Microbiological Quality of Milk Powder Packed in Sudan and Their Antibiotic Susceptibility

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Abstract
In this study the microbiological quality of milk powders packed in Sudan has evaluated by method of enumeration of total viable bacteria, coliform, Escherichia coli and Staphylococcus aureus. Then the antibiotic susceptibility of pattern bacterial isolates has been assessed. Eight samples of different brands packed in Sudan have collected. The highest total bacterial count (5.3×10³ cfu/ml) was found in sample no-8, and the lowest one (3.4×10² cfu/ml) in sample no-4, but they were found free from all other microbes which were tested. Some pathogenic bacteria were isolated from the samples. Bacillus spp. was the most prevalent isolate (46.2%) followed by Streptococcus spp. (30.8%), Staphylococcus spp. (15.4%) and Micrococcus spp. (7.7%). All bacterial isolates were found susceptible to Cefuroxime and Ceftriaxone, but they were resistant to Nalidixic acid, Cotrimoxazole and ampicillin. The practice of packing milk powders should discourage as the final products contaminated with consequent public health risk.

Keywords: Milk; Powder; Brands; Antibiotics; Bacteria; Staphylococcus Aureus; Ceftriaxone; Nalidixic Acid; Escherichia Coli; Buttermilk; Lactic Acid Bacteria

Introduction
Milk powder and cream powder are milk products which can be obtained by the partial removal water from milk or cream. One reason for drying milk is to preserve it. Milk powder has a longer shelf life than liquid milk and does not need to be refrigerated, due to its low moisture content. Another reason is to reduce its bulk for economy ease of transportation. Powdered milk and dairy products include such items as dry whole milk, non-fat (skimmed) dry milk, dry butter milk, dry whey products and dry dairy blends. Milk is a part of daily diet for adults (especially expectant and breast-feeding mothers) and growing children [1]. Milk is highly valued because it provides important source of many of the nutrients required for the proper development and maintenance of the human body [2]. Milk is highly nutritious and serves as an excellent growth medium for a wide range of microorganisms contaminating it. The microbial quality of milk and other dairy products influenced of the initial flora of raw milk, the processing addition, and post-thermal treatment concentration.

Microorganisms that can cause spoilage of dairy products include Gram-negative psychographs, coliforms, Lactic acid bacteria, yeasts, and moulds. Furthermore, various bacteria of public health concern such as Salmonella spp., Listeria Monocytogenes, Compylobacter jejuni, Versinia enterococcalita, Pathogenic strains of Escherichia coli and enterotoxigenic strains of Staphylococcus aureus many also be found in Milk [2,3].

Therefore, the microbiological examination of milk and dairy products cannot be over emphasized. Microbiological quality analysis is crucial for the assessment of quality and safety. Conformation with standards and specifications and regulatory compliance. Pathogen bacteria of public health significance in
milk have been a major concern since the early days of the dairy industry, as many diseases are transmissible via milk products traditionally raw or unpasteurized milk has been giving more attention as a major for transmission of pathogens [1]. According to the standards [4-7]. Whole milk powder should contain more than 10 cfu/gm of coliforms and yeasts and moulds, <1 cfu/gm of E. coli and 50,000 cfu/gm (maximum) of total bacterial count, and both salmonella and Staphylococcus aureus should be absent in 25gm of the product. The consumption of whole milk powder increased in the last few years due to shortage of fresh milk. This situation encouraged some investors import powdered milk in large size bags (25 kg) and repack into small size bags to be distributed in the retail markets. Most plants repack the milk in aluminum bags, while some milk powders are packed metal containers. The study was aimed at evaluating the microbiological quality of milk packed in Sudan and to assess the antibiotic susceptibility pattern of bacteria isolates.

Material and Methods

Sample Collection

A total of eight (8) of milk powder samples 200gm from different commercial brands were purchased from the supermarkets in Khartoum state, Sudan. All the samples were packed (in aluminum foil bags) in Sudan. The milk powder of Nido brand was imported from Netherlands and used as control. The samples were transported to the central Laboratory, Faculty of Agriculture University of Khartoum, Sudan for analysis. The samples were aseptically opened and immediately subjected to microbiological analysis.

Experimental Procedures

Preparation of Milk Samples

A tenfold serial dilution up to 10-6 for each sample were prepared in 0.1% peptone water was obtained [8].

I Numeration of Total Viable Bacteria

For the determination of TVC, 1 ml of each dilution was transferred using sterile pipette and spreader on plate count Agar sample. The Petri dish was then kept in a cubature at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVC. The TVC was expressed as several organisms of colony forming units per ml (CFU/ml) of samples according to [9].

