Original article

Isolation of Bacteria from Raw Milk Collected at Milk Super Markets in Tripoli City

Salah Altwanesy* 🆔**, Abdulfatha Abokridighah**

Libyan Biotechnology Research Center, Tripoli, Libya

ARTICLE INFO

Corresponding Email. salahaltwanesy@gmail.com

ABSTRACT

Globally, billions of people consume milk and dairy products every day. Milk samples were collected at 15 milk collection centers (milk super market). According to stratified random sampling design. Samples were analyzed for Total Plate Count (TPC). The prevalence of selected pathogens such as Listeria monocytogenes, E. coli and Salmonella was determined. The mean counts per ml for TPC, psychrotrophs and thermophiles were 12×10^6, 7.5×10^3 and 9.1×10^3, respectively. A TPC less than 10^6 cfu ml^{-1} was used as a basic standard by MCC in the Price Incentive Programme. From the 150 milk samples tested, approximately 90% were contaminated by coliform bacteria and 65% were E. coli positive, with mean counts ranged from 10^3 to 10^4 cfu ml^{-1}. S. aureus was isolated from more than 60% of the samples and the mean count per ml was 12×10^3. Meanwhile, E. coli was also detected in 20 (33.5%) samples. However, Salmonella was only detected in 1.4% of the samples, with the Central region having the highest frequency of isolation. Thirteen Salmonella serotypes were identified, including S. muenchen, S. anatum and S. agona. A total of 47 strains of Listeria were isolated from 4.4% Listeria-positive samples including L. monocytogenes (1.9%), L. innocua (2.1%) and L. welshimeri (0.6%). The presence of pathogenic bacteria such as E. coli, Salmonella and Listeria spp. in raw milk is of public health concern since drinking raw milk is still considered good for health in rural population.

INTRODUCTION

Milk is a nutritious food for human beings, but it also serves as a good medium for the growth of many micro-organisms, especially bacterial pathogens. Lactococcus, Lactobacillus, Streptococcus, Staphylococcus and Micrococcus spp. are among common bacterial flora of fresh milk [1]. The flora may also predominate by psychrotrophs if the milk is kept cool before further processing. The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used [2].

When fresh milk is extracted from a healthy cow, its microbial load is typically low (less than 1000 ml^{-1}). However, after being stored at room temperature for a while, the loads can rise to 100 times or higher. However, between milking at the farm and transportation to the processing plant, milk stored in clean containers at refrigerated temperatures may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk. Contamination of mastitis milk with fresh clean milk may be one of the reasons for the high microbial load of bulk milk [3,4].

Over time, there has been a significant shift in the relative importance of different etiological agents in milkborne disease. Nonetheless, bacteria remained the cause of over 90% of all documented cases of dairy-related illness, and at least 21 diseases that are currently known to be milkborne or potentially milkborne [5]. Several pathogens, including Listeria monocytogenes, Salmonella, Campylobacter, Staphylococcus aureus, B. cereus, and Cl. botulinum, have been linked to foodborne outbreaks linked to milk consumption. Major public health concerns have emerged regarding the presence of these pathogenic bacteria in milk, particularly for those who continue to consume raw milk [6,7]. With multiple outbreaks documented in developed nations, ranging from mild diarrhea to potentially fatal hemolytic uremic syndrome (HUS), hemorrhagic colitis, and thrombotic thrombocytopaenic purpura, E. Coli 0157:H7 has emerged as a major threat to the dairy industry in recent times [8].

Keeping fresh milk at high temperatures, combined with unsanitary milking practices, can result in microbiologically inferior quality. These appear to be common practices among small-scale farmers in Qasr Bin Ghashir, south of Tripoli, who produce fresh milk and sell it to local consumers or Milk Collection Centers. This study was conducted to investigate the microbiological quality and safety of locally produced raw milk.

