Assessment of Microbial Quality of Cow Milk Products (Kindirimo, Nono and Wara) Sold in Bida Metropolis, Niger State, Nigeria.

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Abstract: The microbial assessment of three cow milk products sold in Bida metropolis, Niger State where analyzed. A total of 150 samples were collected from different individual (the Milkers) from Angwa-Fulani market and other part of Bida metropolis where other milk-products are sold. 50 samples were collected each for, Kindirimo, Nono and wara. Coagulase Negative *Staphylococcus* was the most predominant bacterial isolated with the percentage frequency of 22.0%, 10.0% and 6.0% for Wara, Nono and Kindirimo respectively, follow by *Escherichia coli* which had percentage frequeny of 14.0% for Wara only. *Streptococcus* species had percentage frequency of 12.0% for Wara only, then *Staphylococcus aureus* and *Lactobacillus* species had 10.0% and 2.0% for Wara and Nono respectively. Other bacteria isolated include *Proteus* species 10.0%, *Listeria* species 6.0%, *Pseudomona* species 2.0%, *Klebsiella* species 8.0%, *Enterobacter* species 4.0%, from Wara, *Alkaligens* Species 2.0%, from both Wara and Nono. From the findings of this study its concluded that cow milk product such as Wara are of poor microbial quality and hazardous for human consumption. This is supported by evidence of pathogenic bacteria isolated in this study which may be due to poor or unhygienic practices and surrounding environment during the processing of the product (wara). Also Nono and Kindirimo showed some level of poor microbial quality which may pose pathogenic zoonosis.

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1. Introduction

Milk is an important source of nutrients to human and animals. It is meant to be the essential food for the offspring of mammals as is almost complete food (Pandey and Voskuil, 2011). Cow milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria (Parekh and Subhash, 2008). When it is secreted from a healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter (Omore et al., 2005; Pandey and Voskuil, 2011). After milking environmental contamination occurs, which in turns increases the total bacteria count up to 50,000 per ml or may even reach several million bacteria per milliliter (Pandey and Voskuil, 2011).

Fresh milk drawn from a healthy cow normally contains a low microbial activity particularly with bacterial load of less than 103 cfu/ml (Chatterjee et al., 2006; Lingathurai et al., 2009), but the load may increase up to 100 fold or more once it is stored for some time at ambient temperature (Lingathurai et al., 2009). It contains lipids, proteins (casein, whey), carbohydrates (lactose), amino acids, vitamins and minerals (calcium), essential for growth (Haug et al., 2007; Javaid et al., 2009; Laba et al., 2013). Microbial contamination in cow milk may cause milk-borne diseases to humans while others are known to cause milk spoilage. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occur along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment's and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation and storage. There is tertiary microbial contamination which occurs mainly due to re-contamination of milk after being processed due to unhygienic conditions and/or poor or improper handling and storage of milk during consumption (Parekh and Subhash, 2008).

Nono is local uncontrolled fermented cow milk product which forms a major part of the marketable food in Northern part of Nigeria. They are produced mainly by the nomadic Fulani (Egwaikhide et al., 2014). It is produced from non-pasteurized cow milk collected in a container called calabash and allowed to ferment naturally for 24 hours (Eka and Ohaba, 1977; Olusupo et al., 1996; Makwin et al., 2014). It can also be produced by the addition of large volume of water to the curdle sour milk called "Kindirimo" which is

then stirred with a T-shaped stick to a liquid of fine consistency. The most commonly product often mixed with nono is called "Fura" (a dumping made of millet or maize) to made a preparation called fura da nono. (Egwaikhide et al., 2014).

Kindrimo is produced locally through pasteurization of cow milk which are prepared by heating to boiling point and allowed to cool at room temperature, and then milk butter from the previous day production is added to it at the rate of 0.5-1% of the amount of milk to be processed, and then left overnight to become sour until it coagulate (Odunfa, 1988; Makwin et al., 2014).

