points (Monday Market, Custom Market, Kasuwan Shanu and Tashan Bama). The bacteriological quality of each milk sample were analysed using standard procedures for isolation, identification and enumeration of pathogenic bacteria. The mean total plate count ranged from $1.44x10^8$CFU/mL to $10.31x10^8$CFU/mL, while the mean total coliform count range from $0.57x10^8$*CFU/*mL to $11.17x10^8$*CFU/*mL. Statistically, there was a significant difference ($p<0.05$) in the mean total plate count and coliform count of milk samples collected from the four sampling points.Bacteriological culture and biochemical identification of all the isolates revealed *Escherichia coli*29(72.5%),*Klebsiella* specie3(7.5%),*Salmonella* specie 2(5%),*Staphylococcus aureus* 14(35%) and*Streptococcus* specie15(37.5%)respectively.All the results were above the acceptable limits (1.0 to 3.0×10^5 *CFU*/mL for total plate count and no coliform count per 100ml of milk sample)set by NAFDAC. Therefore the Nono products consumed in Maiduguri was not safe and wholesome for public consumption. Hence, the need to ensure the maintenance of standard hygienic protocols during the collection, storage, processing and marketing of locally fermented cow milk (Nono) in order to minimize microbial contamination.

Key words: fermented cow milk, Nono, Maiduguri, bacterial contamination.

INTRODUCTION

Preservation of milk by fermentation process is an age long tradition in Africa, Middle East, Asia, and Europe (Savadogo *et al.*, 2004). Locally fermented cow milk (Nono) forms part of a normal daily diets of most of the families in the northern part

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of Nigeria, especially among the nomadic Fulani family who owns more than 80% of the cattle population (Obi and Ikenebomeh, 2007). In Nigeria, cow milk is consumed immediately after collection, pasteurized or incorporated as an infant food supplement. Additionally, Nono also serves as an important source of income to many families in northern Nigeria. This is because of the general belief that locally fermented milk is more nutritious than the commercially processed yoghurt (Akabanda *et al.,* 2010; Egwaikhide *et al.,* 2014).

Despite its high nutritive content, cow milk can serve as an excellent culture medium for the growth and multiplication of microorganisms due to its complex nutrients and high water activity (Ashenafi and Beyene, 1994; Chye *et al.,* 2004). The commonly isolated bacterial flora found in fresh milk samples includes, *Streptococcus, Micrococcus*, *Lactobacillus, Lactococcus* and *Staphylococcus* spp. However, when milk is kept at a very low temperature before processing, the bacterial flora likely to be predominantly isolated from the sample are psychrotrophs. The isolation of coliform and other pathogenic bacteria from fresh milk samples usually occur as a result of contamination from processing equipment, hands of milkers or due to mastitis (Bonfoh *et al.*, 2003; Chye *et al.,* 2004). Fresh milk obtained from a healthy cow normally contains a low microbial activity, particularly with bacterial load of less than 10^3CFU/mL but, the bacterial load is likely to increase up to about 100 fold or more, if the milk is stored for some time at ambient temperature (Lingathurai and Vellathurai, 2010).

Contamination of milk by saprophytic bacteria not only reduces the nutritional quality of milk but also, constitute a significant health hazards to the humans beings (Godefay and Molla, 2000). Contamination of fresh milk sample can occur either at the stage of procurement, processing or distribution. It is pertinent therefore to identify the critical control points in order to ensure the production of safe and wholesome milk for human consumption. Important risk factors of milk contamination includes, the health status of the cow, the hygiene of the milking environment, hands of milkers, sources of water during processing , the hygiene of the utensils used to process the milk and lack of refrigeration facilities at both the farm and household (Mosu*et al.,* 2013). In addition, contamination of milk by microbes make it not only grossly unwholesome, but potentially harmful for human consumption. This is because, consumption of raw or unpasteurized milk has been reported to be associated with the occurrence of some important zoonotic and food-borne pathogens such as *Brucella abortus, Mycobacterium tuberculosis, Salmonella enterica, Yersinia* spp., *E. coli* O157 and Staphylococcal enterotoxins(Baylis, 2009).

