American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

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

Nagat A. Elrofaei[1], Kauther H. Elsharif[1], Amna Yousif Mohamed[1], Sara Y. Ali[1], Omnia A. Mohamed[1] and Ahmed Ali Mustafa[2]*

[1]*Biotechnology Department, Faculty of Science and Technology, Omdurman Islamic University, Sudan*

[2]*Botany Department, Faculty of Science and Technology, Omdurman Islamic University, Sudan*

***Corresponding author:** Ahmed Ali Mustafa, Botany Department, Faculty of Science and Technology, Omdurman Islamic University, Sudan.

To Cite This Article: *Nagat A Elrofaei, Kauther H Elsharif, AmnaYousif Mohamed, Sara Y Ali, Omnia A Mohamed, Ahmed Ali Mustafa. Microbiological Quality of Milk Powder Packed in Sudan and Their Antibiotic Susceptibility. Am J Biomed Sci & Res. 2021 - 14(3). AJBSR. MS.ID.001989.* DOI: 10.34297/AJBSR.2021.14.001989.

Received: May 13, 2021; **Published:** October 01, 2021

Abstract

In this study the microbiological quality of milk powders packed in Sudan has evaluated by method of enumeration of total viable bacteria, *coli* form, *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 (5.3×10^3 cfu/ml) was found in sample no-8, and the lowest one 3.4×102cfu/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%), *Micrococcus spp*. (15.4%) and *Staphylococcus aureus* (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*, *Compylobacterjejuni, Yersinia enterocolitica*, 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 transmissiblevia 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, <1cfu/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

Total plate count was used for enumeration 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- 48hours [10].

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 24hours at 37˚C [11].

Yeast and Moulds Counting Method: The Medium for yeasts and moulds was Sabouraud Dextrose Agar (SDA). 1mlof 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 3-5days [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 enstitute 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 ager 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 (5.3×103cfu/ml) was found in sample no-8 and the lowest one was 3.4×102cfu/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 molds 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].

Table 1: Microbiological quality of Milk powder of 8 different brands packed in Sudan

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	4.3×10^2	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	3×10^3	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].

Table 2: Percentage prevalence of bacterial isolation from milk powders packed in Sudan.

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 applicate 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 *Micrococcusspp*, which have not been associated with human infection [25].

Antibiotic Susceptibility Test

All the bacterial isolates were susceptible to cefuroxime and ceftriaxone 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	Bacterial isolates			
		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 presences 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 many 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.

Acknowledgment

We are grateful to Department of Biotechnology, Faculty of Science and technology, Omdurman Islamic University, and Department of Botany, Faculty of Sciences and Technology, Omdurman Islamic University, Omdurman, Sudan.

References

1. El Khier, M Khalid, Abu El Gasim AY (2009) Quality assessment of milk powders packed in Sudan. Pakistan Journal of Nutrition 8(4): 388-391.
2. Deeb AM, Al Hawary II, Aman IM, Shahine DA (2010) Bacteriological investigation on milk powder in the Egyptionarket with emphasis on its safety. Global etenaria 4(5): 424-433.
3. Rajput I R, Khaskheli M, Kaleri HA, Fazlani S A, Devi K, et al. (2009) Determination of total viable cells and enterobacteriaceae in categorized milk powder. Pakistan journal of nutrition 8 (9): 1493-1496.
4. Codex Stan (1999) Codex standard for milk powder and cream powder. Codex stan 207-1999. Codex Alimentarius commission.
5. EAS (2006) Dried whole milk and skimmed milk powder-Specification. EAS 49: 2006. East African community standards.
6. SDS (2008) Milk powder SDS 2008/108. Sudanese Standards and Methodology Organization, Khartoum, Sudan.
7. SVGNS (2004) Standard for milk powders and cream powder.ST Vincent and the grenadines bureau of standards.
8. ICMSF (1978) International Commission on Microbiology for specification for food Microorganism's food. Their significance and Methods of numeration, (2nd Edn) Toronto, Canada University of Toronto press.
9. FAO/WHO (1973) Code of principles concerning milk and milk products (7th Edn). Standard for whole milk powder partly skimmed milk powder and skimmed milk powder Vo. A.S. FAO/WHO.
10. Jahan M, RahmanM, Parvej, M S Chowdhury Z H, Haque M E, et al. (2015) Isolation and characterization of *Staphylococcus aureus* from raw milk in Bangladesh. Journal of Advanced veterinary and Animal Research 2: 49-55.
11. Robinson R K (1983) the Microbiology of Milk. Applied science publishers Ltd. London. Dairy microbiology 1: 209-231.
12. Houghtby A G, Maturin L J, Koenig E (1992) Microbiological count methods. In Standard methods for the Examination of Dairy products, 16thedition, Ed, RT. Marshal. Washington, DC, American public Health Association0020 213-246.

