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BACTERIOLOGICAL QUALITY OF UNPACKAGED POWERED MILK SOLD WITHIN KADUNA

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ABSTRACT

Milk **is a** nutritious food and a excellent source of protein needed for growth and body building. It's costly nature however makes it unaffordable by most people especially in a developing country like Nigeria. The need of this product and the current economic realities has made most Nigerians settle for cheaper alternatives such as those bagged in large quantities and retailed in an unpackaged form in smaller quantities based on the consumer needs. The aim of this study is to evaluate the bacteriological quality of unpackaged powdered milk sold within Kaduna south local government. The objectives are to determine the bacteriological load of three brands of powdered milk and to isolate and identify bacteria from the samples analyzed. This was done using standard microbiological methods. Thirty milk samples from three different brands (A, B and C) were collected from markets within Kaduna south local government. The bacterial load of brand A ranged from $1.26x\ 10^5$–$7.0x10^5$ with a mean viable count of $4.0x10^5 \pm 2.9$ that of brand B ranged from $1.38x10^5$-$2.44x10^5$ with a mean count of $2.0x10^5 \pm 0.5$ while that of brand C ranged from $1.00x10^5$-$2.0x10^5$ with a mean count of $1.3x10^5 \pm 0.5$.Three genera of bacteria (*Bacillus spp Staphylococcus aureus, and Streptococcus spp)* with percentage occurance of 60, 23.3 nd 16.7% respectively were isolated from all the milk samples analysed. The study establish evidence of contamination of all the milk samples with the total viable count in all the samples($>10^4$ CFU/ g). Proper hygienic practices should be enforced and maintained during handling to reduce post pasteurization contamination and spoilage of powdered milk.

Keywords: Powdered milk, Bacteriological quality, *Staphylococcus aureus, Bacillus spp*

INTRODUCTION

Milk is a white liquid food produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals (including breastfed human infants) before they are able to digest solid food (Van Winckel, 2011). The Females of all mammal species can, by definition, produce milk, but cow's milk dominates commercial production. In 2011, FAO estimates 85% of all milk worldwide was produced from cows.(Gerosa and Skoet 2012).

Milk is sterile when synthesized into a healthy cow's udder, however it can become contaminated by microorganisms during milking, processing and transport due to its high nutrient content, therefore strict aseptic techniques must be employed during milking, processing and transport to reduce contamination to the bearest minimum and hence avoid spoilage. Different preservation techniques have been employed to help prolong the keeping time of milk. Techniques such as refrigeration, drying, pasteurization have been used.

Powdered or dry milk, is a manufactured dairy product made by evaporating milk to dryness. Drying milk is one of the ways of preserving milk. The low water activity in dehydrated milk makes it less prone to microbial contamination and spoilage because microbes require water for growth. However, contamination with thermophiles can have significant economic consequences when they exceed specification limits, and may result in down grading of the products, as these have ability to produce extremely heat resistant spores, and thus are important source of pre- and post-pasteurization contamination (Anup and Rupesh, 2012).Microbial pathogens of major concern in dried milk includes *Bacillus cereus, Staphylococcus aureus and Salmonella*. These organisms may remain viable in milk powder for long period of time, and resume growth when the powder is reconstituted and stored at favorable temperature (Hafsa et al., 2013).

As delicious and highly nutritious milk is, it is usually not affordable by most people especially in developing countries. The high nutrient content of milk makes it prone to easy post pasteurization contamination and spoilage by microorganisms if not properly packaged and preserved. The cost of packaging contributes to the overall high cost of this product. In recent times because of the need of this highly nutritious food there seems to be a shift to cheaper alternatives, such as those in bags/sacks where the milk is then retailed in unpackaged and unsealed nylon bags based on the needs of the consumer. However, the milk may become continuously exposed to pathogens during retailing, which involves the opening and exposure of bags/sacks , hands and the utensils used in scooping of the milk and the environment in which the milk is kept before it is retailed .

An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years(WHO, 2022).Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year.(WHO, 2022)

Milk contaminated with pathogenic bacteria is a major cause of foodborne disease, which is a serious health problem for millions of people in the world. Food-related diseases causing mortality or other complications due to contaminated milk increase every day and create a significant burden on the healthcare system (FAO, 2006) Among food poisoning bacteria, salmonella causes the most widespread diseases in the world and is estimated at 1.3 billion gastroenteritis and 3 million deaths worldwide (Ohud et al, 2012). Similarly, food poisoning caused by *Staphylococcus aureus* (SFP) is also the most widespread cause of gastroenteritis in the world (Wang et al., 2007). In addition, *E. coli* O157 : H7 is the other most important food mediator, causing human diarrhea, hemorrhagic colitis, and hemorrhagic therapy syndrome. (Wang et al., 2007)

Food-associated diseases are responsible for 33 to 90% of the deaths of children in Africa, and represent serious problems of the continent (Flint et al.., 2005)

It is therefore important to assess the bacteriological quality of unpackaged dry milk sold in order to determine if the bacterial load and the species of bacteria isolated from such milk fall within acceptable limit.