In Nigeria, fresh milk obtained from cows was usually being processed via traditional method of fermentation by pouring the milk into a clean semi-dried container made from calabash (Egwaikhide *et al.,* 2014). The procedure was crude and unhygienic, hence serving as an important medium for the growth of pathogenic bacteria that could constitute significant public health problem (Emmanuel *et al.,* 2014). In addition, most cow milk sellers

were street peddlers and the milk was sold in an open environment by the road side; thus, potentially increasing the rate of contamination by bacterial pathogens. In some cases, left over milk was mixed with freshly prepared milk product without any special pretreatment to reduce the bacterial load (Okonkwo, 2011). To this end, this study was designed to assess the bacteriological quality of locally fermented cow milk in Maiduguri, Northeastern Nigeria.

MATERIALS AND METHODS

Samples collection

A total of forty (n = 40) freshly prepared fermented cow milk (Nono) samples were randomly purchased from four different milk selling points (10) each within Maiduguri Metropolis (Monday market, Custom market, Kasuwan shanu and Tashan Bama). Two hundred milliliters (200 mL) each of milk samples were collected in a sterile pre-labelled sample bottle, stored in ice and transported within 1-2hour to Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for bacteriological analysis. A total volume of 25 mL of the milk sample was poured into a sterile plastic bag containing 225 mL of sterile distilled water and then homogenized with the aid of a stomacher.

Microbiological analysis

The milk samples were assessed for their bacteriological quality and as well as the occurrence of selected pathogenic bacteria. Enumeration of bacteria of interest using total plate count (*E. coli*, *Salmonella* spp and *Staphylococcus* spp) was carried out as per the guidelines of American Public Health Association(Vanderzant, 1992). Each sample was serially diluted using sterile distil water as described by (Obande et al., 2017) with little modification. Enumeration of the total numbers of *E. coli* and coliform bacteria in each milk sample was carried out using the three tube Most Probable Number (MPN) technique. Positive tube samples were cultured onto Eosin methylene blue agar (EMB) and then incubated at 37 ºC overnight. Colonies were confirmed based on colonial morphology, Gram staining for cellular morphology and biochemical test as described by (Cheesebrough, 2004; Egwaikhide *et al.,* 2014).

RESULTS AND DISCUSSION

The present study investigated the bacteriological quality of locally fermented cow milk (Nono) sold in Maiduguri, northeastern Nigeria. Cow milk is known to serve as a very good medium that supports the growth and multiplication of bacterial pathogens with undesirable consequences to human health. The presence of coliform in milk samples indicates gross contamination. Coliforms are considered as normal flora of the intestinal tract of human and animals, their presence in milk is indicative of fecal contamination. (El-Bakri *et al.,* 2009).

The result obtained showed that the milk samples collected in Tashan Bama had the highest mean Total plate count (TPC) ($10.31x10^8$CFU/mL) than those collected from Monday market ($1.44x10^8$CFU/mL) (Table 1). Additionally, there was a significant difference ($P<0.05$) between the mean TPC of milk samples collected from

Custom market, Kasuwan shanu market and those from Monday market and Tashan Bama. However, no significant difference ($p>0.05$) was observed between the mean TPC of milk samples collected from Custom market and Kasuwan shanu market. The mean TPC (of $5.5x10^8$CFU/mL) obtained in this study is above the accepted standard value 1.0 to *3.0 x 10^5 CFU*/mL for TPC set by National Food and Drug Administration and Control (NAFDAC) 2005. It was also higher than($3x10^3$CFU/mL - $25x10^3$CFU/mL)reported by(Egwaikhide *et al.,* 2014). This higher values could be due to one of the following reasons, the difference in the quality of the milk sample, the farm management practice, the method of collection, the health status of the cow, the geographical location and season, the method of processing, storage and distribution. This was because contamination of milk could occur through the hands of milkers, the use of unhygienic utensils and water during collection, storage and processing of the milk (Egwaikhide *et al.,* 2014). Furthermore, the result of this study also showed that milk samples collected from Tashan Bama had the highest mean total coliform count (TCC) ($11.17x10^8$CFU/mL) than those collected from Monday market ($0.57x10^8$CFU/mL) and the result was statistically significant at ($P<0.05$). However, there was no significant difference ($p>0.05$) between the mean TCC of milk samples collected from Monday market and Custom market. The mean TCC ($5.27x10^8$CFU/mL) observed in this study was not in line with standard set by NAFDAC that no *E. coli*or coliform shloud be isolated per 100mL of processed milk. This was higher than (13.9×10^6 CFU/mL) reported by (Lingathurai and Vellathurai, 2010) in the province of Madurai in Southern India. Coliforms bacteria were considered as normal flora of the intestinal tract of human and animals, where by majority of them do not pose danger to consumers but some pathogenic strains of *E. coli* could cause serious systemic condition. The presence of coliform in milk indicates direct fecal contamination (Okiki *et al.,* 2018).