13. Holt J G, Krieg N R, Sneath, Staley J T, Williams S T, et al. (1994). Bergey's Manual of Determinative bacteriology, 9thedn. Lippincott Williams and Wilkins, Baltimore, M.D.

14. Clinical and Laboratory standards institute (CLSI) (2007) Performance standards for Antimicrobial susceptibity Testing, 17thInformational supplement (CLSI document M 100-S17).

15. Oyeyipo OO, Iwuji C A, Owhoeli O (2012) Public health implication of mycotoxin contaminated paw paw (Carica papaya L) on sale in Nigerian markets. International journal of Health research 5(1) 23-27.

16. Ahmed S, Anwar M N (2006) Microbial counts of dried powder Milk availed in local markets in Bangladesh. Bangladesh journal of Microbiology 23(2): 162-164.

17. Fernandes De oliveira, C A Mestieri, L Santos, M V Moreno, Spers A, et al. (2000) Effect of microbiological characteristics of raw milk on the quality of whole milk powder. Brazilian Journal of Microbiology 31: 95-98.

18. USDEC (2001) US Dairy Export council with the collaboration of Dr. N. Farkye, California polytechnic san luis Obispo, Dr. K. Smith, Wisconsin center for Dairy Research and F. Tracy schonrock, Schonconrock consulting.

19. Oyeyi T I, Lum nwi (2008) Bacteriological quality of some street vended foods in Bayero University compuses, Kano, Nigeria. Journal of biological and Environmental Science for the Tropics 5(4): 239-2243.

20. Osuntogun B, Aboaba O O (2004) Microbiological and Physico-chemical evaluation of some non-alcoholic beverges. Pakistan Journal of Nutrition 3(3): 188-192.

21. Terranova W, Blake P A (1978) *Bacillus cereus* food poisoning. North England journal of Medicine 298-144.

22. Shamsuddeen, U, Ameh J B, Oyeyi T I (2008) Survey on the possible critical control point during the production of dambunnama in Kano. Journal of biological and Environmental science for Tropics 5(4): 1-5.

23. Kawo A H, Abdulmumin F N (2009) Microbiological quality of re-packaged sweets sold in metropolitan Kano, Nigeria Bayero Journal of pure Applied Science 2(1): 154-159.

24. Wieneke A A, Roberts D, Gilbert R J (1993) *Staphylococcal* food poisoning in the United Kingdom. (1969-1990). Journal of Epidemiology and Infectious Diseases 110(3): 519-531.

25. Falegan C R, Oluwaniyi T T (2015) Microbial composition, antibiotic sensitivity and proximate composition of popular imported powdered infant milk formulas sold in Abo-Ekiti, Nigeria. International journal of Microbiological Genetics and Molecular biology research 1 (1): 10-24.

26. Klein E, Smith D L, Laxminaryan R (2007) Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus in United states, 1999-2005. Imerging infections disease 13(12): 1840-1846.

27. Scott K F, Jeff Hageman, Linda K McDougal, Jasmine Mohammed, William R Jarvis, et al. (2003) Epidrmialogical and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to Vancomycin, United state: 1971-2001. Clin Infect Dis 36(4): 429-439.

28. Foster T (1996) *Staphylococcus*, In: Barrons Medical Microbiology, 4th edition. University of Texas Medical branch ISBN 0-9631172-1-1.