METHODS

Study design and setting

This was an experimental study design. A total of 60 raw cow milk samples were collected from150 dairy farmers who send their milk to the milk collection centers in Qasr Bin Ghashir area of Tripoli city, Libya. Farmers involved in the study were chosen according to stratified experimental design,

Sample collection

Samples were collected in the early morning. Approximately 100–300 ml milk was aseptically collected into a sterile Scotch bottle. It was collected immediately after milking using hand or machine into bulk milk containers at ambient temperature (28–30C). Samples were delivered to the laboratory in a cool box at less than 4C within 1–2 h of collection and tested immediately upon arrival. Initially, 25 ml of sample was dispensed into a sterile bag. Subsequent serial decimal dilutions of milk were prepared in saline water.

Microbiological analysis

Samples were analysed for their microbiological quality and safety as well as the prevalence of selected bacterial pathogens. Enumeration of Total Plate Count mesophiles, psychrotrophs and thermophiles, coliform. To enumerate the numbers of coliform bacteria and E. coli in milk, a three-tube Most Probable Number (MPN) technique was employed. Positive tube from MPN was streaked onto eosin methylene blue (EMB) agar and then incubated overnight at 35C.

Detection of Salmonella was carried out according to the International Standard Organization protocol (ISO,1990), and typical Salmonella colonies were confirmed using API 20E test kit Positive analyse samples for the presence of E. coli. Milk samples (25 ml) were inoculated into 225 ml modified Tryptic Soy broth with Novobiocin and incubated overnight at 35C. Approximately 0.1 ml of the broth then was streaked onto the surface of Sorbitol MacConkey agar (SMAC, Merck).

Colorless colonies from SMAC agar were streaked onto a modified EMB agar before confirmed with E. coli latex test (Oxoid) method was used to detect Listeria spp. in raw milk as outlined by Westoo and Peterz (1992). Five colonies from each plate of Oxford Listeria selective agar and Palcam Listeria selective agar were picked at random and streaked onto Trypticase Soy agar (TSA, Merck). Presumptive Listeria spp. isolates were confirmed according to Gram reaction, catalase test and umbrella motility in MIO medium (Difco). Isolates that were Gram-positive, catalasepositive and motile were sub-cultured and then identified with API Listeria) and CAMP Test.

RESULTS AND DISCUSSION

Generally, fresh raw milks collected from farms and milk super mark were heavily contaminated by bacteria with a mean total cfu ml1 plate count (TPC) of 12x 106 reasons for the high counts could be due to infected possible udders of the cows, unhygienic milking procedures or equipment, and/or inferior microbiological quality of water used for cleaning utensils and animals.

Counts for psychrotrophs and thermophiles ranged between 103 and 104 cfu ml1 with an average count of & 9.1x103 cfu ml1 respectively 7.5× 103. Generally, psychrotrophic organisms were represented by both Gram-negative and Gram-positive bacteria, such as Pseudomonas, Flavobacterium, Bacillus, Clostridium and Mycobacterium. Practicing very good hygiene principles at the farms, in handling and transportation of milk, is a must [9]. It is important to cool raw milk quickly and to store it no longer than necessary. Equipment that is poorly designed with respect to cleaning and

potential for fouling represent another hidden source of psychrotroph and thermophilic bacteria, as well as the destructive enzymes [10].

Nearly 90% of the samples collected were contaminated by coliform bacteria with a mean count of 1.7x105. The existence of coliform bacteria may not necessarily indicate a direct fecal contamination of milk, but more precisely as an indicator of poor hygiene and sanitary practices during milking and further handling [11].
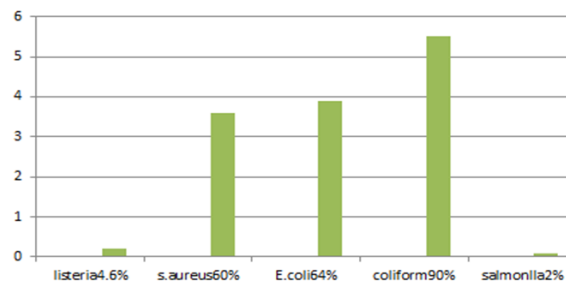
Figure 1. Microorganism identified in tested samples

