Microbiological Safety Concerns of Raw Milk

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Abstract

Milk is an ideal medium for the growth and multiplication of diverse microorganisms resulting in its early deterioration. Consumption of raw milk should be discouraged as numerous epidemiological outbreaks even death have been recorded. Microbiological quality of raw milk is resultant of various factors including health of the animal to handling and storage. Microbiological quality of pasteurized milk or other milk products is dependent on microbiological quality of raw milk therefore hygienic practices should be implemented at farm level for quality improvement. The various factors affecting the microbiological quality of raw milk and hygienic practices to be implemented at farm level for quality improvement have been highlighted. Poor microbiological quality of raw milk around the world is a resultant of various factors such as the health of the animal, extent of environmental contamination at the producing farm and storage conditions. Modification of payment policy, adoption of pre-milking hygienic practices, improvement in barn environment and better infrastructure/technical support have been suggested for improvement in the microbiological safety of raw milk. Adoption of pre-milking hygienic practices, improvement in barn environment and better infrastructure/technical may lead to an improvement in microbiological safety of raw milk.

Keywords: Raw milk; Hygiene; Microbiology; Safety

Introduction

Food spoilage is an enormous worldwide economic problem and approximately one-fourth of the world's food supply is deteriorated due to microbial activity alone [1]. Based upon shelf-life, food can be broadly categorized in two groups as shelf-stable foods and perishable foods. Perishable foods must be processed and stored under controlled conditions to achieve maximum shelf-life and to ensure product safety [2].

Fluid nature of milk, its chemical composition [3,4] coupled with its high water content, a pH close to neutral render milk as an ideal medium for the growth and multiplication of diverse microorganisms resulting in its early deterioration [5] and may also cause intoxications in consumers [6,7]. Diverse factors are responsible for poor microbiological quality of raw milk. Mastitis infected cows (streptococcal and coliform), soiled cows, unclean milking practices, and failure to cool milk rapidly to < 4.48°C contribute to high total viable flora in raw milk [8-10] resulting in spoilage of milk during storage and transportation [11-14].

Numerous epidemiological outbreaks due to consumption of raw milk have been reported [15,16]. Scientific evidence indicated an increased risk of serious milk-borne illness and even death associated with the consumption of raw milk [17], however consumer’s inclination towards consumption of un-pasteurized raw milk due to higher nutritional qualities, taste, and health benefits have been noted [18].

In recent years, food safety has emerged as an important global issue to ensure safe food throughout the entire food chain from primary producer to the ultimate consumers. Low bacterial numbers, the absence or very low numbers of potential human pathogens and avoidance of residues from veterinary drugs and feeds should be the major food safety criteria for milk and milk products [19]. Raw milk is generally pasteurized to render it safe for human consumption as fluid milk or it is subjected to different procedures to produce a wide range of dairy products. Final quality of any dairy product is influenced by the initial quality of raw milk therefore attempt must be taken to produce best quality raw milk. For commercial success of a dairy product, industries are facing challenges in providing foods which are healthy, tasty and safe to sustain in the international market and therefore immediate attention towards quality improvement is emerging. In the present endeavour, attempts have been made to highlight microbiological considerations for the safety of raw milk.

Microbiological Quality of Raw Milk

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder [20] and may get contaminated with commensal microflora on the teat skin or on the epithelial lining of the teat canal comprising of Staphylococcus, Streptococcus, Bacillus, Micrococcus, and Corynebacterium and coliforms [21]. During milking higher bacterial content were noted in the first milk withdrawn from the udder (foremilk) in contrast to that drawn later, while the stripping may show a somewhat higher count than the latter [22,23]. Freshly drawn milk from animals possess natural antibacterial system which exhibits ‘germicidal’ or ‘bacteriostatic’ properties but are short lived and therefore growth of microorganisms is inevitable unless it is subjected to freezing, heat treatment or irradiation [24,25].