In this study, the frequency of occurrence of the different types of bacterial contaminants isolated from all milk samples analyzed revealed *Escherichia coli* 29(72.5%), *Klebsiella* specie 3(7.5%), *Salmonella* specie 2(5%), *Staphylococcus aureus* 14(35%) and *Streptococcus* specie15(37.5%)respectively(Table 3; Figure 1). The presence of these bacteria in milk sample could be detrimental to human health. This was because some of these bacteria had been implicated in cases of milk-borne gastroenteritis (Lingathurai and Vellathurai, 2010). *Escherichia coli* was the most frequent bacteria isolated from all the milk samples analyzed. The level of contamination by *E. coli* reported in this study was higher than the standard set by NAFADAC as no *E. coli* should be isolated per 100mL of milk sample. However this contradict what reported by(Maduka *et al.,* 2013) where none of the commercial yoghurt analyesd were contaminated by *E. coli*. The presence of *E. coli* in milk sample wasusually accompanied by the presence of other bacteria like *Klebsiella* spp and *Staphylococcus aureus* which might be associated with milk handlers, fecal contamination or mastitis (Okonkwo, 2011). In this study, *Klebsiella* spp was also isolated from all the samples with low frequency

of occurrences 7.5%. The presence of this bacteria in milk sample was usually due to contamination by feces. This pathogen could cause detrimental infection in humans, most especially in people who consume Nono. Muhammad *et al.*, (2009) reported that approximately 60-80% of all *Klebsiella* species isolated from feces and clinical specimen were*klebsiella pneumoniae*. Furthermore, *Salmonella* species were also identified in milk samples collected from two sampling locations. This was lower than 9.8% and 23% reported by (Okonkwo *et al.,* 2012) and (Godwin and Emmanuel, 2013) respectively. These pathogens had been reported as one of the most important causes of nosocomial infection worldwide and were resistant to almost all classes of clinically relevant antibiotics (Patel *et al.,* 2008). Their presence in milk sample was worrisome, because *Salmonella*spp was one of the most important sources of food-borne hospitalization worldwide (Emmanuel *et al.,* 2014). Other bacteria isolated from milk samples in this study were*Staphylococcus aureus* and *Streptococcus* species having35% and 37.5% frequency of occurrence respectively. Their presence in milk could be associated with the health status of the cows. It was possible that these milk samples were obtained from mastitic cows. *Staphylococcus aureus* and *Streptococcus* species have been reported as the most predominant causes of bovine mastitis in cow (Workineh *et al.,* 2002). Consumption of milk contaminated with these pathogens exposed human to serious health risk through the transmission of pathogenic bacteria that were resistant to a wide compendium of antibiotics. Thus, increasing healthcare cost, prolonged hospital admission stay and in severe cases death.

CONCLUSION

The findings of this study showed that the bacterial count of locally fermented cow milk (Nono) retailed in Maiduguri is above the acceptable limit (1.0 to *3.0 x* 10^5 *CFU*/mL for total plate count and no coliform count per 100ml of milk sample) set by NAFDAC. Hence, rendering the milk unfit for human consumption. Nono samples containing high level of bacterial contamination could exposes the consumer to a lot of health hazards.

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Conflicting interest

The authors had read and approved the final draft of this manuscript and wishes to declare that there is no conflicting interest with regards to the publication of this manuscript.

