RESEARCH ARTICLE

BACTERIOLOGICAL EXAMINATION OF PACKAGED MILK MARKETED IN ZARIA, NIGERIA

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ABSTRACT

Milk has an outstanding nutritional quality, but is also an excellent medium for bacteria growth and an important source of bacterial infection when consumed without pasteurization. This study was conducted to evaluate the hygienic quality of packaged milk and identify predominant bacteria in milk marketed in Zaria. Two hundred packaged milk samples were bought from five locations (forty samples from each) in Zaria. Isolation and identification of the bacteria species were carried out using standard bacteriological procedures. The study revealed that the four brands of packaged milk sampled in this study were contaminated. The bacterial load obtained from four brands of milk ranged from $19.40 - 1.10 \times 10^6$ cfu/ml. The bacteria identified and their prevalence rates were *Escherichia coli* (13.1%), *Proteus species* (2.6%), *Salmonella specie* (0.65%), *Providencia species* (3.26%), *Enterobacter species* (36.6%), *Citrobacter spp..* (0.65%), *Klebsiella species* (1.31%), *Yersinia specie* (0.65%), *Pseudomonas species* (37.9%) and *S. aureus* (3.28%). One hundred and fifty-three bacterial isolates were identified from the two hundred milk samples, 27.5% were obtained from the first brand of milk sample, 21.6% from the second brand, 12.4% from the third brand and 38.6% from the fourth brand of milk samples. Most of the organisms identified belonged to enterobacteriaceae family, thus indicating probable faecal contamination of the milk as a result of poor hygiene during production processes. This shows that packaged milk is a potential hazard of pathogenic food borne bacteria that may have public health implications. This can pose health hazard to consumers of such milk products. There is the need for some additional food safety measures to be applied before the consumption of milk.

INTRODUCTION

Milk and milk products constitute important nutritional components for human diet and plays a prominent role in human nutrition (Javaid *et al.,* 2009). Good quality milk meets the nutritional needs of the body better than any single food as it contains essential food constituents such as fat, proteins, carbohydrates, minerals, vitamins (Sharm and Joshi 1992; Medhammar *et al.,* 2012). As a result of the presence of these nutritional components, milk is an excellent culture medium for many microorganisms, especially bacterial pathogens (Henry and Newlander 1997; Saeed *et al.,* 2009). In order to extend the shelf life of milk for human consumption and prevent growth of spoilage organisms, as well as prevent transmission of diseases via milk, this highly nutritious food is

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expected to be pasteurized (Edema and Akingbade 2007).Unfortunately, many workers have reported post-pasteurization contamination of milk with resistant pathogenic bacteria (Brisabois *et al.,* 1997; Oliver *et al.,* 2005). For instance, some potential human pathogens, such as *Mycobacterium paratuberculosis*, *Bacillus cereus*, *Clostridium spp., Listeria monocytogenes* and *Salmonella spp.* have been reported to survive conventional heat pasteurization in milk (Stabel *et al.,* 1997; Smith *et al.,* 2002; Torkar and Teger 2008). Microbial contamination of milk has been reported to be responsible for deterioration of the quality of packaged milk (Frazier and Westoff 1986; Guerra *et al.,* 2003). Approximately 50% of the milk produced is consumed as fresh or pasteurized, one sixth ($^1/_6$) as yoghurt or curd and the remaining utilized in the production of varieties of milk products such as ice cream and butter (Anjum *et al.,* 1989; Lindmark *et al.,* 2003). Bacterial contamination can generally occur from three main sources; within the udder, outside the udder, and from the surface of equipment used for milk handling and storage (Oliver *et al.,* 2005). Cow health, milking

procedures, equipment sanitation and environment such as; water and personnel also can influence the level of microbial contamination of raw milk (Farzana *et al.,* 2009). Equally important is the milk holding temperature and length of time milk is stored before testing and processing that allow bacterial contaminants to multiply. These factors will influence the total bacterial count and the types of bacteria present in milk. Another source of contamination by bacterial pathogen is unclean teats (Altug and Bayrak 2003). The use of unclean milking and transporting equipment contributes to poor hygienic quality of milk (Bonfoh *et al.,* 2003). In order to produce milk of good hygienic quality, it is therefore important to have clean healthy cows and clean utensils for milking and storage of the milk. Unfortunately, the consumption of unpasteurized milk in most developing countries including Nigeria has not attracted the desired attention.

