International Journal of Pathogen Research

6(1): 29-35, 2021; Article no.IJPR.63976
ISSN: 2582-3876

Bacteriological Quality of Raw Cow's Milk Sold in Minna Central Market, Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AF and AME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JIA, GDE and and EA managed the analyses of the study. Authors AF, AME, JIA, GDE and EA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2021/v6i130153
Editor(s):
(1) Dr. Khadiga Ahmed Ismail Eltris, Ain Shams University, Egypt.
Reviewers:
(1) Anna Szosland-Fałtyn, Institute of Agriculture and Food Biotechnology, Poland.
(2) Ayokunnumi Funke Obajuluwa, Kaduna State University, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63976

Original Research Article

Received 25 October 2020
Accepted 31 December 2020
Published 22 January 2021

ABSTRACT

Milk is a non-transparent, yellowish-white substance secreted by the mammary glands of all mammals. It contains proteins, minerals, carbohydrates, fat, and vitamins that meet the dietary requirements of the body than any food in a single diet. As a result of their highly nutritious nature, milk supports the rapid growth of many microorganisms, including bacterial pathogens. The aim of this study was to determine the bacteriological quality of raw cow's milk sold by different vendors in Minna central market, Niger state, Nigeria by pour plate method. The highest bacterial count was 7.5×10^7 CFU/mL and the lowest bacterial count was 2.5×10^7 CFU/mL. Bacteria isolated from five different cow's milk samples were identified through their cultural and biochemical properties to be *Bacillus badius*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Salmonella typhi*. The frequency occurrence of the bacterial isolates were *Bacillus spp.* (61.9%), *Staphylococcus spp.* (19.0%), *E. coli* (14.3%)and *Salmonella spp.* (5%). The presence of these bacterial isolates and the colony forming unity count observed in these samples indicated poor hygiene and sanitation during milking and post milking processes. Therefore, efforts should be intensified to pasteurize the milk before consumption in order to guarantee the safety of the consumers.

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Keywords: Milk; contamination; hygiene; bacteria and cow.

1. INTRODUCTION

Milk is a non-transparent, yellowish-white substance secreted by the mammary glands of all mammals [1]. It is the main source of protein and special food for mammalian offspring before they can consume and digest other food forms [1]. Milk and its products are main components of the Nigerian economy, about 90 percent of cattle producing milk belong to the agricultural pastoralist Fulani, and their women process and sell the milk [2].

Cow milk contains other vital food composition such as minerals, carbohydrates, fat, and vitamins and as a result meets the dietary requirements of the body than any food in single diet [3]. As a result of their highly nutritious nature, they facilitate the proliferation of many microorganisms, including disease causing bacteria [4].

Bacterial contamination of milk may occur mainly through cow's microflora, improper udder washing, breast infection, milking and milking conditions, equipment for handling and storage facilities [5]. The presence and growth of microbes cause changes in the milk property, thus reducing the shelf-life that damages the economy and public health [6].

Microorganisms found in milk can be either pathogenic (*Salmonella species*) or spoilage organisms (*Bacillus species*), although some can behave as both for example *Bacillus cereus*, known as a food spoilage organism, can also be a pathogenic organism, as some can cause food poisoning by secreting toxic metabolites [7]. In recent years, the biggest public health problem has been the pathogenic and spoiling microbial populations in milk [8].

There is a steady public demand to minimize or avoid contamination and successive growth of bacteria in milk from those involved in dairy farming and processing [9]. This is primarily due to the importance of high aseptic milk production, which is significant in reducing the incidence of foodborne diseases and prolonging the shelf life of milk, resulting in a safe and stable commodity for consumers [9]. As soon as the milk is processed out of the cow's udder, the preservation of milk needs cooling and neatness [10].

In Nigeria, the incidence rate of foodborne diseases is alarming [11]. There is a need for foods with longer shelf life [12]. Adequate monitoring and control measures are essential to prevent spoilage of milk and ensure consumer safety [13].

It is therefore imperative to create public awareness of health implication of consuming raw cow's milk that does not follow proper hygienic protocols in order to take preventive measures against cow's milk-borne diseases and as well as to eliminate food spoilage species present in cow's milk. The aim of this study was to determine the bacteriological quality of raw cow's milk sold by different vendors in Minna central market.

