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Research Article
Evaluation of Pathogenic Bacteria in Packaged Milk Products Sold in Sokoto Metropolis, Nigeria

M.I.O. Raji and M.H. Jiya

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Abstract

Background and Objective: Packaged milk products mostly being referred to as yogurts are fermented milk products produce by bacteria fermentation of milk and are being consumed all over the world. Fermented milk products are liable to microbial contamination as well as the fresh milk from which they are produced and consumption of contaminated milk is of great health risks. The current study investigated pathogenic bacteria contaminating packaged milk products sold in Sokoto metropolis, Nigeria in order to create awareness for people on the inherent health risks associated with consumption of contaminated milk products. **Materials and Methods:** Twenty seven packaged milk product samples were bought from three different locations (9 samples for each location) in Sokoto metropolis and analyzed. The bacterial load of packaged milk samples obtained by standard plate count method showed that Brand 2 milk samples from Runjin Sambo area had the highest counts ranging from 24.0-368.0×10^6 CFU mL^{-1}. Isolation of bacteria was achieved by culturing on different selective media while identification of the isolated bacteria was done by cellular morphology under a light microscope, colonial morphology on selective media and biochemical tests. **Results:** Forty five pathogenic bacteria comprising 9 different species namely: *Salmonella* spp., *Shigella* spp., *E. coli*, *Serratia* spp., *Providencia* spp., *Pseudomonas* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Bacillus subtilis* were identified from the milk samples. Majority of the pathogenic bacteria were Gram-positive organisms with 30.4% *Bacillus subtilis* and 21.8% *Staphylococcus aureus*. Among Gram-negative organisms, *E coli* had the highest percentage of 15.2%. **Conclusion:** The findings of the study showed that packaged milk products sold in certain areas of Sokoto metropolis contained pathogenic bacteria and therefore, pose a serious health risk to the consumers. To ensure proper quality of packaged milk products, there should be a thorough check and control on the methods through which milk products are being produced and sold in local markets and major streets.

Key words: Pathogenic bacteria, packaged milk products, microbial contamination, health risk, Sokoto metropolis

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Corresponding Author: M.I.O. Raji, Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria Tel: +2348032845124

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Packaged milk products popularly referred to as yogurts are fermented milk products produced by bacteria fermentation of milk and are consumed throughout the world. The French called it 'la lait de la vieeternelle'-the milk of eternity as it was believed to have therapeutic powers and gave long life to those who consumed it. Yogurt is high in protein and calcium and may be easier for people with lactose intolerance to digest because of its active cultures. Yoghurt is made by the fermentation or the addition of healthy bacteria and live cultures to milk. It is a means of protein intake for an improved healthy living[1]. The bacteria used to make yogurt are known as yogurt cultures. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and its characteristics. *Lactobacillus delbrueckii* subsp*. bulgaricus* is commonly used alongside *Streptococcus thermophilus*[2] as a starter for making yoghurt. The two species work in synergy with *Lactobacillus delbrueckii* subsp. *bulgaricus* producing amino acid from milk proteins which are then used by *Streptococcus thermophilus*[2]. Both species produce lactic acid which gives yogurt its tart flavor and acts as a preservative. The resulting decrease in pH also partially coagulates the milk proteins, such as casein, resulting in yogurt thickness[3].

Milk is one of the most important sources of energy, calcium and protein that are considered for infants and young children who have no any other alternative for these nutrients. Apart from its beneficial effects, milk borne illnesses have been recognized since last two decades[4].

Fermented milk, like the fresh milk from which they are produced is liable to contamination. Molds and yeast are the primary contaminants in yogurt produced commercially in Nigeria. Molds and yeasts growing in yogurt utilize some of the acid and produce a corresponding decrease in the acidity, which may favour the growth of putrefactive bacteria[5].

Yogurts are frequently contaminated by bacteria and this often leads to food intoxication/poisoning. The bacteriological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions and post-heat treatment contamination.

In Nigeria, many researchers have reported milk products to be contaminated with several bacterial pathogens such as *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Salmonella* spp[6].