Bacteria are widely distributed in nature and may be introduced into milk easily. Consequently, a broad spectrum of bacteria such as *Staphylococcus aureus, Escherichia coli, Salmonella spp., Pseudomonas spp., Enterobacter spp., Klebsiella spp., Proteus spp.* and *Yersinia spp.* have been recovered from milk (Ayebo *et al.,* 1976; De Buyser *et al.,* 2001; Sivapalasingams *et al.,* 2004) and some of these have been determined to be potentially pathogenic and toxicogenic, and implicated in milk-borne gastroenteritis (Bergdoll 1979; Maguire *et al.,* 1992). However, some of them including *Proteus spp.* and Klebsiella *spp.* are rarely associated with food-borne infections. Pathogenic Klebsiella pneumoniae causes pneumonia while *Proteus spp.* has been reported to be mainly associated with wound and urinary tract infections. Thus, the occurrence of these organisms in milk may pose risk to consumers. Most of the other bacteria identified in milk have been implicated in milk and other food related infections (Kivaria *et al.,* 2006). The presence of coliform organisms in milk has been linked to a wide variety of human infections such as endocarditis, urinary and genital tract infections, meningitis and septicemia (Mannu *et al.,* 2003). Evidence indicates that *Salmonella spp.* is one of the most etiological agents responsible for several outbreaks associated with the consumption of milk (De Buyser *et al.,* 2001). Pathogenic bacteria in milk have been a major public health problem due to the number of diseases caused by them (Grant *et al.,* 1995). Milk helps in fighting against diseases such as gout, kidney stones, breast cancer, rheumatoid arthritis, migraine headaches, amongst others. In Nigeria and other developing countries where surveillance and reporting of food-borne diseases are not properly evaluated, it is extremely difficult to estimate how far milk products contribute to infection and diarrhea associated diseases (Ehiri *et al.,* 2001). In view of the health hazard associated with the consumption of contaminated milk, the findings from this study will help in evaluating the quality of milk within the study area, in order to safeguard the health of the people and also evaluate the microbial contamination and quality of packaged milk sold in the area of study.

MATERIALS AND METHODS

The study was carried out in the Department of Pharmaceutical Microbiology Laboratory, Ahmadu Bello University, Zaria, Nigeria.

Sample collection

Four brands of liquid pasteurized milk were selected from eight brands of packaged milk commonly sold in Zaria. Samplings for this study were purchased randomly from five markets in Zaria, in these locations namely; Samaru, Sabon-Gari, Tudun Wada, Wusasa and Zaria city. Brands 1 and 2 were in plastic bottles while brands 3 and 4 were in paper packs and were all stored at room temperature at the time of purchase. The four brands of milk samples used in this study were all manufactured in Lagos State, Nigeria. A total of two hundred (200) packaged pasteurized milk samples were bought from five major markets in Zaria with different batch numbers, date of manufacture and expiry dates. The four brands of packaged milk samples were manufactured in August, September and October, 2010 while their expiration dates did not exceed May, 2011. Ten packs of four brands of milk samples were bought making a total of forty samples per market. The samples were transported to the laboratory for bacteriological analysis.

Isolation of organisms

The milk packs were swabbed with 70% ethanol before opening. Using sterile syringe, 1.0ml of milk sample was aseptically withdrawn from the packages to make ten-fold serial dilutions using sterile normal saline. Using pour plate method, 1.0 ml of appropriate diluted sample was mixed with 19.0 ml of melted nutrient agar (40°C) and poured into sterile plates aseptically. The plates were incubated at 37°C for 24 hours. Total viable counts were carried out on Plate count agar using colony counter. The numbers of colony forming units (CFU) per millilitre were counted and recorded after 24 hours. Viable colonies were aseptically picked from nutrient agar plates and purified using prepared sterile nutrient broth. Microscopic examination of the selected colonies was carried out to determine cell morphology and Gram staining reactions of the bacteria isolates using standard microbiology method.