2. MATERIALS AND METHODS

2.1 Samples Collection

The samples of cow's milk were bought from five (5) different vendors in Minna Central Market, Niger State, Nigeria between the Month of April and June. The samples were collected into five different sterile plastic containers and transported immediately to Department of Microbiology, Federal University of Technology, Minna for the laboratory analysis.

2.2 Preparation of Media

The media used were prepared according to their manufacturer's guidelines and protocols.

2.3 Total Bacterial Count

Ten test tubes containing distilled water were sterilized and used for serial dilution using an autoclave at 121°C for 15 minutes. Upon dilution of each sample, 1 ml of 10^6 dilutions was plated out by pour plate method and incubated for 24 hours at 37°C in order to obtain the total colony count. The nutrient agar plates were examined and colonies present were counted and recorded as colony forming unit per mL (CFU/mL).

2.4 Isolation of Bacteria

One millilitre of aliquots each sample from dilution factor of 10^6 was inoculated by pour plate method using Nutrient agar. The plates were incubated at 37°C for 24hours. After incubation, the isolate was sub cultured using a sterile wire

loop into a freshly prepared nutrient agar plate and then incubated for 24 hours at 37°C to obtain pure cultures. The Pure isolates were persevered in the refrigerator at 20°C for identification.

2.5 Identification of the Organisms

Diagnostic growth media, morphological, microscopic appearance and biochemical tests (catalase test, coagulase test, methyl red test, Voges Proskauer test, urease test, citrate utilization test, hydrogen sulphide utilization, Indole test) were used to classify isolated microbes, as described by Susan et al. [14].

3. RESULTS

Total viable mesophilic count was highest in sample B (7.5×10^7) followed by A (5.5×10^7), D (4×10^7) and E (3.35×10^7) while sample C (2.5×10^7) had the least value as shown in Table 1.

The result of the morphological and biochemical characteristics of bacteria isolates were shown in Table 2.

The isolates were *Bacillus badius*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Salmonella typhi*.

Table 1. The result of total viable mesophilic bacterial counts

Samples	(CFU/mL), 10^{-6}	Rep. (CFU/mL), 10^{-6}	Average CFU/Ml
A	5×10^7	6×10^7	5.5×10^7
B	8×10^7	7×10^7	7.5×10^7
C	3×10^7	2×10^7	2.5×10^7
D	3.5×10^7	3.2×10^7	3.35×10^7
E	5×10^7	3×10^7	4×10^7