Undesirable bacteria that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria. In addition, various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli*, *Bacillus subtilis* and enterotoxigenic strains of *Staphyloccocus aureus* may also be found in milk and dairy products[7]. Recently, there have been numerous outbreaks of milk-borne diseases in humans with pathogens such as *S. aureus*, *E. coli*, *Salmonella* spp., *Yersinia* spp. and *Enterobacter spp* being implicated, especially since mass production came into effect[8]. Milk can act as a vehicle for the transmission of bacterial diseases such as, salmonellosis, *E. coli* infections, cholera, brucellosis, streptococcal infections and listeriosis. Infection with enteropathogenic *E. coli* usually, results in mild illness, however, some serotypes are enterohemorrhagic and can lead to hemolytic uremic syndrome. *Escherichia coli* 0157:H7 is the most common enterohemorrhagic strain[9], which have been reported to cause acute kidney failure in children in the United States[10]. Because *Salmonella* and *E. coli* 0157:H7 are shed in the animal's feces, there is a risk of these pathogens entering the milk[9]. Diarrhea disease has been a major public health problem causing high morbidity and mortality among children for many years[11]. *Salmonella* causes diseases ranging from diarrhea to septicemia. As milk and milk products play an important role in human nutrition throughout the world, the products must be of high hygienic quality. In less developed areas and especially in hot tropics, high quality of safe product is most important but not easily accomplished[12]. This is required since milk is also a suitable substrate for microbial growth and development, thus the reason why milk and milk products are more prone to the harbouring and proliferation of micro-organisms. And this is the reason for embarking on this study to evaluate the pathogenic bacteria in milk products sold in some areas in Sokoto metropolis in order to create awareness for people on the high risk associated with consuming substandard or unhygienic milk products containing pathogenic organisms and the health complications associated with it.

MATERIALS AND METHODS

Study area: The research was carried out in Sokoto metropolis located in the northwest of Nigeria on latitudes 11030°N and 14000°N and longitudes 4000°E and 6040°E.

Study location: Three different brands of yogurt were obtained from three different retail outlets in Sokoto metropolis namely Unguwar rogo Market, Runjin Sambo Market and Sokoto Central Market.

Sampling: Three different brands of yogurt in plastic bottles were randomly selected among yogurts sold in Sokoto metropolis. Three units of 3 brands of the yogurt sample were purchased making a total of 9 samples per location. A total of 27 yogurt samples were purchased from the three different markets with different batch number, manufacturing and expiry dates. The three brands, two of which were manufactured in Kaduna state and the 3rd in Sokoto state, were still within their expiry date. Samples were collected using sterile polythene bags and transported to the laboratory for analysis.

Media preparation and sterilization of culture media: Each bacteriological medium used was prepared from commercially available powder according to the manufacturer's instructions. Sterilization was by autoclaving at 121°C for 15 min.

Determination of bacterial load: Each plastic bottle was swabbed with 75% ethanol before opening. Total aerobic count was carried out using standard plate count method. With the aid of a sterile pipette, 1 mL of the sample was transferred aseptically into the first tube containing 9 mL of diluents (normal saline) to make a ten-fold serial dilution. One milliliter of the appropriate dilutions (10^{-4}-10^{-6}) were pipetted and poured correspondingly into triplicate of appropriately labeled Petri-dishes. Melted Nutrient agar (15 mL) at 45°C was then carefully poured onto the sample within the dish and the plate was swirled gently for the sample to mix with the agar. This was repeated for the remaining samples. The plates were then incubated at 37°C for 24 h. Colonies obtained were counted using a colony counter and value multiplied by dilution factor to get real bacterial count expressed in colony forming units per milliliter (CFU mL^{-1}).

Identification of isolates: Pure cultures of bacterial isolates were obtained from the primary culture after sub culturing using NA plates which were incubated overnight at 37°C. Upon sub-culturing, bacteria that grew as discrete colonies were stored on nutrient agar slants for further characterization and identification[13]. The cellular morphology under a light microscope, colonial morphology on selective media and biochemical test were carried out.

Microscopic examination of isolates: The morphology of the isolates was determined by carrying out Gram's stain procedure which under microscope distinguishes organisms into Gram-positive or Gram-negative.