MATERIALS AND METHODS

Collection and preparation of samples

Powdered milk samples from three popular brands were bought from different sales outletst in markets within Kaduna south local government area. Ten (10) samples of each brand (A, B and C) was randomly collected from Central market, Kasuwan Barchi market and kakuri market. The samples were kept in a sterile polythene bag for further analysis.

Preparation of homogenate/stock

Five (5) gram of each of the samples was weighed and transferred aseptically into a beaker containing 45ml of sterile water. It was shaken thoroughly after which seven fold dilution was prepared from it.

Total Viable Count

One(1) ml of sample from 10^{-4} dilution was dispensed onto the sterile petri dish containing 20ml sterile nutrient agar .This was done in duplicate. The plate was rocked gently to ensure even distribution of inoculum after which it was then allowed to solidify and was incubated at 37°C for 24hours.After the period of incubation, only plates with colony count within the range of 30- 300 colonies were considered.

Coliform count

One(1) ml of sample from 10^{-4} dilution was dispensed into sterile petri dish containing 20 ml of Macconkey agar in duplicate. The plate was rocked gently to ensure even distribution of inoculum. it was then allowed to solidify and was incubated at 37°C for 24hours.

Examination of cultures

After the period of incubation, the plates were examined for the detection of growth, pigmentation, colonial morphology as well as changes in the media. Plates which showed visible growth was subjected to further biochemical tests while those which did not show visible growth were incubated for further 48hours and then discarded when no growth was detected.

Purification of Cultures

The primary isolates were subcultured on nutrient agar. The subculture was repeated until pure colonies were obtained.

Preparation of Smears

Smears were prepared by emulsifying small inoculums of the bacterial colony in a drop of normal saline on clean slide and spreading it. The smears were allowed to dry and then fixed by gentle heating.

Grams Staining Technique

The prepared smear was flooded with crystal violet for 60 seconds and then washed with water, followed by Lugol's iodine for 30 seconds and then washed with water. Alcohol was added for 10 seconds and quickly washed and safranin was added for 30 seconds and washed. The slide was allowed to air dry.

Microscopic examination of isolates.

All isolated bacteria were subjected to microscopic examination under oil immersion using x100 objective lens. The shape, arrangement of cells and Gram's reaction were recorded.

Biochemical Tests

Catalase Test

A portion of the isolate was placed on a drop of 3% hydrogen peroxide on a clean slide using a sterile loop. Production of air bubbles indicated a positive result.

Indole Test

The isolate was inoculated in peptone water and incubated at 37^{o}C for 48hours.Two to three drops of Kovac's reagent was added to culture and mixed properly.. Production of pink color on the upper layer of the reagent was considered for indole production.

Coagulase Test

A portion of the isolate was placed on a drop of normal saline on a slide and a drop of serum was dropped and emulsified. Presence of agglutination indicated positive result.

Oxidase Test

The filter paper was moistened with a (1% of solution of tetramethyl-phenylene diamond-dihydrochloride). The colony to be tested was picked with a platinum loop and smeared in the filter paper. Inoculated areas of the paper was observed for colour change.

RESULT

The bacterial load of powdered milk sold within Kaduna south local government is presented in Table 1. The bacterial count of brand A ranged from $1.26x10^5$ to $7.0x10^5$ with a total mean count of $4.0x10^5$±2.9. That of brand B ranged from $1.38x10^5$ to $2.44x10^5$ with a total mean count $2.0x10^5$±0.5.While that of brand C had a bacterial count range of $1.00x10^5$ to $2.0x10^5$ with a total mean count 1.3x105±0.5.The morphological, cultural and biochemical characteristics of the isolates is shown on Table 2.The isolated bacteria are *Bacillus spp*, *Streptococcus spp* and *Staphylococcus spp*. The percentage occurance of the three isolates is shown on Table 3. *Bacillus spp* was the most prevalent, with percentage occurance of 60% , while *Streptococcus spp* was the least prevalent with percentage occurance of 16.67% The coliform count for all the milk samples analysed is presented on Table 4 all the milk samples analysed were free from coliforms.

Table 1: Total mean viable count of the milk samples

BRANDS	Range of count	Total mean viable count (CFU/g) ±SD
A	$1.26x10^5$x$7.0x10^5$	$4.0x10^5$ ± 2.9
B	$1.38x10^5$-$2.44x10^5$	$2.0x10^5$ ± 0.5
C	$1.00x10^5$-$2.0x10^5$	$1.3x10^5$± 0.5

Table 2: Morphological, cultural and biochemical characteristics of Bacteria isolate.