Key: Rep = replicate

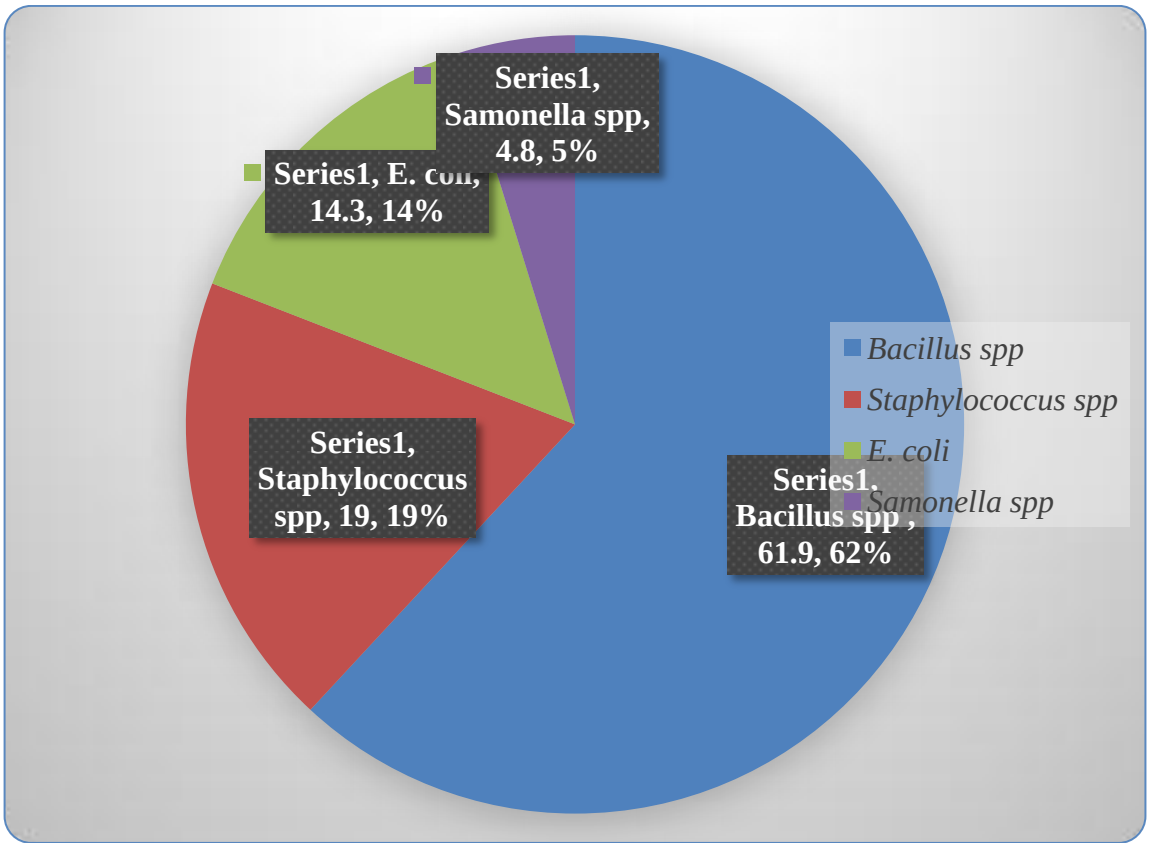

Fig. 1. The percentage occurrence of the 21 identified bacterial isolates from the milk

Table 2. The result of the morphological, microscopic and biochemical characteristics of bacteria isolates

Sample	GR	SH	CA	CO	ST	CI	UR	IN	MSA	MR	VP	H2S	MO	Probable organisms
A_1	+	Rod	+	-	+	-	-	-	-	-	-	-	+	*Bacillus badius*
A_2	-	Rod	+	-	-	-	-	+	+	+	-	-	+	*Escherichia coli*
B_1	-	Rod	+	-	-	-	-	-	-	+	-	+	+	*Salmonela typhi*
B_2	+	Rod	+	-	+	+	-	-	-	-	+	-	+	*Bacillus subtilis*
C_1	+	Cocci	+	+	-	-	+	-	+	+	$_+$	-	-	*S. aureus*
C_2	+	Cocci	+	-	-	-	+	-	+	-	+	-	-	*S. epidermidis*
D_1	-	Rod	+	-	-	-	-	+	+	+	-	-	+	*Escherichia. Coli*
D_2	+	Rod	+	-	-	-	-	-	-	-	-	-	+	*Bacillus badius*
E_1	+	Rod	+	-	+	+	-	-	-	-	-	-	+	*Bacillus badius*
E_2	+	Rod	+	-	+	+	-	-	-	-	+	-	+	*Bacillus subtilis*

Keys: GR= Gram reaction, SH= Shape, CA= Catalase, CO= coagulase, ST= Starch hydrolysis, MR= Methyle Red, VP= Voges Proskauer, IN= Indole CI= Citrate, UR= Urease, MSA= Mannitol salt agar, H_2S= Hydrogen sulphide, MO= Motility, + = Positive and - = Negative

The result of percentage occurrence of 21 bacteria isolates which was grouped into four namely: *Bacillus spp.* (61.9%), *Staphylococcus spp*. (19%), *Escherichia coli* (14.3%) and *Salmonella spp*. (4.8%) were shown in the Fig. 1.

4. DISCUSSION

The result of the mean average of total bacteria counts per milk samples varied. This might be as a result of environmental factors and improper hygienic condition of the handlers or health condition of the animals. This discovery was in agreement with Mohammed et al*.* [1] who reported such varied bacterial count from goat milk and dairy farm within Kaduna North.

