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Original Article

BACTERIOLOGICAL SAFETY ASSESSMENT OF MILK AND MILK PRODUCTS SOLD IN SOME PARTS OF KADUNA STATE, NIGERIA

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ABSTRACT

A total of 320 fresh milk and milk products comprising of 80 samples each of fresh milk, "nono", "kindrimo" and "manshanu" respectively were collected from four Local Government Areas of Kaduna state. The samples were subjected to microbiological techniques for the isolation of pathogenic bacteria. The isolated organisms were identified by standard bacteriological methods. The organisms identified were; *Bacillus* spp. 11.25%, *Staphylococcus aureus* 8.75%, *Staphylococcus* spp. 8.75%, *Proteus* spp. 6.88%, *Providencia* spp. 2.50%, *Pantoea agglomerans* 1.25%, *Escherichia coli* 0.94%, *Acinetobacter lwoffi* 0.94%, *Hafnia alvei* 0.63%, *Salmonella pullorum* 0.31%, *Acetobacter haemolyticus* 0.31% and *Citrobacter diversus* 0.31%. The study showed that these milk and milk products could be an important source of infection with a wide range of organisms particularly Enterobacteria. There is therefore the need to institute effective control measures and improved hygiene handling of milk and milk products to protect public health.

Keywords: Bacterialogical safety, Fresh milk, milk products.

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INTRODUCTION

Milk has been described as a nearly perfect food because it contains the essential nutrients required by the body in appropriate proportions (Karshima *et al*., 2013). It is an aqueous colloidal suspension of proteins, fats, and carbohydrates that contains numerous vitamins and minerals (Ogbolu *et al.,* 2014). Equally, it can be a vehicle for

several pathogenic bacteria such as S*taphylococcus aureus* and *Salmonella spp*. (Esona *et al.,* 2004; Jorgensen *et al.,* 2005;Rall*et al*. 2008).

Fresh milk and other dairy products are traditionally staple food commodities for the nomadic population of Northern Nigeria and many other part of Africa (Okeke *et al.,* 2014). Cow milk is utilized in the production of at least 400 different fermented products all over the world (Robinson and Tamime, 2006). Manshanu, Kindirmoand Nono are fermented milk products mostly consumed by the Hausas (Fulanis) in northern Nigeria. Nono is a crude cultured whole milk whose fermentation may be brought about by a number of bacterial species from various sources. Kindirmois a full fat or partially skimmed cultured milk while Manshanu is fat from milk. In Nigeria about 90% of the dairy cattle belong to the Fulani agro-pasteuralist and their women strictly control the processing and marketing of their milk and milk products (Chukwuma, 2009). Due to the low educational status of these women, these dairy products are handled poorly during processing and marketing exposing the products to microbial contamination. These informally marketed milk and milk products could then be sources of milk borne disease such as tuberculosis, diphtheria, listeriosis, brucellosis, and staphylococcal food poisoning (Ijah *et al.,* 2002), especially among urban residents who drink fresh milk sold by the Fulani women.

This research was therefore conducted to investigate the bacteriological safety of fresh cow milk and milk products (Manshanu*,* Nono and Kindirmo) in four local government areas of Kaduna state, Nigeria.

MATERIALS AND METHODS

Study area

The study area included four (4) Local Government Areas (Giwa, Kaduna North, Soba and Chikun Local Government Areas) in Kaduna state.

Sample collection

A total of 320 samples; comprising of 80 samples each of fresh milk, Manshanu, Nono and Kindrimo (20 from each local government) were collected. Fresh milk samples were collected from farm steads or Fulani settlements while, Manshanu, Nono and Kindrimo samples were obtained from motor parks and markets. Samples were collected in sterile containers and placed in ice- packed coolers and taken to the laboratory, for analysis.

Bacteriological Analyses

Isolation of *Staphylococcus* spp. was conducted according to the procedure described by Imanifooladi *et al.* (2010). Each fresh milk and milk product sample was diluted in the ratio 1/100 in normal saline. From each solution produced, 1ml was transferred to 9mls cook meat media culture with 9% NaCl and incubated at 37^0 C for 48 hr. In the second phase, 0.1ml from each previously cultured medium was then transferred to Baird-Parker agar (BPA) and incubated for 24hr. Black colonies with transparent zone on Baird-Parker agar were considered as presumptive *Staphylococcus* species. They were picked and stored on nutrient agar slants for further confirmation tests.
The isolation method for *Sallmonella* spp. followed a three- stage procedure as described by Esona *et al*. (2004) and

Henzlar *et al.* (1994). Pre- enrichment was done by adding 25 ml of sample to 225ml of buffered peptone water and incubated at 37^0 C for 16 hours. Enrichment was done by inoculating 1ml of the pre-enriched samples into enrichment broths, selenite F broth (Merck) for Salmonella. The plates were incubated at 37^0 C for 48hr.

For the isolation of *Escherichia coli* and other coliforms, 25 ml of each sample were dispensed into a prepared 225 ml of normal saline. The content was shaken for a homogenous mixture and 0.2 ml of sample homogenate was plated using spread plate technique onto Eosin Methylene Blue (EMB) agar (Oxoid). All inoculated plates were allowed to dry, inverted and incubated at 37^0 C for 24 hr. Typical colonies were picked and stored on nutrient agar slants for further confirmation tests.