Wara is a white soft non-ripened cheese produced by the addition of extract from the plant (*Calotropis procera*) to the non-pasteurized whole milk from cattle (Adeyemi and Umar, 1994; Uzeh, et al., 2006). Common bacteria reported to be isolated from cow milk include *Staphylococcus* species, *Listeria* species, *Salmonella* species, *Escherichia coli*, *Campylobacter* species, *Mycobacterium* species, *Brucella* species, *Coxiella burnetii*, *Yersinia* species, *Pseudomonas aeroginosa* and *Corynebacterium ulcerans*. Others are *Proteus* species, *Leptospira* species, *Clostridium* species, *Streptococcus* species, *Klebsiella* species, *Enterobacter* species. and *Bacillus* species. (Shirima et al., 2003; Sivapalasingams et al., 2004; Al-Tahiri, 2005; Donkor et al., 2007; Parekh and Subhash, 2008).

Few examples of milk-borne diseases which are transmitted to consumers and pose a risk to public health are bovine tuberculosis, brucellosis, anthrax, listeriosis, salmonellosis, leptospirosis, Q fever, campylobacteriosis and E. coli O157:H7 as an emerged new milk-borne bacterial pathogen reported recently with a very serious health effects (Sivapalasingams et al., 2004).

According to U.S. Food and Drug Administration (FDA), theses harmful bacteria can seriously affect the health of anyone who drinks raw milk, or eats foods made from raw milk. However, the bacteria in raw milk can be especially dangerous to people with weakened immune systems, older adults, pregnant women, and children. In fact, the CDC analysis found that foodborne illness from raw milk especially affected children and teenagers.

Therefore, proper milking, cleaning and sanitizing procedures of equipment and environment used are important tools to ensure quality of milk and milk products. Many countries have implemented laws and regulations concerning the composition and hygienic quality of milk and milk products to protect both the consumers and the public health (Pandey and Voskuil, 2011).

2. Material and Methods

A total of 150 samples were collected from different individual (the Milkers) from Angwa-Fulani market and other part of Bida metropolis where other milk-products are sold. 50 samples were collected each for, Kindirimo, Nono and wara. Approximately 10mls of each samples were aseptically collected into sterile screw capped sterile universal sample bottle. Cow milk products (Kindirimo, Nono and Wara) was drawn from pooled containers containing milk products which are sold to the public within Bida Metropolis. All samples were coded with random numbers for identification and stored in a cool box with ice packs and transported to the Laboratory for analysis.

One milliliter of each sample (Kindirimo, Nono and Wara) was inoculated into a Nutrient broth and incubated at 37^{o}C for 18-24 hours. After incubation, the inoculated Nutrient broth was sub-cultured onto plates of blood agar, Chocolate agar, MacConkey agar, and salmonella and shigella agar, and further incubated at 37^{o}C for 18-24 hours. The colonial appearance of bacteria colonies isolated were evaluated, gram stained and further subjected to different biochemical test for complete analysis.

Catalase Test: This test differentiates catalase-positive micrococcal and staphylococcal species from catalase-negative streptococcal species. It is also used to defferntiate some other organism such as Listeria specias, Enterobacteriaceae (*Citrobacter* species, *Escherichia coli*, *Enterobacter* species,) etc.

Coagulase Test: The test was used to differentiate *Staphylococcus aureus* (positive) from coagulase-negative *staphylococci* (negative).

Indole Test: This test was used to identify organisms that produce the enzyme tryptophanase. E.g. *Escherichia coli* etc.

Oxidase Test (Kovac's Method): This test determines the presence of cytochrome oxidase activity in microorganisms for the identification of oxidase-negative Enterobacteriaceae, differentiating them from other gram-negative bacilli. E.g. *Pseudomonas aeruginosa*, *Alcaligens,* and *Campylobacter* species.

Triple Sugar Iron Agar (TSI): TSI was used to determine whether a gram negative rod ferments glucose and lactose or sucrose and forms hydrogen sulfide (H_2S). The test is used primarily to differentiate members of the Enterobacteriaceae family from other gram-negative rods. E.g. *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri, Proteus mirabilis* etc.

Auramine O: is a diarylmethane dye used as a fluorescent stain. it was used for identification of *Mycobacterium* species.

