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Microbiology of milk: Public health aspect

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Abstract
Milk is also an excellent medium for bacterial growth and an important source of bacterial infection when consumed without pasteurization. Microbial contamination might generally occur from within the udder, exterior to the udder and from the surface of milk handling and storage equipment. Some of the disease causing bacteria in the milk are Salmonella spp., *M. bovis*, *Coryno bacterium* spp., *C. perfringens*, *Yersinia enterocolitica*, *Coxiella burnetii*, Brucella, Staphylococcus spp., *Campylobacter jejuni*, *M. avium*, Listeria spp., *E. coli*, and other coliforms that cause human health hazard. So from collection point to consumption milk should be handled very carefully.

Introduction

Milk is the lacteal secretion of the mammary glands of a mammal. It is the first natural food of all young mammals during the period immediately after birth (Gebra-Emanuel, Teka, 1997) [13], an outstanding source of calcium and phosphorus for bones and teeth, and contains vitamin B6, A and B1 in significant amounts (O"Mahony, 1988) [22]. It contains colloidal suspension of tiny solid casein particles (micelles), an emulsion of globules of milk fat and fat soluble vitamins which stay in suspension, a solution of lactose, water soluble proteins, minerals, salts and other substances (Homan and Michael, 1996) [18]. Its complex biochemical composition and high water activity, milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms (Ashenafi and Beyene, 1994) [2]. Nearly all the changes that take place in the flavor and appearance of the milk after it is drawn from the cow, are the result of the cow, are the result of the activities of microorganism, therefore, it is very essential to control these microorganisms. In many countries, a poorly developed dairy industry entails the frequent consumption of unpasteurized milk (Yazdankhah *et al*., 2004) [30]. Therefore, knowledge of the microbial load as well as the microbial groups present in raw milk will be crucial to assess the hygienic quality. High population of bacteria in milk samples or detection of presence of harmful pathogenic microorganisms is an evidence of unhygienic milk production conditions (Abrahamsen *et al*., 2007) [1]. In developing countries due to factors such as poor hygiene and sanitation during milking and milk handling, unclean water, lack of cooling facilities and inadequate infrastructures for milk transportation to the processing facilities (Bille *et al.,* 2000) [4]. In this paper, we discuss different disease causing organisms that are present in raw milk.

Sources of milk microorganisms

The organisms enter into milk from a number of sources including disease condition like mastitis, external udder surfaces and from the milking plant (Hagstad and Hubert, 1986) [16]. There are several bacteria that can cause a mastitis infection including, *Streptococcus, Staphylococcus, Enterococcus* and coliforms such as *Escherichia coli, Klebsiella pneumonia* and *Enterobacter aerogenes.* During milking, the major source of bacteria in milk is the milk contact surfaces of milking equipment and milk cans or bulk tanks (Ashenafi and Beyene, 1994) [2]. Similarly, milking machines can contain a reservoir of microorganisms, and thus, unsurprisingly, differences between machines and related practices can influence the microbial population of the milk collected (Michel *et al.,* 2006) [20]. Milk handling personnel may contribute various organisms including pathogens especially when they are careless, uninformed, or willfully negligent, directly to milk. The soils, while the cows are in pasture, manure, the animal tails etc. are some of the possible sources of contamination of milk (Gebra-Emanuel, 1997) [13].

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Other bacterial contaminants generated from soil, water, animal feed and animal faeces including gram negative rods (Alcaligenes, Acenitobacter, Aeromonas, and Flavobacterium), gram positive bacteria (Bacillus, Clostridium, Lactobacillus, Streptococcus, and staphylococcus), yeasts, and molds. However, most strains of bacteria are not able to reproduce after pasteurization under refrigerated storage conditions like Bacillus, Micrococcus, Enterococcus, Corynebacterium, Microbacterium, Arthrobacter, and Lactobacillus (Champagne *et al.,* 1994) [5].

The organisms present in milk are categorized as per their property

1) Lactic acid bacteria (LAB): These ferment lactose to lactic acid and other end products and are important in cheese making. LAB prefer temperatures greater than 30 ^{0}C, so, depending on initial relative counts, psychrotrophic bacteria including some coliform and pseudomonas bacteria are able to outgrow LAB at room temperature. The most common LAB genera in milk include Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and Enterococcus.

2) Proteolytic bacteria: Bacteria degrade protein and cause bitterness and putrefaction. Most important species are *Pseudomonas* which are psychrotrophic and produce heat stable lipases, *Bacillus* which form heat stable spores and survive pasteurization.

3) Lipolytic bacteria: The organisms digest fats and produce lipolytic rancidity. Again, the most common example in milk is the genus *Pseudomonas*. Several psychrotrophic species of *Pseudomonas* produce heat stable lipases as well as proteases.