The presence of the bacteria in milk indicates possible contamination by manure, soil and contaminated water E. coli and coliform bacteria are often used as indicator microorganisms, and the presence of E. coli implies a risk that other enteric pathogens may be present in the sample [12]. Nearly 61% of the milk samples analyzed were positive for S. aureus. The incidence of Salmonella spp. in local raw milk was still low, as only 2 of 60 milk samples were found positive for this organism. Thirteen Salmonella serotypes were identified from samples positive for Salmonella spp. The most frequently identified serotype was S. muenchen (23.1%), followed by S. agona (15.4%) and S. anatum (15.4%). Other serotypes isolated were S. typhimurium, S. hadar and S. Newport. A total of 4.6% milk samples were contaminated by Listeria spp. with a higher incidence of Listeria in raw Milk samples. Only four species of Listeria were identified, namely L. monocytogenes, L. innocua, L. seeligeri and L. welshimeri. Eighteen samples (1.9%).

Generally, fresh raw milks collected from farms were heavily contaminated by bacteria with a mean TPC of 12x106 cfu ml -1. Possible reason for the high counts could be due to infected udders of the cows, unhygienic milking procedures or equipment, and/or inferior microbiological quality of water used for cleaning utensils and animals [13].

Results of the study clearly indicated that microbiological quality of raw milk produced by local farmers. High microbial counts and the occurrence of pathogens is likely to affect. Keeping quality samples collected were contaminated bacteria by coliform 90% of the positive for S. aureus of the milk samples analyzed were salmonella spp 2% E. coli 64%. A total of 4.6% milk samples were contaminated by Listeria spp.

CONCLUSION

The presence of pathogenic bacteria such as E. coli, Salmonella and Listeria spp. in raw milk is of public health concern since drinking raw milk is still considered good for health in rural population. Since the microbiological limits of raw milk are not established in this country, it is very likely that milk may often be tested, found positive for pathogens and withheld from human consumption. Therefore, the production of high-quality milk and safe milk should be of great importance to the economy of the farmer and the sustainability of the dairy industry.

Conflict of interest

The authors declare no conflict of interest in this study.

REFERENCES

1. Calahorrano-Moreno MB, Ordoñez-Bailon JJ, Baquerizo-Crespo RJ, Dueñas-Rivadeneira AA, B S M Montenegro MC, Rodríguez-Díaz JM. Contaminants in the cow's milk we consume? Pasteurization and other technologies in the elimination of contaminants. F1000Res. 2022 Jan 25;11:91. doi: 10.12688/f1000research.108779.1.
2. Hantsis-Zacharov E, Halpern M. Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Appl Environ Microbiol. 2007 Nov;73(22):7162-8.
3. Knight-Jones TJ, Hang'ombe MB, Songe MM, Sinkala Y, Grace D. Microbial Contamination and Hygiene of Fresh Cow's Milk Produced by Smallholders in Western Zambia. Int J Environ Res Public Health. 2016 Jul 21;13(5):737.