Safety of raw milk depends upon the numbers and types of organisms present [26,27] and its potentiality to cause health hazards, which can be judged by its microbial population and the presence bacterial pathogens [28]. Raw milk was recognized as a source of food-borne illness and disease [29] and epidemiological reports on food-borne outbreaks due to consumption of raw milk infected with potential pathogens have been reported [18]. Mycobacterium avium sub sp. paratuberculosis causes John’s disease in ruminants and may contribute to Crohn’s disease in humans [30] and its detection at a high frequency in milk suggests that raw milk ingestion represents a potential risk of health hazard [31]. Campylobacter infection reported in humans from Hungary [32] and in children from Netherlands [16] due to consumption of fecal contaminated milk evidenced from the presence of C. jejuni in rectal swabs of cows [15].

Microbiological analysis of raw milk indicated presence of pathogenic organisms like coliforms [32], S. aureus [33], E. coli [34], E. aerogene sp., Salmonella typhi [35], Salmonella sp. [36] from India, Klebsiella sp., Proteus sp., Enterobacter sp., Mycobacterium sp. from Ghana [37], E. coli, Aeromonas, Salmonella sp. [38] from Bangladesh,
Factors Affecting Microbiological Quality of Raw Milk

Milk is the normal mammary secretion of milking animals obtained from one or more milking without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing. Raw milk is defined as milk, which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect [48]. Scientific evidence indicates that bovine raw milk is not microbiologically safe for direct human consumption and must be boiled or pasteurized prior to its consumption. Since the final quality of any product is directly dependent on the quality of the raw materials, it becomes imperative to evaluate various factors responsible for poor microbiological quality of raw milk to undertake necessary steps for its betterment. Various factors affecting microbiological quality of raw milk are delineated underneath.

Health of the animal

Mastitis is defined as an inflammation of the mammary gland caused by a wide spectrum of pathogenic agents including Staphylococcus aureus, Streptococcus uberis, Mycoplasma sp. and Escherichia coli which penetrate the teat canal and multiply in the udder cistern [49]. Milk from sub-clinically infected cows is more critical than those obtained from clinically infected cows as no visible signs are reflected in the former milk [50] and hence cannot be segregated and mixed up with non-infected milk [51]. Mastitis induced alteration in milk composition through enzymatic breakdown of casein and milk fat [52] and may contribute up to 10^6 microorganisms into the milk depending on the strains of the infecting microorganism, the stage of infection as well as the percentage of the herd that is infected [24]. Pathogenic gram-negative bacteria can gain access to raw milk from infected mammary glands [53] and animals suffering from sub-clinical mastitis excrete pathogens in milk [54] like S. aureus, S. agalactiae, E. coli and E. faecalis [55]. Mastitis infected milk poses a threat to human health as it contains enterotoxigenic S. aureus of animal origin [56] causing nausea, vomiting and abdominal cramps upon ingestion [57].

Somatic cell count (SCC) is internationally recognized as a parameter for assessing milk quality and udder health [58] and milk from healthy cows should have SCC < 400,000 cells/ml [59]. Milk obtained from healthy cows in a dairy herd have a SCC < 50,000 cell/ml [60] but individual udder quarters with a SCC > 200,000 cell/ml are considered to have sub-clinical mastitis [61]. An increase in SCC is correlated with increased amounts of heat-stable protease (plasmin) and lipase (lipoprotein lipase) in milk [60]. Santos et al. [62] observed earlier development of off-flavour resulting from lipolysis or proteolysis in pasteurized milk obtained from raw milk having higher SCC (1,000,000 vs. 100,000 cells/ml) and when stored at higher temperature of 6°C (35 vs. 61 days) than those at 0.5°C (≥ 61 vs. > 61 days) [62]. Changing from once- to once-daily milking lowered milk production and increased SCC [63] and milking intervals between eight and ten hours is suggested to obtain lowest SCC [64]. Mastitis induced elevated SCC levels and alters protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity and pH of raw milk [65-67]. Mastitis non infected milk had a somatic cell count (log CFU/ml) of 5.55 ± 0.03 which increased to 5.80 ± 0.03 with 46.9% Staphylococcus aureus infected quarters, 5.75 ± 0.05 with 14.3% Streptococci infected quarters, 6.01 ± 0.05 with 14.3% E. coli infected quarters, 5.62 with 4.1% Klebsiella sp. and Bacillus sp. infected quarters [68].