4) Gas producing microorganisms which cause cheese openness, floating curd in cottage cheese, and gassy milk. This include-
a. Coliform bacteria are always present in milk but their numbers can be minimized by good sanitation. Also, coliform bacteria compete poorly with lactic acid bacteria, so their numbers rapidly decrease in the presence of a rapidly growing lactic acid culture.
b. *Clostridium tyrobutyricum* is a thermoduric (survives pasteurization) spore forming organism of legendary fame among cheese makers. C. tyrobutyricum causes gas formation (carbon dioxide) during the later stages of ripening of Swiss and Dutch type cheeses and causes 'late gas defect'. European cheese makers frequently check raw milk for thermoduric and/or spore forming bacteria to estimate potential for late gas defects.
c. *Propioni bacterium* produces the desirable gas formation in Swiss type cheese.
d. Some lactic cultures, called heterofermentative, also produce carbon dioxide

(5) Ropy bacteria cause stringy milk due to excretion of gummy polysaccharides. Usually the bacteria like *Alcaligenes viscolactis* are undesirable. However, in some fermented dairy products, ropy lactic acid bacteria such as certain subspecies of *Lactococcus lactis* are used to develop texture.

(6) Sweet curdling bacteria produce rennet-like enzymes which may coagulate milk. Common examples are the psychrotrophic spore formers *Bacillus subtilis* and *Bacillus cereus.*

Milk borne infection
There are several bacteria which can be responsible for milk-borne diseases and it includes Brucella spp, *Campylobacter jejuni, Bacillus cereus*, Shiga toxin-producing E. coli (E. coli O157:H7), Coxiella burnetii, *Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis,* Mycobacterium avium subspecies *paratuberculosis, Salmonella spp, Yersinia enterocolitica*, and certain strains of *Staphylococcus aureus* which are capable of producing highly heat-stable toxins. Coliform contamination ranks high among the most common types of contamination in the dairy industry. Microorganisms such as *Escherichia coli, Pseudomonas aeruginosa, Citrobacter spp, Klebsiella spp* and *Proteus mirabilis* can multiply in the normal summer temperatures and hence unpasteurized milk has every chance of containing *E coli*. Brucellosis is one classical example of milk-borne infection, *Brucella spp* being transmitted from goats to humans either through direct contact or through the milk of the infected animal, particularly since the appearance and taste of the milk are rarely affected by the presence of the bacteria. Once transmitted to humans, *Brucella* is responsible for a type of granulomatous hepatitis or an acute febrile illness that progress to a chronically incapacitating disease with serious complications. For S.aureus, humans and dairy cows are the main carriers of this microbe, presenting mucosal or cutaneous lesions such as impetigo or cattle mastitis. The source of infection is generally a human carrier among dairy industry workers. Pasteurization is the best way of destroying *Salmonella typhi* and *paratyphi.* Another relatively rare milk-borne pathogen is *Bacillus anthracis,* a Gram-positive, spore-forming rod which has been shown to pass into the milk when it is present in cattle in large amounts. *Corynebacterium diphtheriae* can contaminate milk during the handling process if infected dairy workers sneeze or cough the bacilli into milk.
Viruses can also be involved in milk-borne infections. Infections with polioviruses is correlated with milk contamination. Some other agents which can potentially contaminate milk are tick-borne encephalitis viruses, found more often in the milk of sheep, goats and less often in cow milk (Cisak *et al.* 2010) [6]. Hepatitis viruses, particularly hepatitis A virus (HAV) and hepatitis E virus (HEV) can also contaminate milk (Bidawid *et al.* 2000; Drobeniuc *et al.* 2001) [3, 10]
Certain parasites such as *Taenia spp* or *Toxoplasma gondii* can contaminate milk and be transmitted to humans. Soil contamination may also lead to the presence of soil-borne parasites in milk (e.g., *Ascaris lumbricoides, Trichuris trichiura.* Fungus *Nocardia asteroides* has been found to cause bovine mastitis (Cook and Holliman, 2004) [7]. Other fungal species such as *Nocardia brasiliensis, Candida tropicalis, Candida albicans* or *Candida krusei* have also been shown to cause bovine mastitis and therefore can be transmitted to humans through incorrectly processed milk (Zaragoza *et al* 2011. Zhao *et al.*2010; Seker 2010) [31, 32, 28]

Methods employed to determine the microbial composition of milk
The ultimate identification of these cultured microorganisms involves phenotypic and/or genotypic methods (Quigley *et al.* 2013) [26]. Traditionally methods involve the growth of microorganisms in microbiological media (either general or selective) supplemented with morphological, biochemical or physiological characterisation (Quigley *et al.,* 2011) [25].