Identification of Isolates and Biochemical Test

Isolation of specific bacteria was done by streaking on selective media. Overnight cultures were grown on nutrient broth and a loopful of inoculum from nutrient broth was streaked on selective agar and incubated at 37°C for 24 hours. Mannitol salt agar (MSA) was used for isolation of *S. aureus*, Cetrimide agar for isolation of *Pseudomonas species*, Eosin Methylene Blue agar for *E. coli*, Salmonella Shigella Agar for *Salmonella* species and MacConkey agar for isolation of other Enterobacteriaceae present in the milk sample as described by Chessbrough (2000). Presumptively identified organisms were sub-cultured on nutrient agar slant, incubated at 37°C for 24 hours and stored in refrigerator at 4°C pending further studies. The biochemical evaluation of the identified isolates were carried out using standard microbiological method (Cowan and Steel 1993; Barrow and Feltham 1993; De Silva *et al.,* 2001; Ellis and Goodacre 2006).

RESULTS AND DISCUSSION

The bacteria load from packaged milk samples obtained in various locations were as follow; Samaru (1.10 – 19.20 x 10^6

cfu/ml), Sabo Gari (1.20 – 17.20 x 10^6 cfu/ml), Tudun Wada (1.80 – 14.80 x 10^6 cfu/ml), Wusasa (1.10 – 16.80 x 10^6 cfu/ml) and Zaria City (1.40 – 19.40 x 10^6 cfu/ml), while the mean counts were 5.98 ± 4.0 x 10^6cfu/ml, 3.98 ± 2.7x 10^6cfu/ml, 4.45 ± 3.1 x 10^6 cfu/ml, 4.08± 4.7 x 10^6 cfu/ml and 3.32 ± 4.5x 10^6 cfu/ml respectively. The mean count from the four brands of packaged milk showed that brand 4 had the highest counts while mean count based on location showed that Samaru had the highest count (Table 1 and 2). Based on morphological and biochemical characteristics of the organisms isolated, a total of ten (10) bacterial species comprising one hundred and fifty-three (153) isolates were identified in the 200 milk samples studied.

Table 1. Mean of Total Aerobic Bacterial Counts of four Brands of Packaged Milk Samples in Zaria

Milk Brands	Number of Samples	Packaging Material	Mean Count (Cfu/ml)
Brand 1	50	Plastic Packs	3.57 ± 1.8 x 10^6
Brand 2	50	Plastic Packs	3.13 ± 2.3 x 10^6
Brand 3	50	Paper Packs	1.26 ± 0.5 x 10^6
Brand 4	50	Paper Packs	9.62 ± 2.1 x 10^6
Mean Total Count			4.40 ± 3.6 x 10^6

Table 2. Mean Count (Cfu/ml) of Brands of Packaged Milk based on Location

Brands	Samaru	Sabo-Gari	Tudun Wada	Wuzaza	Zaria City
Brand 1	5.49 x 10^6	5.56 x 10^6	2.67 x 10^6	2.67 x 10^6	1.48 x 10^6
Brand 2	4.43 x 10^6	2.78 x 10^6	6.40 x 10^6	1.38 x 10^6	0.68 x 10^6
Brand 3	2.28 x 10^6	0.82 x 10^6	1.03 x 10^6	1.18 x 10^6	1.00 x 10^6
Brand 4	11.7 x 10^6	6.77 x 10^6	7.71 x 10^6	11.1 x 10^6	10.1 x 10^6
Mean Count	5.98± 4.0 x 10^6	3.98 ± 2.7x 10^6	4.45±3.1x 10^6	4.08± 4.7 x 10^6	3.32±4.5x 10^6

Escherichia coli (13.1%), *Proteus species* (2.6%), *Salmonella specie* (0.65%), *Providencia species* (3.26%), *Enterobacter species* (36.6%), *Citobacter spp.* (0.65%), *Klebsiella species* (1.31%) and *Yersinia specie* (0.65%) were bacteria from Enterobacteriaceae Family. Other bacteria isolated were *Pseudomonas species* (37.9%) and *S. aureus* (3.28%) (Table 3).