Selective culturing and identification of isolates: The results obtained from the gram stain reaction were then subjected to further identification by streaking on selective media. Overnight cultures were grown on nutrient broth and a loopful of inoculum from the nutrient broth was streaked on selective agar and incubated at 37°C for 24 h. Mannitol salt agar (MSA) was used for isolation of *Staphylococcus aureus*, Cetrimide agar for isolation of *Pseudomonas species*, Eosin Methylene Blue (EMB) agar for *E. coli*, *Salmonella shigella* Agar (SSA) for *Salmonella* species and MacConkey agar for isolation of other Enterobacteriaceae present in the milk sample. On MSA, colonies that appeared yellowish were presumptively identified as *Staphylococci* while greenish colonies on cetrimide agar were identified as *Pseudomonas* species. Colonies that produced greenish metallic sheen on EMB were presumptively regarded as *E. coli* while colourless colonies with black spot on SSA were identified as *Salmonella* species. Based on lactose fermentation properties Enterobacteriaceae organisms were isolated on MAC agar.

Presumptively identified organisms were sub-cultured on nutrient agar slant, incubated at 37°C for 24 h and stored in refrigerator at 4°C pending further analysis.

Biochemical tests: The following biochemical tests were carried out to identify the isolated organisms using the method described by Cheesbrough[14] and Oyeleke and Manga[15], sugar fermentation test, indole test, oxidase test, catalase test, coagulase test, citrate utilization test, Methyl Red Voges Proskauer test, triple sugar iron (TSI) test and H_2S production. A 24 h, overnight culture of the test organisms were prepared on either nutrient agar or in nutrient broth before caring out any of the biochemical tests.

RESULTS

Bacterial load: The bacterial load of packaged milk samples obtained from three different locations namely Unguwar Rogo, Runjin Sambo and Sokoto Central Markets ranges from 8.0-104.0×10^6, 24.0-368.0×10^6 and 24.0-128.0×10^6 CFU mL^{-1}, respectively for Brand 1, Brand 2 and Brand 3 milk samples as shown in Table 1. It was further shown in the table that the mean counts for the three brands of packaged milk products were 140.1±0.9×10^6, 652.9±0.8×10^6 and 260.0±0.0×10^6 CFU mL^{-1}, respectively with Brand 2 having the highest counts.

Table 1: Total bacteria count of packaged milk samples from three locations in sokoto metropolis, Nigeria

	Location 1		Location 2		Location 3			
Milk brands	Milk sample	Bacteria count ($\times 10^6$ CFU mL^{-1})	Milk sample	Bacteria count ($\times 10^6$ CFU mL^{-1})	Milk sample	Bacteria count ($\times 10^6$ CFU mL^{-1})	No. of samples	Mean count ($\times 10^6$ CFU mL^{-1})
Brand 1	B 1,1	24.0	B 1,2	28.0	B 1,3	40.0	3	30.7±0.8
	B 2,1	76.0	B 2,2	104.0	B 2,3	8.0	3	62.7±0.3
	B 3,1	44.0	B 3,2	52.0	B 3,3	44.0	3	46.7±0.8
Total							9	140.1±0.9
Brand 2	S 1,1	54.0	S 1,2	92.0	S 1,3	176.0	3	107.3±0.0
	S 2,1	368.0	S 2,2	39.0	S 2,3	76.0	3	432.3±0.6
	S 3,1	264.0	S 3,2	24.0	S 3,3	52.0	3	113.3±0.2
Total							9	652.9±0.8
Brand 3	H 1,1	128.0	H 1,2	120.0	H 1,3	112.0	3	120.0±0.5
	H 2,1	68.0	H 2,2	100.0	H 2,3	68.0	3	78.7±0.1
	H 3,1	80.0	H 3,2	80.0	H 3,3	24.0	3	61.3±0.4
Total							9	260.0±0.0

CFU: Colony forming unit

Table 2: Distribution of bacterial organisms in different brands of packaged milk samples