Biochemical Characterization of isolates

Presumptive *Staphylococcus* species that were gram positive cocci in clusters were subjected to some biochemical tests as described by Cheesbrough (2009). These included; coagulase, catalase, fermentation of glucose and Mannitol. These were further confirmed using Microgen™ STAPH- identification system (Microgen Bioproducts, United Kingdom). Single colonies of the presumptive isolates were tested with 12 standardized biochemical substrates following the manufacturer's instructions.Typical colonies on Salmonella- Shigella agar (SSA) and Eosin Methylene Blue (EMB) agar plates were identified by Gram staining. Presumptive isolates (Gram negative rods) were subjected to series of biochemical tests such as; indole, motility, urease, citrate utilization and triple sugar iron tests. Microgen™ Enterobacteriaceae GnA-Identification System was used for further biochemical distinction of the coliforms and *Salmonella* spp. The test organisms were identified by interpreting the permutations of metabolized substrates using the microgen identification system software (MID-60).

RESULTS

A profile of some potential pathogenic and opportunistic bacteria from fresh milk and milk products is shown in Table 1. A total of 45 Enterobacteriaceae species, 34 *Bacillus* species and 56 Staphylococcal Species were isolated. The Enterobacteriaceae and Staphylococcal species were identified to the genus level and their frequency of occurrence in relation to the total number of organisms isolated.

Fresh milk samples were mostly contaminated with *Bacillus*spp. while *Staphylococcus* spp. were mostly found in Manshanu. *Acetobacter haemolyticus* and *Salmonella pullorum* were found only in Nono samples. Kindrimo were the least contaminated devoid of most enterobacteria isolated (Figure 1).

The occurrence of bacterial isolates in the different sampling locations is displayed in Table 2. Kaduna North had the highest occurrence of bacterial isolates 39 (28.9%) while samples from Chikun were the least contaminated 28 (20.74%). Organisms such as *Acetobacter haemolyticus*, *Citrobacter diversus, Hafnia alvei* and *Salmonella pullorum* were only present in samples from Chikun while *Acinetobacter lwoffi* was found only in Kaduna North.

Table 1: Frequency of occurrence of potential pathogenic bacterial species isolated from fresh Milk and Milk Products.

Bacterial isolates	Frequency	Percentage (%)
Bacillus species	34	25.2
Staphylococcus aureus	28	20.7
Staphylococcus chromogens	04	3.0
Staphylococcus capitis	01	0.7
Staphylococcus haemolyticus	03	2.2
Staphylococcus hyicus	06	4.4
Staphylococcus xylosus	14	10.4
Pantoea agglomerans	04	3.0
Escherichia coli	03	2.2
Acetobacter haemolyticus	01	0.7
Salmonella Pullorum	01	0.7
Proteus vulgaris	02	1.5
Proteus mirabilis	20	14.8
Acinetobacter lwoffi	03	2.2
Providencia stuartii	06	4.4
Providencia rettgeri	02	1.5
Citrobacter diversus	01	0.7
Hafnia alvei	02	1.5
Total	135	100

Key: n= 135.

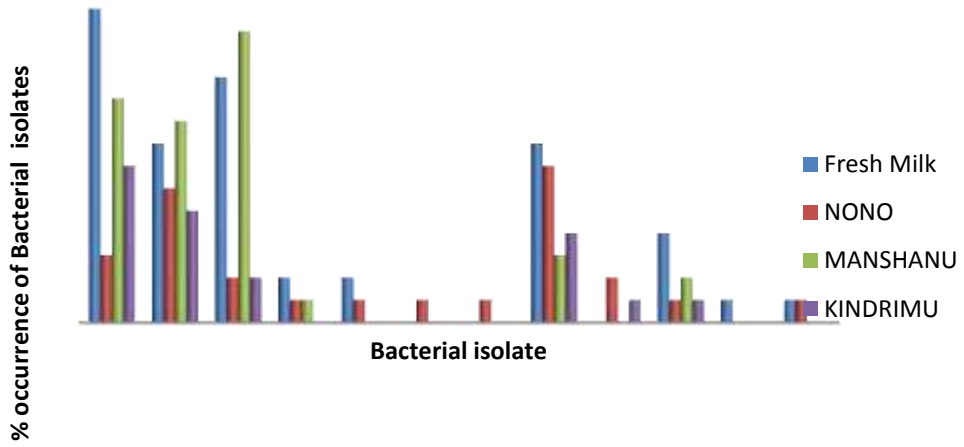

Figure 1: Distribution of bacterial isolates from fresh milk and milk products.

Table 2: Occurrence of Bacterial isolates in the different sampling locations.