3. Results

Table 1 shows the percentage occurrence frequency of bacteria isolates from three different cow milk products (Wara, Nono and kindirimo). Coagulase Negative Staphylococcus was the most predominant bacterial isolated with the percentage frequency of 22.0%, 10.0% and 6.0% for Wara, Nono and Kindirimo respectively, follow by *Escherichia coli* which had percentage frequeny of 14.0% for Wara only. *Streptococcus* species had percentage frequency of 12.0% for Wara only, then *Staphylococcus aureus* and *Lactobacillus* species had 10.0% and 2.0% for Wara and Nono respectively. Other bacteria isolated include *Proteus* species 10.0%, *Listeria* species 6.0%, *Pseudomona* species 2.0%, *Klebsiella* species 8.0%, *Enterobacter* species 4.0%, from Wara, *Alkaligens* Species 2.0%, from both Wara and Nono.

Table 1: Frequency of Bacteria Isolates identified in 150 Cow milk products (Kindirimo, Nono and Wara) samples collected within Bida metropolis.

Isolates	Wara (%) (N=50)	Nono (%) (N=50)	Kindirimo (%) (N=50)	Total (%) (N=150)
Escherichia coli	14.0	0	0	14.0
Proteus species	10.0	0	0	10.0
Pseudomonas species	2.0	0	0	2.0
Staphylococcus aureus	10.0	2.0	0	12.0
Coagulase Negative *Staphylococcus*	22.0	10.0	6.0	38.0
Klebsiella species	8.0	0	0	8.0
Enterobacter species	4.0	0	0	4.0
Streptococcus species	12.0	0	0	12.0
Lactobacillus species	10.0	2.0	0	12.0
Listeria species	6.0	0	0	6.0
Alkaligens species	2.0	2.0	0	4.0