The total aerobic bacterial per each of this sample were very high. Sample B (7.5×10^7 CFU/mL) had the highest total bacterial count while sample C (2.5×10^7 CFU/mL) had the least. According to European Commission (EC) 2001, the total plate count for raw cow's milk should not exceed 10^5 CFU/ML [15]. Susan et al. [14] equally reported that raw milk (Grade A) ($<10^5$ CFU/mL) and Grade B (milk from local producers) ($<10^6$ CFU/mL). All total viable bacterial count in this experiment exceeded the limit for both local and standard raw milk (Susan et al. [14].

The identified bacterial as followed: *Bacillus spp*, *Escherichia coli*, *Salmenella typhi*, *Staphyloccocus aureus* and *Staphyloccocus epidermidis* had been revealed through Gram reaction and biochemical results that some were positive while others were negative to the various test carried out. The presence of these organisms from the studied cow's milk sample indicated a serious public health problem as some were known to be human pathogens.

Bacillus spp in cow's milk had been reported as one of the bacteria commonly found [13]. However, *Bacillus* cereus has been linked to food poison [13]. The presence of *Bacillus spp* in raw milk might emanated from various sources; soil, milking container, air and food source [16].

The presence *Staphylococcus spp* in raw milk sold is an evidence hygienic compromise because milk is virtually a sterile fluid secreted from alveoli of udder [17]. *Staphylococcus epidermidis* had been reported as a causative agent of chronic mastitis (breast infection) in human and animals [18]. while *Staphylococcus aureus*, coagulase positive and non-motile bacteria causes acute mastitis [18] and food poison [19]. According to the report of Olatunji et al. [20], some strains of *Staphylococcus aureus* produce a potent exotoxin. Ingesting of a food containing toxin producing strains may result in severe gastroenteritis [20].

The isolation of *E. coli* and *Salmonella typhi* in these raw milk showed poor hygienic state of the milk products and presents a potential hazard to the consumers [17]. *E. coli* and *Salmonella typhi* were among the reported foodborne pathogens [21]. The 1985 food regulatory acts specified that coliform, *E. coli* should not be present in one milligram of sample [1]. *E. coli* can find its route into milk through faece, manure and soil [22]. However, the evidence of fecal contamination was indicated by presence of the coliforms bacteria and *Bifidobacteria* species [16].

Salmonella typhi cause gastroenteritis in humans and other animals worldwide and can sometimes lead to systemic infection and even death in severe cases. Contaminated udders, contaminated water, poor sanitation practices, contaminated containers and milk handlers themselves may be the source of *Salmonella* in the raw cow's milk. Since the milk is transported at an ambient temperature and handled [17].

The frequency occurrence of the bacterial species in Fig. 1 showed that *Bacillus* species was the major contaminant of the raw milk sample as reported by Gopal et al. [13]. Probably, they are the most abundant bacterial found everywhere owing to their ability to withstand stress and ability to produce spore. The next, is *Staphylococcus spp*. According to Roberts et al. [23], *Staphylococcus spp* were found on human, animal and environment. Olatunji et al. [20] equally isolated *S*. *aureus* from cow's milk. It was hypnotized that cross contamination might have occurred since most of the people handling cow's milk are uneducated and may lack hygienic protocols [20]. The least occurrence bacteria were *E*. *coli* and *Samonella spp* (14% and 5% respectively) owing to their sources of contamination.

5. CONCLUSION

From bacteriological study of raw cow's milk sold in Minna Central market in Niger State, the presence of *E. coli, Salmonella typhi.* and *Staphylococcus spp* indicated poor handling of milk. This will no doubt pose great health risk to

the populace especially those people patronizing the milk. In all the studied samples, the bacterial load exceeded the recommended ($1x10^5$ CFU/mL) value. Since these products were sold to the public, it is therefore become imperative to educate the milkers and the handlers of the dangers of poor hygienic practice and as well as source of their water used in washing their milking instruments.

ACKNOWLEDGEMENT

We give thanks to Professor Abalaka, M.E. over his guidance and we are not forgetting laboratory technicians for their unrelenting efforts in providing all necessary assistance in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63976