Environmental contamination at the producing farm

Milk is subsequently contaminated with diverse sources like interior and exterior of the udder, surrounding environment [69], animal skin [54], polluted water supplies [70], air [71], extraneous dirt [8], cow shed [72], feed, soil, faeces and grass [73], milking machines [74], milking techniques, methods of disinfection of milking machines [75], milk handling equipment [76], milking utensils [77], milk handlers, storage equipments [78,14] and storage conditions [79]. Source of contamination differs with the housing system of dairy cattle. Identified source of contamination of cow’s teat is soil when on pasture and faeces and bedding material during housing in the barn [80,81]. Deep sawdust bedding of housed animals [82] and the soil of grazed areas [80] are the major source of spores and the spore content of milk was influenced by the degree of soil contamination of teats, dirtiness of the cow access lane [80].

At herd level, the effect of shedding on the bulk tank bacterial count depends on the size of the herd, number of mastitic cows, and ratio of mastitic to nonmastitic milk [8]. Yirsaw [83] reported diversity in total viable flora (cfu/ml) and coliform (cfu/ml) count of raw milk from udder (2.10 × 10^5, 4.04 × 10^3), bucket (1.50 × 10^7, 1.37 × 10^5), storage container (1.50 × 10^6, 1.67 × 10^6) and upon arrival at processing plant (5.67 × 10^9, 1.26 × 10^7). Gradual increase in viable population of aerobic mesophilic bacteria, coliform and yeast and mould of raw milk at different stages from the udder (4.57, 2.47, 3.03 log10 cfu/ml), storage containers at farm gate (7.28, 4.93 and 5.44 log10 cfu/ml) and selling points upon arrival (10.28, 6.52 and 7.13 log10 cfu/ml) indicated contamination of milk due to unhygienic conditions of milking, unclean milk handling equipment and use of contaminated cleaning water [76]. Duangpan and Suryaphan [84] reported higher incidence of mesophilic bacteria (2.52 - 5.76 log cfu/ml), psychrotrophic bacteria (2.52-5.76 log cfu/ml), coliform (2.83 - 3.17 log cfu/ml) and E.coli (0.67-1.73 log cfu/ml) in raw buffalo milk due to its contamination with microflora of worker’s hand comprising of mesophilic bacteria (510 - 546 cfu/in2), coliform (29 - 40 cfu/in2) and E.coli (4 - 11 cfu/in2). Microbial flora (log cfu/ml) of raw milk from Ethiopia consisted of 9.82 aerobic mesophilic bacteria, 4.03 coliforms and 4.15 Enterobacteriaceae and heat treatment and adequate sanitary measures at all stages of milk handling are suggested to render it safe for human consumption [85]. Presence of coliform bacteria and pathogens in raw milk indicated contamination either from hair dropping in to milk during milking, udder washed with unclean water, dirty towels and not dried before milking and improperly cleaned and sanitized milking equipment [12]. Unhygienic practices during pre-milking udder preparation, sub-optimal hygiene of milk handlers and poor sanitation of milking equipments, storage and transportation containers were detected as the critical points for the contamination of raw milk [43].
Storage conditions

Fresh milk from healthy animal contains relatively few bacteria (102-103 cfu/ml) which increase up to 100 fold [86] or double in less than three hours during its storage at normal temperature, depending on the initial microbial population and the temperature of storage [11]. Higher microbial population (> 10⁵ cfu/ml) in aseptically drawn milk [12] or detection of pathogenic microorganisms [87] in raw milk is an indicative of unhygienic milk production. Sarkar and Misra [88] noted lowering of methylene blue reduction time (5.5 - 2.00 hours) with an elevation in viable population (96 × 10³ - 3 × 10⁵ CFU/ml) resulting from holding of raw milk at 35°C for eight hours.