Table 3. Distribution of Organisms in different Brands of Packaged Milk Samples

Isolates	Milk Samples				Frequency no. (%)
	Brand 1	Brand 2	Brand 3	Brand 4	
E. coli	3	1	2	14	20 (13.1%)
Proteus spp.	3	–	1	–	4 (2.6%)
Salmonella spp.	–	–	–	1	1 (0.65%)
Providencia spp.	2	–	–	3	5 (3.27%)
Enterobacter spp.	6	28	5	17	56 (36.6%)
Citrobacter spp.	1	–	–	–	1 (0.65%)
Klebsiella spp.	1	–	–	1	2 (1.31%)
Yersinia spp.	–	–	1	–	1 (0.65%)
Pseudomonas spp.	23	4	9	22	58 (37.9%)
Staphylococci spp.	3	–	1	1	5 (3.27%)
Total	42	33	19	59	153

Gram's reaction revealed that the isolates were made of predominantly Gram negative rods (96.7%) and few Gram

positive cocci in clusters (3.3%). A total of 42 (27.5%) isolates were obtained from the first brand of milk sample, made of predominantly *Pseudomonas species* (Table 3). 21.6% isolates were isolated from the second brand of milk sample, made of predominantly *Enterobacter species* (Table 3). The third brand of milk sample consisted of 12.4% isolates and was predominantly *Pseudomonas spp.* (Table 3), while 38.6% isolates were obtained from the fourth brand of milk sample made of predominantly *Pseudomonas species* (Table 3). *Pseudomonas species* (37.9%) was the most frequently isolated organism followed by *Enterobacter species* (36.6%), *and Escherichia coli* (13%). The least isolated organisms includes *Salmonella spp.* (0.7%), *Citrobacter spp.* (0.7%), *Yersinia spp.* (0.7%) and *Klebsiella specie* (1.3%) (Table 3).

Table 4 shows milk sample analysis from five locations in Zaria. The analysis of the data showed that 33.3% of the isolates were from Samaru, 12.4% from Sabo-Gari, 19% from Tudun-wada, 19.6% from Wusasa and 15.7% from Zaria city.

Table 4. Distribution of Organisms in Milk Samples based on Sampling Locations in Zaria

Isolates	Location				
	Samaru	Sabo Gari	Tudun-wada	Wusasa	Zaria-city
E. coli	3	–	3	6	8
Proteus spp.	4	–	–	–	–
Salmonella spp.	1	–	–	–	–
Providencia spp.	2	2	–	1	–
Enterobacter spp.	11	1	16	16	12
Citrobacter spp..	–	–	1		
Klebsiella spp..	–	–	–	1	1
Yersinia spp..	1	–	–		
Pseudomonas spp.	26	16	8	6	2
S. aureus	3	–	1	–	1
Total	51(33.3%)	19 (12.4%)	29 (19%)	30 (19.6%)	24 (15.7%)

Escherichia coli were isolated from four of the locations except Sabo Gari. *Proteus spp.*, *Salmonella spp.* and *Yersinia spp.* were found only in Samaru. *Citrobacter spp.* occurred only in Tudun-wada while the two *Klebsiella spp.* isolated were found in Wusasa and Zaria city. Higher rate of isolation of *Pseudomonas spp.* were from Samaru followed by Sabo Gari. *Enterobacter spp.* were found to be present in all the locations of study with high rates occurring in samples from Tudun Wada and Wusasa. The results obtained from this study showed that the products were grossly contaminated with bacteria of public health concern. The bacteria load count from milk products in this study area ranged from 19.4 x 10^6cfu/ml to 1.1 x 10^6cfu/ml. The high total aerobic bacteria counts in the milk products examined could be a consequence of the low level of hygiene maintained during the processing of the milk products. It has been reported that the unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating bacterial contamination of milk products beside the post manufacturing contamination (El-Mahmood and Doughari 2007). The high numbers of the isolated bacteria observed in this study could be due to the fact that milk being a good nutritive medium enhanced the growth of bacteria contaminant in the milk investigated (International Dairy Federation 1994; Adesiyun *et al.,* 1997).