4. Lafarge V, Ogier JC, Girard V, Maladen V, Leveau JY, Gruss A, Delacroix-Buchet A. Raw cow milk bacterial population shifts attributable to refrigeration. Appl Environ Microbiol. 2004 Sep;70(9):5644-50.
5. Berhe G, Wasihun AG, Kassaye E, Gebreselasie K. Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health. 2020 Jun 9;20(1):894.
6. Bintsis T. Foodborne pathogens. AIMS Microbiol. 2017 Jun 29;3(3):529-563.
7. Elbehiry A, Abalkhail A, Marzouk E, Elmanssury AE, Almuzaini AM, Alfheeaid H, et al. An Overview of the Public Health Challenges in Diagnosing and Controlling Human Foodborne Pathogens. Vaccines (Basel). 2023 Mar 24;11(4):725.
8. Travert B, Rafat C, Mariani P, Cointe A, Dossier A, Coppo P, Joseph A. Shiga Toxin-Associated Hemolytic Uremic Syndrome: Specificities of Adult Patients and Implications for Critical Care Management. Toxins (Basel). 2021 Apr 26;13(5):306.
9. Berge AC, Baars T. Raw milk producers with high levels of hygiene and safety. Epidemiol Infect. 2020 Jan 31;148:e14. doi: 10.1017/S0950268820000060. Erratum in: Epidemiol Infect. 2020 Apr 06;148:e77.
10. Yuan L, Sadiq FA, Liu TJ, Li Y, Gu JS, Yang HY, He GQ. Spoilage potential of psychrotrophic bacteria isolated from raw milk and the thermo-stability of their enzymes. J Zhejiang Univ Sci B. 2018 Aug.;19(8):630-642.
11. Martin NH, Trmčić A, Hsieh TH, Boor KJ, Wiedmann M. The Evolving Role of Coliforms as Indicators of Unhygienic Processing Conditions in Dairy Foods. Front Microbiol. 2016 Sep 30;7:1549.
12. Cabral JP. Water microbiology. Bacterial pathogens and water. Int J Environ Res Public Health. 2010 Oct;7(10):3657-703.
13. Deddefo A, Mamo G, Asfaw M, Amenu K. Factors affecting the microbiological quality and contamination of farm bulk milk by Staphylococcus aureus in dairy farms in Asella, Ethiopia. BMC Microbiol. 2023 Mar 7;23(1):65.

عزل البكتيريا من الحليب الخام الذي تم جمعه في أسواق الحليب في مدينة طرابلس

صلاح التوينسي*، عبد الفتاح أبو كردغة

المركز الليبي لبحوث التقنيات الحيوية، طرابلس، ليبيا

المستخلص

على مسـتوى العالم، يسـتهلك مليارات الأشـخاص الحليب ومنتجات الألبان كل يوم. تم جمع عينات الحليب من 15 مركزًا لجمع الحليب (سـوبر ماركت الحليب). وفقًا لتصـميم أخذ العينات العشـوائية الطبقية. تم تحليل العينات لمعرفة إجمالي عدد الأطباق (TPC). تم تحديد انتشــار مسـببات الأمراض المختارة مثل Listeria monocytogenes و E. coli و Salmonella. كان متوسط الأعداد لكل مل لــ TPC والبكتيريا المحبة للحرارة والبكتيريا المحبة للحرارة 12 × 106 و 7.5 × 103 و 9.1 × 103 على التوالي. تم استخدام TPC أقل من 106 cfu ml-1 كمعيار أسـاسـي من قبل MCC في برنامج الحوافز الســعرية. من بين 150 عينة حليب تم اختبارها، كان ما يقرب من 90٪ ملوثًا بالبكتيريا القولونية و 65٪ كانت إيجابية لـــE. coli ، مع متوسط الأعداد يتراوح من 103 إلى 104 cfu ml-1. تم عزل S. aureus من أكثر من 60٪ من العينات وكان متوسط العدد لكل مل 12 × 103. وفي الوقت نفسه، تم الكشف عن الإشريكية القولونية أيضًا في 20 عينة (33.5%). ومع ذلك، تم الكشف عن السـالمونيلا في 1.4% فقط من العينات، حيث كانت المنطقة الوسطى هي الأعلى تواترًا للعزل. تم تحديد ثلاثة عشر نوعًا مصلًا من السـالمونيلا، بما في ذلك S. muenchen و S. anatum و S. agona. تم عزل ما مجموعه 47 سـلالة من الليسـتيريا من 4.4% من العينات الإيجابية للليسـتيريا بما في ذلك L. monocytogenes (1.9%) وL. innocua (2.1%) و(0.6%) L. welshimeri. إن وجود البكتيريا المسـببة للأمراض مثل الإشـريكية القولونية والسـالمونيلا والليسـتيريا في الحليب الخام يشـكل مصـدر قلق للصـحة العامة حيث لا يزال شرب الحليب الخام يعتبر جيدًا للصحة في المناطق الريفية.

الكلمات المفتاحية. البكتيريا، الحليب الخام، أسواق الحليب، مدينة طرابلس.