Storage of raw milk at lower temperature is a prerequisite practice on farms and in processing plants which reduces the chances of spoilage induced by mesophilic microorganisms but could not prevent deterioration by psychrotrophic microorganisms [1]. In fresh milk, Pseudomonas sp. constitutes < 10% of the total viable flora [89] but had a short lag phases at 2 - 7°C [90] and emerged as the most significant spoilage causing psychrotroph in both raw and pasteurized milk [90-92] when stored at 10°C [89,93]. During extended refrigerated storage microbiological problems are associated with psychrotrophic microorganisms which can grow slowly at <7°C with an optimum growth temperature of 25-30°C [94,95] and mesophilic pathogens which can survive under refrigeration with an optimum growth temperature of 30 - 40°C [96]. Total bacterial count (log 10 cfu/ml) and psychrotrophic counts (log 10 cfu/ml) of raw milk samples collected from farms (5.38 ± 0.10, 3.66 ± 0.05), milk vendors (6.53 ± 0.14, 4.96 ± 0.06) and processing dairies (6.25 ± 0.08, 5.03 ± 0.11), respectively indicated microbial growth during transportation of milk from farm to the processing dairies and storage of milk at lower temperature [97]. During refrigerated storage and transportation, microbiota of raw milk shifts from predominantly Gram-positive to predominantly Gram-negative organisms, accounting for >90% of the microbial population composed mainly of psychrotrophic species of Pseudomonas, Achromobacter, Aeromonas, Serratia, Alcaligenes, Chromobacterium, Flavobacterium and Enterobacter [98-101].

Kumaresan et al. [97] noted psychrotrophic counts (log 10 cfu/ml) in raw milk (4.04 ± 0.19 cfu/ml) to attain highest values after 14 days of storage of milk at 7°C (7.19 ± 0.23 cfu/ml) in contrast to 4°C (5.99 ± 0.19 cfu/ml) and 2°C (5.05 ± 0.19 cfu/ml). Duangpan and Suryapan [84] noted highest increment (log cfu/ml) in the psychrotrophic count (3.46 ± 0.01, 2.96 ± 0.20, 3.03 ± 0.08) and coliform count (4.05 ± 0.35, 3.00 ± 0.13, 3.31 ± 0.06) in raw buffalo milk after three hours of storage at ambient temperature (29 ± 1°C), less for those stored at ambient temperature after being cooled to 7 ± 1°C and least being noted at cold storage (7 ± 1°C). Pseudomonas that had been incubated in raw milk for three days at 7°C had greater growth rates and greater proteolytic and lipolytic activity than those isolated directly from the milk shortly after milking [102]. During refrigerated storage, microflora in the milk remains at low numbers [90-92] and at a temperature less than 7°C psychrotrophs produce enzymes [103] which are not inactivated by HTST pasteurization or by ultra-high temperature treatment [105] resulting in a decline in the organoleptical quality and shelf life of processed milk [106]. A significant enhancement in total viable population (log CFU/ml) from 5.08 ± 0.05 to 5.96 ± 0.12 during transit of milk from farm to milk collecting centre was attributed to prolonged holding of milk in unhygienic containers or utensils at farm at a temperature ≥ 4 - 5°C [107]. Mixing of fresh milk with previous day milk could be attributed to high bacterial growth in raw milk [108]. Therefore stress should be given towards hygienic milk production, minimization of retention period of raw milk at refrigerated temperature and to process the milk at the earliest to result in safe processed milk with extended shelf-life.

Hygienic Practices for Microbiological Safety of Raw Milk

Hazard Analysis and Critical Control Point (HACCP) has become the internationally recognized system for the management of food safety for all companies involved in the production, processing, storage, and distribution of food for human consumption. HACCP is a science-based analytical tool that enables management to introduce and maintain a cost-effective ongoing food safety program involving systematic assessment of all steps involved in a food operation for identification of those steps that are critical to the safety of the product.