The detection of bacteria from enterobacteriaceae group such as *Escherichia coli, Enterobacter spp.*, *Klebsiella spp.*, *Proteus spp.*, *Citrobacter spp.*, *Yersinia spp.*, *Salmonella spp.* and *Providencia spp.* in the studied milk products, probably indicates possible faecal contamination (Talaro and Talaro 2006). However, bacteria contaminations of milk products have been previously reported (Yagoub *et al.*, 2005; Oranusi *et al.*, 2007). Being enteric bacteria, their presence indicates poor hygienic practices among handlers of these products. Due to the significance of the faecal-oral route transmission for many bacteria food borne diseases, basic hygiene measures assume a decisive importance in food safety management (Utermann 1998). The presence of these bacteria in milk also suggests contamination from various sources, which may include animal, human, environment, utensils and others (Murphy and Boor 2000). Isolation of *E. coli* could be due to faecally contaminated water used in milk production and raw materials storage environment. *Escherichia coli* have been reported to be linked to diarrhea diseases, urethrocystitis, prostatitis and pyelonephritis (Kurt and Wolfgang 2000; Leflon-Guibouta *et al.*, 2002).

Other bacteria isolated in this study include *Pseudomonas species* and *Staphylococcus aureus*. *Pseudomonas species* have been implicated in the spoilage of milk and its products at even refrigerator temperatures (Gilmour and Rowe 1990), also in localized/generalized infections following surgery or burns, nosocomial infections e.g. Urinary tract infections following catheterization, eye and ear infections which may be serious in hospitalized patients or those with cancer who consume unpasteurized milk (Okpalugo *et al.*, 2008). The detection of *Pseudomonas spp.* can also be due to the low temperature of storage of pasteurized milk, which might have supported the growth of psychrotrophs as reported by Holm *et al.* (2004). Valbuena *et al.* (2004) also reported detection of *Pseudomonas spp.* in milk products. The detection of *Staphylococcus aureus* is also of public health importance because of its ability to cause a wide range of infections especially food-borne intoxication. Isolation of *Staphylococcus aureus* from milk products have been reported in other works (Teale 2002; Jayarao and Wolfgang 2003; Sato *et al.*, 2004). *Staphylococcus aureus* has been linked to gastroenteritis by producing chemical enterotoxins. As little as 1.0µg of the toxin in contaminated food produces symptoms of illness. This level of the toxin has been found at 10^5 cells/g of food (Ananthanarayan and Panikaran 2001). *Staphylococcus aureus* has also been reportedly linked to boils, skin infections, (pneumonia, deep abscesses and meningitis in debilitated persons). *Staphylococcus aureus* has been reported highly vulnerable to destruction by heat treatment and nearly all sanitizing agents; therefore, the presence of this bacterium in milk is an indication of poor sanitation during processing, handling and packaging or post pasteurization contamination (Ahmed *et al.*, 2009).

Conclusion and Recommendation

Findings from this study have showed that packaged milk samples sold in Zaria metropolis, Nigeria were contaminated with bacteria majorly of Enterobacteriaceae group,

Pseudomonas spp. and *S. aureus.* Based on the results obtained from this study, strict hygienic measures should be applied during production, processing and distribution of milk and its products to avoid contamination by bacteria pathogens. The key to preventing contamination of milk products is to prevent post-pasteurization contamination through well designed quality assurance. It is also the key responsibility of both consumers and suppliers to adequately store milk at suitable temperatures in order to control the levels of bacteria and to retard the rate of milk spoilage. Effective measures to ensure safe milk for human consumption such as the phosphatase and methylene blue reduction tests should be routinely performed on each batch of milk processed by dairy plants. Since public perception of food quality is critical in the marketing of any product, it is very important that the Nigerian milk products industries should maintain high processing standards.

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