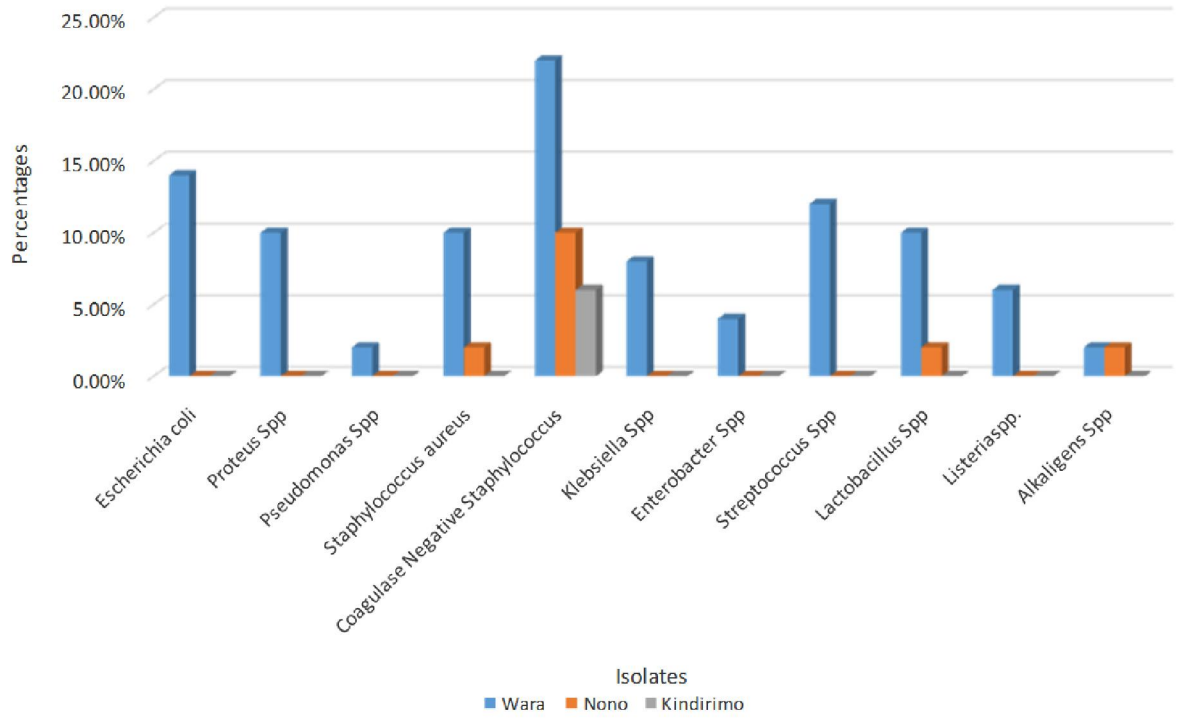

Figure 1. Bar Chart showing the occurrence of bacteria isolated from three different cow milk products (Wara, Nono and kindirimo).

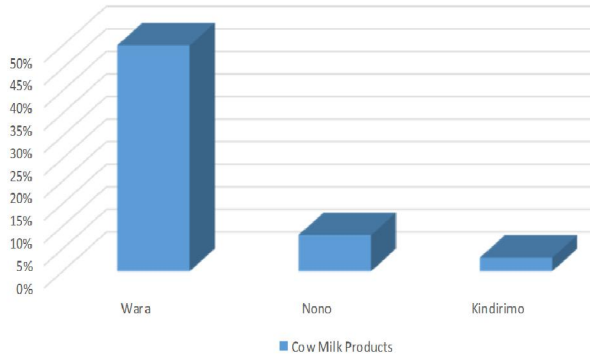

Figure 2. Bar Chart showing the incidence of bacteria isolate in Wara, Nono and Kindirimo.

4. Discussions

This study was designed to assess the bacterial quality of cow milk products (Wara, Nono and Kindirimo) sold within Bida metropolis, Niger state, Nigeria. This is due to the fact that cow milk products produced and sold by nomadic Fulanis in bida and other parts of Nigeria are not regulated by any agency e.g. NAFDAC (National Agency for Food and Drug Administration and Control), SON (Standard Organization of Nigeria), FDA (Food and Drug Administration) etc. and such products may pose health hazard due to the contamination with pathogens. Findings showed that there had been a misconception regarding the pathogenic contamination associated with cow milk products among the consumers and non-consumers. This is due to the fact that Fulani women who market the products are known to be unhygienic in some of their domestic practices such as water used in cleaning hands and milking equipment, storage containers and duration of storage which may increase the risk of contaminating the products.

This study isolated bacteria that are members of the enterobacteriaceae (Such as *Escherichia coli* 14.0%, *klebsiella* species 8.0%, *Proteus* Species10.0% and *Enterobacter* species 4.0%) which are example of probable fecal contamination from water used to process the products. Coagulase negative *staphylococcus* has the highest percentage (22.0%, 10.0% and 6.0% in Wara, Nono and Kindirimo respectively) which may be due to contamination from hand and containers used for storage. The presence of bacteria such as *Alkaligenes*, *Bacillus*, *Enterobacter* and *klebsiella* specias shows similarity with these findings of Roseline et al., 2006 (Microbiological and Nutritional Qualities of Dairy Products: Nono and Wara) in Lagos, Nigeria.

The isolation of *Listeria* species 6.0% (from Wara) in this study are similar to previous studies that reported presence of *Listeria* species in raw cow milk by (Yakubu et al., 2012) in Nigeria and (Sharma et al., 2012) India. All these bacteria contaminations are causative agent of disease condition such as Gastroenteritis, Listeriosis etc. and also food born intoxication which pose a major threat to public health.

From the findings of this study its concluded that cow milk product such as Wara are of poor microbial quality and hazardous for human consumption. This is supported by evidence of pathogenic bacteria isolated in this study which may be due to poor or unhygienic practices and surrounding environment during the processing of the product (wara). It's advisable that individual who consume this product should heat it (the Wara) to boiling point as it reduced the microbial Also Nono and Kindirimo showed some level of poor microbial quality which may pose pathogenic zoonosis.

Routine assessment of microbial quality of cow milk products consumed by the general public has to be encourage in other to safeguard the public from zoonotic infection. This should be done through the involvement of authorities such as NAFDAC, WHO, SON, FDA etc. with the help of Medical Laboratory Scientist.

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