I Numeration of Total Coliform Bacteria

It was performed by most probable number (NPN) Technique (Harrigan and Mac Cance,1976) using Mac Con key broth. And (MPN) value was calculated from a most Probable Number (MPN) table.

I Numeration of Escherichia coli:

E. coli was enumerated on selective medium Eosin Methylene Blue (EMB)Agar and incubated at 37°C for 24 hours.

I Numeration of Staphylococcus Aureus

Enriched samples were streaked on baited parked Agar (BPA) and the plate was incubated at 37°C for 24-48 hours [10].

I Numeration of Salmonella Spp:
The medium used for salmonella spp. Was Sabouraud Dextrose Agar (SDA). 1ml of each dilution milk sample was transferred using sterile pipette into two tubes of (1) Selenite Cysteine broth inoculated for 24hr at 35°C. (2) Tetrathionate Brilliant Green Broth (TBG) inoculated for 24hr at 42.5 After incubation, broth was streaked onto xylos Lysine. Desoxychollate (XLD) agar and incubated for a further 24 hours at 37°C [11].

I Yeast and Moulds Counting Method:
The Medium for yeasts and moulds was Sabouraud Dextrose Agar (SDA). 1ml of each dilution milk samples were transferred using sterile pipette on (SDA), using a sterile petri dish for each sample. The dishes were than kept in an incubator at 27°C for 3-5 days [12]. All plates in all experiments mentioned above were in duplicate.

Characterization and Identification of Isolates:

Characterization and identification of the isolated bacteria were carried out based on either colonial, morphological or biochemical characteristics with reference to bergeys Manual of Systematic bacteriology [13].

Antibiotic Susceptibility Testing:

Antibiotic susceptibilities of bacterial isolate were determined according to method recommended by the clinical and laboratory standards institute 2007 [14]. Briefly, inocula were prepared for each bacterial isolate by adjusting, the turbidity to 0.5 MC farland standard and pried on muller-Hinton agar plates. Antibiotic discs (Himedia and Mumbai, India) were placed on the agar plates and incubated overnight at 7°C for 24 hours. The 20nes of inhibition were measured and the isolates were classified as sensitive, moderately sensitive, intermediate, and resistant according to CLSI tables and guidelines.

The following antibiotics with the disc strength in parentheses were used:

Ciprofloxacin (5mg), Nalidixin acid (30mg), Cefotaxime (30mg), Cefuroxime (30mg), Azithromycin (15mg), Imipenem (10mg), Cephalexin (30mg), Ceftriaxone (30mg), Cotrimoxazole (25mg), Ampicillin (10mg), Erythromycin (15mg). A control strain of E. coli ATCC25922 was included in each plate.
Results and Discussion

Total Viable Bacterial Count

The highest total viable bacterial count (5.3 × 10^3 cfu/ml) was found in sample no-8 and the lowest one was 3.4 × 10^2 cfu/ml in sample no-4 compared to the count of bacteria in Nido (3 × 10) table 1. The variations in the microbial composition of the sample in this study either regards to location may be due to the level of unhygienic environmental conditions and handling of the repacked milk powder milk powder and their multiplication would however depend on favorable environmental conditions like time, temperature, relative humidity, and duration of storage as well as parameters such as potential water activity (aw), moisture content, nutrients present [15,16]. Total coliforms, E. coli, S. auers, Salmonella spp. and yeasts and moulds were not found in all milk powders which have been tested (Table 1), thus, they were considered microbiologically safe. Milk powder is generally considered a product of good microbiological quality [17] it made from good quality milk and containing low microbial count and the moisture content is kept low [18].

| Sample | Total viable count (cfu/ml) | Total coliform count (MPN/100ml) | E. coli count (MPN/100ml) | Staphylococcus count (cfu/100ml) | Salmonella count (cfu/ml) | Yeasts and moulds count (cfu/ml) |
|--------|-----------------------------|---------------------------------|--------------------------|---------------------------------|--------------------------|-------------------------------|
| 1      | 5.0 × 10^2                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 2      | 3.8 × 10^2                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 3      | 3.3 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 4      | 3.4 × 10^2                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 5      | 3.6 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 6      | 4.3 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 7      | 4.5 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 8      | 5.3 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |
| 9      | 3.1 × 10^3                  | Nil                             | Nil                      | Nil                             | Nil                      | Nil                           |

Key: 9=Nido sample (control)