Colour	Surfaces	Edge	Texture	Gram reaction	Shape	Catalase	Coagulase	Oxidase	Indole	Bacteria isolate
Milky	Rough	Entire	Dry	+ve	Rod	+	-	-	-	*Bacillus spp*
Creamy	Smooth	Entire	Moist	+ve	Cocci in clusters	+	+	-	-	*Staphylococcus aureus*
Light yellow	Glossy	Entire	Moist	+ve	Cocci in chains	-	-	-	-	*Streptococcus spp*

Key: + = positive
- = Negative

Table 3: Percentage prevalence of bacterial isolates

ISOLATES	NUMBER	PERCENTAGE (%) PREVALENCE
BACILLUS SPP	18	60
STAPHYLOCCUS AUREUS	**7**	23.3
STREPTOCCUS SPP	5	16.**7**

Table 4: Coliform count for powered milk samples

BRANDS	Mean Coliform count(CFU/g)
A	Nil
B	Nil
C	Nil

DISCUSSION

Bacteriological quality of three brands of unpackaged powdered milk sold within Kaduna south local government area were analyzed. All the brands analyzed were contaminated evident from the total viable count that was above the acceptable limit of 10^4 CFU/g This is similar to the findings reported by Nazia and Rasheda (2018).The products might have been contaminated after unsealing of the bags during retailing through contaminated hands, environment, scooping equipment's and people involved in selling of products. Milk is a highly nutritious food and a good medium for microbial growth and proliferation when contaminated, therefore hygienic practices must be strictly adhered to during retailing to minimize the risk of contamination. In this study, three genera of bacteria (*staphylococcus aureus, Bacillus spp and Streptococcus spp)* with percentage occurance of 60, 23.3 and 16.7% respectively were isolated from all the milk samples analyzed . These finding is in agreement with Vaisanen et al.,(2001), CoghillJuffs (2007) ,Ahmad et al., (1997) and Naget et al., (2021*)*, Ibrahim et al., (2022), Madika et al., (2020). Pathogens of major concern in both dried and infant milk formula includes *Bacillus cereus, Staphylococcus aureus and Salmonella*. These organisms may remain viable in milk powder for long period of time, and resume growth when the powder is reconstituted and stored at favorable temperature (Hafsa et al., 2013). *Staphylococcus aureus* is a normal flora of human and animals therefore contamination can occur before pasteurization during milking from the teat of the cows and from personals involved in the milking process . Contamination can also occur post pasteurization especially with products that involves handling by human when aseptic techniques are not followed and maintained. *Staphylococcus aureus* though a normal flora of humans has been implicated in a number of food borne illnesses, one of which is Staphyloccal food poisoning transmitted through contaminated food or water. If enterotoxigenic staphylococci are able to grow in food to high numbers (more than 10^5 to 10^6cfu/g or ml)before they are killed, there is a risk of intoxication with consumption of such food (Hafsa et al., 2013).The disease is usually fatal in children under five years, the elderly and immuno compromised patients .Presence of *Bacillus spp* in the sample could be hazardous since the resistant spores might germinate under appropriate conditions and liberate substantial amount of toxins. Bacillus species are found in raw milk and at all stages of dairy processing (Kalogridou-Vassiliadou 1992; Scheldeman et al. 2006). They are ubiquitous in soil, in the farm environment (feed, manure) and in various unprocessed foods (Crielly et al. 1994). These spore-forming micro-organisms can survive pasteurization and ultra high temperature (UHT) treatment and in this way find their way into final dairy products (Vyletělová and Hanuš 2005) . The presence of *Streptococcus spp* in powdered milk is similar to the findings reported by Nagat et al., (2021), these might have been spread through mucus from the nose or throat of infected persons or through the air by sneezing or coughing. (Reves et al., 2004). Researchers (Shamsudeen et al., 2008; Kawo and Abdulmumin, 2009) have reported that the presence *Streptococcus spp*. as possible contaminants from handlers. *Streptococcus spp.* is implicated in human infections like Pharyngitis, scarlet fever, and pneumonia (Shamsudeen et al., 2008; Kawo and Abdulmumin, 2009).

CONCLUSION

From the study, the unpackaged powdered milk analyzed were found to be contaminated evident from the total viable count ($>10^4$CFU/g). Three genera of bacteria :*Bacillus spp, Staphylococcus aureus* and *Streptococcus spp* with percentage prevalence of 60, 23.3 and 16.7% respectively were isolated from the milk samples analysed. .Measures to reduce pre -pasteurization and post pasteurization contamination should be strictly adhered to and maintained to reduce risk of the infections that comes with consuming contaminated powdered milk,

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