Bacterial isolate	Location n(%) Giwa	Kaduna North	Chikun	Soba
Bacillus spp.	9 (6.67)	9 (6.67)	8 (5.9)	8 (5.93)
S.aureus	9 (6.67)	9 (6.67)	5 (3.7)	5 (3.7)
Other *Staphylococcus* spp.	9 (6.67)	8 (5.93)	1 (0.74)	10 (7.41)
Pantoea agglomerans	-	1 (0.74)	1 (0.74)	2 (1.48)
Escherichia coli	-	-	1 (0.74)	2 (1.48)
Acetobacter haemolyticus	-	-	1 (0.74)	-
Salmonella Pullorum	-	-	1 (0.74%)	-
Proteus spp.	1 (0.74)	8 (5.93)	4 (2.96)	9 (6.67)
Acinetobacter lwoffi	-	3 (2.22)	-	-
Providencia stuartii	3 (2.22)	1 (0.74)	2 (1.48)	-
Providencia rettgeri	1 (0.74)	-	1 (0.74)	-
Citrobacter diversus	-	-	1 (0.74)	-
Hafnia alvei	-	-	2 (1.48)	-
Total (%)	32 (23.70)	39 (28.9)	28 (20.74)	36 (26.67)

DISCUSSION

The results of this study revealed high incidence of contaminating organisms especially the enterobacteria. Enterobacteria are common inhabitants of the intestinal tract of various domestic animals including cow, and are commonly found in cow dung which was observed to be abundant at the milking environments, and therefore easy contamination of the milk as a result of poor sanitation of the milking environments. *Bacillus* and *Staphylococcus* are associated with food borne intoxication through production of enterotoxins, and the main agents involved here are *Bacillus cereus* and *Staphylococcus aureus* (both had high frequency of occurrence) (Ryser,1998). *Bacillus* spp. may have been introduced into milk from the soil; cattle feed, milking equipment, and the udder, while *Staphylococcus* spp. could come from the udder or skin of humans. *Bacillus spp*. has been implicated as the main reason for significant economic losses in the dairy industry (Brown, 2000). *Bacillus* spp. such as *B.licheniformis, B. cereus, B. subtilis, B. mycoides* and *B.megaterium* are said to be able to produce spores which can survive pasteurization (Ledenbach and Marshal,2009). Similarly, the enterotoxin produced by *Staphylococcus aureus* and some Coagulase negative Staphylococci (CNS) are also heat stable and can also survive pasteurization (Ledenbach and Marshal,2009). Apart from *Escherichia coli*, which has some toxin producing strains, the other food related pathogens identified in the milk samples such as *Proteus* spp. hardly produce toxin, neither do they form spores. *Proteus* spp. though isolated, their presence in milk sample is less frequent. However, being coliforms they may have occurred in milk samples probably as contaminants. Other bacteria species isolated include, *Acinetobacter,*

Enterobacter, Providencia, *Citrobacter* and *Hafnia alvei. Acinetobacter* species are known to cause ropiness of milk and secretion of extracellular enzymes both at psychrophilic and mesophilic temperatures (Uzeh *et al*., 2006).

The results also indicated *Staphylococcus aureus* as the most prevalent bacteria being implicated. The isolation of *Staphylococcus aureus* is of public health significance since it is said to be a commonly recovered pathogen in outbreaks of food poisoning due to milk and milk product (Junaidu *et al*., 2011).

The high frequency of occurrence of bacterial isolates in the four locations may not be unrelated to improper public health measures, sanitary and poor cleaning of people concerned with milk marketing. In addition, the primitive system of transportation and marketing practiced in these locations may also be predisposing factors. *Staphylococcus aureus* may originate from mastitic animals or human sources (Akram *et al*., 2013; Oliver *et al*., 2005). Soba had the highest occurrence of bacteria. This may have been due to the primitive systems of storage, transportation and marketing practices at the sampling sites which were the motor parks and the market places. Obviously, the quantities of bacterial contaminants in food products are related to these factors.

The presence of *Salmonella* in 'Nono'is in agreement with that of Junaida *et al*. (2011) who isolated 2(2.17%) in clinically mastitic cow and 2.6% overall prevalence reported by Van Kessel *et al.* (2004) from bulk tank milk. This was however higher than 0.0% prevalence reported by Mhone *et al.* (2012). The presence of this organism may indicate fecal contamination of milk but more importantly an indicator of poor sanitary practice during milking. The *Salmonella* spp. isolated was *S.pullorum. S.pullorum* is of the *Salmonella* serovar *Gallinarum* and is responsible for fowl typhoid and Pullorum disease of breeding flocks (Proux *et al.,* 2002). Although *S.pullorum* are considered to be adapted to birds, a few infections have been reported in mammals (Anderson *et al., 2006*). *S.pullorum* occasionally causes acute, self limiting enteritis in people who eat massively contaminated food (CFSPH, 2009) hence *S.pollurum* is not considered to be a serious public health concern.

CONCLUSION

The growth of these pathogenic organisms and their toxins in local dairy cattle products is an indication of poor sanitary practices in the production of fresh milk and its products. It is however noted that the types of organisms and their concentration in these fresh milk and milk products from the four studied locations should be of great concern to the health authorities as these pose serious public health problems to consumers. Safety of food consumers is however of utmost importance. All hands must be on deck to have this assured at all times.

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