Percentage of Prevalence Bacterial Isolates

Four genera of bacteria have been isolated from the packed milk powder, Bacillus spp. was the most prevalence pathogens (46.2%) followed by Streptococcus spp. (30.8%), Micrococcus spp. (15.4%) and Staphylococcus aureus (7.7%). (Table 2). Isolation of bacillus spp.is attributed to the presence of them in the environment. In addition, they are spore- forming bacteria, which made them to survive high temperatures of processing. Also, they are able to inhabit soils and vegetation and have been repeatedly isolated in several countries from wide variety of foods [19,20]. Most strains of bacillus are pathogenic for man, affect them incidentally. A notable exception is Bacillus anthracis, which caused anthrax in human and domestic animals and Bacillus thuringiensis, which caused disease in insects [21].

| Isolates       | No. of isolates | Percentage (%) |
|----------------|-----------------|----------------|
| Bacillus spp.  | 6               | 46.2           |
| Streptococcus spp | 4              | 30.8           |
| Micrococcus spp | 2               | 15.4           |
| Staphylococcus aureus | 1             | 7.7            |

Many researchers [22,23] have reported that the presence of Staphylococcus and Streptococcus spp. were possible contaminants from handlers. Streptococcus spp. has been implicated in Human infections like Pharyngitis, scarlet fever, and pneumonia. Staphylococcus aureus, a mesophile bacteria have been beplicate in food poisoning outbreak of some good material, special interest is the ability of S.aureus to elaborate enterotoxins food, which is dangerous to human and other animal health [24]. All bacterial isolates in this study have a health implication for man except Micrococcus spp, which have not been associated with human infection [25].

Antibiotic Susceptibility Test

All the bacterial isolates were susceptible to cefuroxime and ceftaxone but were found resistant tonalidixic acid, cotrimoxazole and ampicillin. Varied results of resistance, intermediate susceptible
and moderate susceptibilities are observed with the other tested antibiotics (Table 3). Multiple antibiotics resistance (MAR) has been widely reported in Bacteria [26-28]. Indexes of multiple antibiotics resistance are revealed the spread of resistance in each microbial population, it implied that the strains of such bacteria originate from an environment where several antibiotics are used. Resistance to antimicrobial agents is a public health threat and increasingly coming a global problem. Also, it limited therapeutic options and lead to increase of mortality and morbidity. Infection with antibiotic resistant bacteria make the therapeutic treatment against infection extremely difficult or virtually impossible in some instances [25] (Table 1-3).

Table 3: Antibiotic susceptibilities of bacterial isolation from milk powder packed in Sudan.

| Antibiotics       | Disk | Bacillus Spp | Streptococcus | Micrococcus Spp | Staphylococcus Spp |
|-------------------|------|--------------|---------------|-----------------|-------------------|
| Ciprofloxacin     | 5mg  | 13®          | 10®           | 27(s)           | 26(s)             |
| Nalidixic acid    | 30mg | 0            | 0             | 0               | 0                 |
| Cefotaxime        | 30mg | 0            | 17(MS)        | 21(s)           | 25(s)             |
| Cefuroxime        | 30mg | 25(S)        | 24(S)         | 29(s)           | 38(s)             |
| Azithromycin      | 15mg | 12®          | 0             | 22(S)           | 24(s)             |
| Imipenem          | 10mg | 10®          | 21(S)         | 25 (s)          | 28(s)             |
| Cephalexin        | 30mg | 0            | 10®           | 20 (s)          | 21(s)             |
| Ceftriaxone       | 30mg | 23(S)        | 19(S)         | 32(s)           | 39(s)             |
| Cotrimoxazole     | 25mg | 0            | 0             | 12®             | 11®               |
| Ampicillin        | 10mg | 0            | 0             | 17®             | 11®               |
| Erythromycin      | 15mg | 0            | 14(I)         | 12®             | 13®               |

Keys: mg=Micro gram; mm: millimetre; R= Resistant; I= Intermediate; S=Susceptible; MS= Moderately Susceptible; O=No Zone of inhibition.

Conclusions and Recommendation

This study has revealed the presence of pathogenic/toxigenic bacteria in milk powder packed in Sudan. Also, the study has explained that development of multiple antibiotics resistance is the major public health threat to consumers, especially in children. And they administered on them during illness. Infections may be prolonged a may even lead to increase in mortality therefore, we recommended the following:

a. Improvement of personal hygiene of sellers.

b. To use small package of milk powder to reduce the duration of exposure and hence decrease chance of contamination.

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