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Table1: Mean total plate count (TPC) of locally fermented cow milk (Nono) samples collected from four locations in Maiduguri Metropolis

Locations	No. of samples	Mean x10^8/mL	95% Confidence interval
MMKT	10	1.44 [a]	0.36 - 2.52
CMKT	10	4.14 [b]	1.18 - 7.10
KMKT	10	6.11 [b]	3.69 - 8.53
TBM	10	10.31 [c]	9.03 - 11.59
TOTAL	40	5.50	3.57 -7.44

Values along the same column differently superscripted differs significantly (P<0.05); MMKT (Monday Market), CMKT (Custom Market), KMKT (Kasuwan Shanu Market), TBM (Tashan Bama)

Table 2: Mean total coliform count (TCC) of locally fermented cow milk (Nono) collected from four locations in Maiduguri metropolis

Locations	No. of samples	Mean x10^8CFU/mL	95% confidence interval
MMKT	10	0.57 [a]	0.36 - 2.52
CMKT	10	3.09 [a]	1.18 - 7.10
KMKT	10	6.25 [b]	3.69 - 8.53
TBM	10	11.17 [c]	9.03 - 11.59
TOTAL	40	5.27	3.57 - 7.44

Values along the same column differently superscripted differs significantly (P<0.05); MMKT (Monday Market), CMKT (Custom Market), KMKT (Kasuwan Shanu Market), TBM (Tashan Bama)

Table 3: Frequency distributionof occurrences of the different bacteria isolated from locally fermented cow milk (Nono) samples collected from four locations within Maiduguri metropolis

Locations	Sample size	*Escherichia coli*	*Klebsiella* spp	*Salmonella* spp	Staphylococcus spp	Streptococcus spp
MMKT	10	5	2	1	2	5
CMKT	10	7	0	0	5	2
KMKT	10	8	1	1	3	3
TBM	10	9	0	0	4	5
Total	40	29	3	2	14	15

MMKT (Monday Market), CMKT (Custom Market), KMKT (Kasuwan Shanu Market), TBM (Tashan Bama)

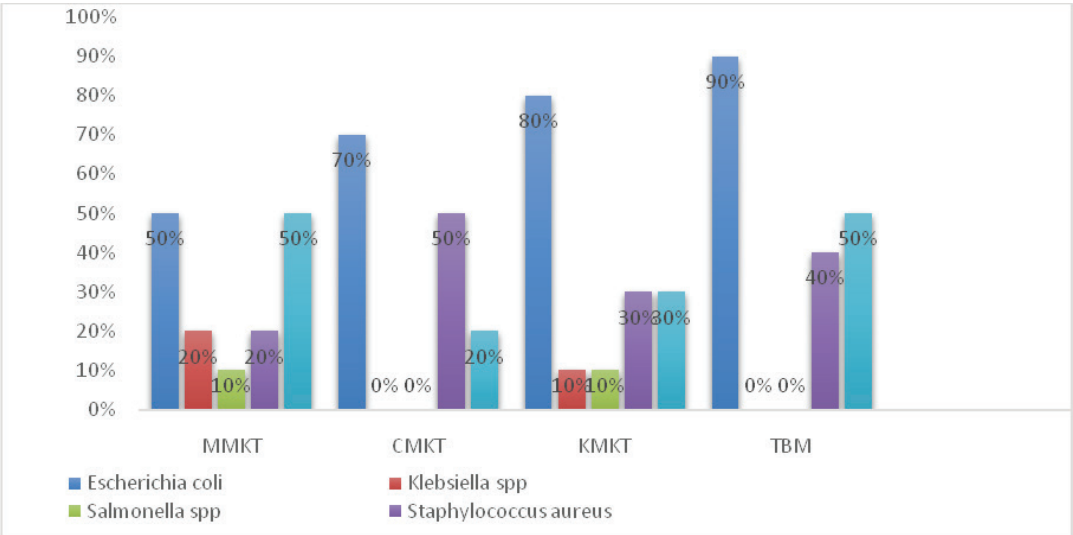

Figure: 1: Distribution of the overall mean (%) frequency of occurrences of the different bacteria isolated from fermented cow milk (Nono) samples.