Significant difference in the coliform, E. coli and S. aureus counts for raw milk from different small holder dairy schemes of Zimbabwe indicated differences in milking hygiene and those farms which are more access to training and monitoring of microbiological quality of milk had lower counts [109]. HACCP system during milk collection, processing and storage and microbial exposure assessments and risk analysis should be implemented to ensure safe and healthy milk products [110-112]. Abdalla and Elhagaz [113] denoted a decline in counts (log CFU/ml) of total bacteria, S. aureus and coliform due to rubbing of cow’s udder, teats and milker’s hands with wet clean towels prior to milking (8.13 ± 0.121, 1.75 ± 0.124 and 0.44 ± 0.136 respectively) or due to use of clean coat, covering of hair, washing of hands with soap and water by milker’s and rubbing of cow’s udder, teats and hands with wet clean towel before milking (7.19 ± 0.121, 0.99 ± 0.124 and 0.72 ± 0.136, respectively) in contrast to those noted without applying any hygienic practices (8.93 ± 0.121, 3.45 ± 0.124 and 2.23 ± 0.136, respectively). Lower microbial load was noted in individual cow milk (10-10⁵ cfu/ml), which increase to 10⁶ cfu/ml at farm tank milk and to 10⁷ cfu/ml in tank milk at dairy processing units indicate contamination of milk after milking [114].

Implementation of HACCP system at the dairy farm and during milk handling [43] resulted in a significant improvement in the microbiological quality of raw milk which in turn resulted in a decline in total viable count (log cfu/ml) of pasteurized milk (3.32 ± 0.48 to 3.11 ± 0.30). Recommendations for improving the microbiological safety of milk are delineated below.

Payment Policy

i. Implementation of premium quality payment programs for milk with low Somatic Cell Count [114].

ii. Identification and segregation of milk source with high somatic cell count to prevent their intermixing with bulk milk with low somatic cell count [60].

iii. Implementation of payment systems for milk with good microbial quality [116].

Pre-Milking Hygienic Practices

i. Cleaning of udder with water or disinfection with a germicidal solution prior to milking.

ii. Regular clipping of udders and tails of cows prior to milking, cleaning and drying off of udder and teats with disposable towels, clean and dry hands of milker [117].

iii. Educating the dairy farm owners about sanitary practices for udder preparation and at collection centres [109,43].

iv. Awareness regarding heat treatment of milk and good hygiene practices in the dairy chain [77].

Heat-treatment of water to be used for udder washing and cleaning of milk handling equipment [85].

Medical examination of milk handlers to reduce chance of contamination from infected handlers [118].

**Barn Environment**

i. Protecting the cattle from contamination with manure, soil and water by cleaning manure from the barns or milking parlours [70].

ii. Pre-milking udder preparation with pre-dip followed by drying being superior to other methods in reducing the bacterial counts [119].

iii. Introduction of automatic milking system to replace conventional milking system should not be encouraged due to challenges like automatic detection of subclinical and clinical mastitis and cleaning the teats before milking resulting in slight deterioration in udder health [120].

**Infrastructure/Technical Support**

i. Microfiltration represents one possible processing tool for removal of bacterial spores, with the goal of extending milk shelf life [121].

ii. Provision of cooling systems at milk collection centres and quick dust proof transportation systems [109,43].

iii. Infrastructure at the raw milk collection points and removal of bacterial spores, with the goal of extending milk shelf life [120].

**Conclusion**

Milk being a good media for microbial growth and therefore must be protected from extraneous contamination during production, transportation and storage to render it safe for human consumption. Microbial contamination of milk arising from unhygienic conditions coupled with improper processing and handling result it unsafe products causing several disease outbreaks. A worldwide growing inclination of consumers towards high quality, natural food containing no artificial preservatives and contaminating microorganisms has been noticed. Hence Good hygiene practices during milking and subsequent handling of milk are essential to reduce the risk of contamination at the farm level and HACCP may appear as a most suitable technique. Cleaning and disinfection of milking machine is one of the critical control points for determining the hygienic quality of raw milk. Introduction of price incentive based on microbiological quality of raw milk may be a tool to enhance the hygienic and microbiological quality of raw milk.

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