	Milk samples			
Isolates	Brand 1	Brand 2	Brand 3	Frequency
Salmonella spp.	-	2	-	2 (4.3)
Shigella spp.	-	3	1	4 (8.7)
E. coli	1	4	2	7 (15.2)
Serratia spp.	-	1	-	1 (2.2)
Providencia spp.	-	2	-	2 (4.3)
Pseudomonas spp.	2	2	1	5 (10.9)
Enterobacter spp	1	-	-	1 (2.2)
Staphylococcus aureus	2	6	2	10 (21.8)
Bacillus subtilis	7	4	3	13 (30.4)
Total	13	24	9	45.0

Identification and distribution of isolated organisms: Based on cultural, morphological and biochemical characteristics of the organisms isolated, a total of 9 bacterial species comprising 45 isolates were identified in the 27 packaged milk samples studied.

Bacillus subtilis among Gram-positive organisms had the highest percentage (30.4%) followed by *Staphylococcus aureus* (21.8%) while *Escherichia coli* (15.2%), *Pseudomonas aeruginosa* (10.9%), *Shigella* spp. (8.7%), *Salmonella* and *Providencia* spp. (4.3%), *Serratia* and *Enterobacter* species (2.2%) were bacteria from Gram-negative organisms in descending order as shown in Table 2. Gram's reaction revealed that the isolates were made up of Gram-positive (52.2%) pre-dominantly and Gram-negative rods (47.8%) were within a close range as further shown in Table 2.

DISCUSSION

The results obtained from the bacteriological analysis of packaged milk samples obtained from Unguwar Rogo, Runjin Sambo and Sokoto Central Market showed that the products were grossly contaminated with bacteria of public health concern. The bacterial load counts from yogurt samples in this study range from 8.0×10^6-368.0×10^6 CFU mL^{-1}. The high level bacterial counts in the yogurt samples could be as a result of low level 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[16]. On the other hand, 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 yogurt samples studied as stated in International Dairy Federation[17] and Adesiyun *et al.*[18]. The detection of Gram-negative organisms such as *Escherichia coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Serratia* spp., *Pseudomonas* spp and *Providencia* spp. in the studied milk samples, probably indicates possible faecal contamination[19] due to poor hygienic practices among handlers of these products since the organisms are enteric bacteria. Isolation of similar bacteria from milk products has been previously reported[8,20]. The presence of these bacteria in milk also suggests contamination from various sources, which may include animal, human, environment, utensils and others[21].

Isolation of *E. coli* could be due to faecal contaminated water used in milk production, raw materials or storage environment. Consumption of milk products contaminated with *Escherichia coli* is capable of causing diarrheal diseases, urethrocystitis, prostatitis and pyelonephritis[22,23].

Other bacteria isolated in this study include *Pseudomonas* species and *Staphylococcus aureus*, similar to the finding of Gilmour and Rowe[24]. *Pseudomonas* species have been implicated in the spoilage of milk and its products at even refrigerator temperatures[24]. *Pseudomonas* has been implicated 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 pasteurized milk[25]. Detection of *Pseudomonas* spp. could 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*.[26]. The detection of *Staphylococcus aureus* and *Bacillus subtilis* implied contamination from personnel and the environment respectively and this is of public health importance because of their ability to cause a wide range of infections especially food-borne intoxication. This is buttressed by the assertion made in several reports that *Staphylococcus aureus* has link with gastroenteritis by producing toxic chemical enterotoxins[27-29]. *Staphylococcus aureus* has been reported to be 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 production practices, handling and packaging or post pasteurization contamination[30].

The current study supports many existing literature to ascertain presence of pathogenic bacteria in packaged milk products and also creates awareness for people on the high risk associated with consuming contaminated milk products because of the health complications associated with it.

CONCLUSION

The findings of the study revealed that packaged milk products sold at Unguwar Rogo, Runjin Sambo and Sokoto Central Markets in Sokoto metropolis contained pathogenic bacteria namely *Salmonella* spp., *Shigella* spp., *E coli*, *Serratia* spp., *Providencia* spp., *Pseudomonas* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Bacillus subtilis* which have been reported capable of causing various illnesses and thereby pose serious health risks to the consumers. It is therefore recommended that in order to ensure proper quality of packaged milk products, there should be a thorough check and control on the methods through which packaged milk products are being produced, handled and sold in local markets and major streets.

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