Identification of initial microbial load is important and different methods are used at field and laboratory level. Methylene blue dye reduction test (MBRT) has been used as rapid alternative method to determine whether milk is acceptable or not. Standard plate count (SPC) is a procedure that allows microbiologists to estimate the quantitative population density of microorganism in liquid milk. Tests commonly employed to determine the quality of milk include dye-reduction (Methylene blue reduction and resazurine reduction), Alcohol test, Standard plate count, Coliform count, Somatic cell count, Titrable acidity, and phosphatase tests.

a. Methylene blue reduction test

This is a rapid test to find relative number of bacteria in a milk sample. Methylene blue is a blue colored reagent which is used to estimate the bacterial population of a given milk sample. A known dilution of the methylene blue solution is added to the milk sample and observation is made at fixed intervals until the blue color disappears. Normally, if the number of bacterial organisms is greater, the time required to decolorize the blue color is shorter. This test is usually used for grading the quality of raw milk before pasteurization. On the basis of this test, raw milk is graded as follows (Kurwijila *et al*., 1992) [19]:

- Very good: not decolorizing in 5 hours.
- Good: decolorized in less than 4 hours, but not less than 3 hours.
- Fair: decolorized in less than 2 hours, but not less than 1 hour.
- Poor: decolorized in less than ½ hour.

b. Resazurine reduction test

The procedure is similar to that of the methylene blue test, except that this test is quicker and the result is obtained in much less time. Resazurin imparts blue color to milk which when reduced to resorufin changes to pink and finally to white when reduced to dihydroresorufin. The time required for complete decolorization, reduction of the resazurin and the degree of colour change is directly related to the number of bacterial organisms in the milk (Ombui *et al*., 1995) [24]. A comparator disc reading value of 4 and above for 10 minutes resazurin test indicates good quality but while a comparator disc reading value of less than 4 at 10 minutes indicates poor quality milk (Ombui *et al*., 1995) [24]

c. Alcohol test

When milk contains more than 0.21% acid, or when calcium or magnesium compound are present in greater than normal compounds, it coagulates on the addition of alcohol

d. Coliform bacteria count

It includes all aerobic and facultative anaerobic, gram-negative, non-spore forming rods able to ferment lactose with the production of acid and gas at 35°C within 48 hours. Most of them belong to the genera *Escherichia coli, Enterobacter and Klebsiella* (Godefay and Molla, 2000) [14]. The presence of coliform organisms in milk indicates unsanitary conditions of production, processing or storage. Hence, their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumer's health. A count less than 100 cfu/ml is considered acceptable for milk intended to be pasteurized before consumption. Counts of 10

cfu/ml or less are desirable if raw milk will be consumed directly (Ruegg, 2003) [27].

e. Standard plate count (SPC)

The standard plate count is a basis for grading milk Milk samples are plated on standard plate count agar media and then incubated for 48 hours at 32 °C to encourage bacterial growth. The standard plate count of raw milk gives an indication of the total number of aerobic bacteria present in the milk. All plate counts are expressed as the number of cfu/ml. SPC doesn't indicate the quality of microbial populations in terms of pathogens and non-pathogens. generally accepted as the most accurate and informative method of testing bacteriological quality of milk (Kurwijila *et al*., 1992; Godefay and Molla, 2000) [19, 14]

Bacterial cfu/ml	Grade
Not exceeding 200,000	Very good
200,000 – 1,000,000	Good
1,000,000-5,000,000	Fair
>5,000,000	Poor

Grade of raw milk based on standard plate count

Somatic cell counts (SCC)
Somatic cell counts levels are monitored to ensure compliance with set milk quality standards. Most markets in developed countries pay a premium for low SCC, good quality milk.

Aerobic spore forming count
Spore forming bacteria are divided into two main genera. The first, the genus *Bacillus*, comprises aerobic and facultative anaerobic species, whilst the second, the genus *Clostridium,* contains mainly obligate anaerobic species (Hayes, 1981). *Bacillus* species present in raw milk and are the most common cause of sweet curdling, bitter flavour and bitty cream in pasteurized milk. These defects occur because the spores of these organisms survive pasteurization. In pasteurized products held at ambient temperatures the spores, can germinate and grow to produce vegetative cells in large numbers. These organisms gain entrance to milk from unsterile utensils, and the dust of hay, straw and grains.

Titrable acidity test
Determine the sourness of milk, we use titration using sodium hydroxide (NaOH) and the degree of sourness is given by Soxhilet-Henkel Degree (SH0). Generally the sourness of normal milk is 6 to 7 SH0. If the milk sourness is 4 to 5 SH0, it indicates that either the milk is adulterated or there is mastitis (Kurwijila *et al.,* 1992) [19].

Phosphatase test
Phosphatase is an enzyme, which is normally present in raw milk. When milk is pasteurized by any of the recognized processes, the enzyme is completely inactivated. Therefore, a positive phosphatase test will indicate that the milk is not properly pasteurized.
More recently, considerable efforts have been made to develop more rapid, high-throughput tests, that rely on DNA-based, genotypic analysis. Such technologies, which usually rely, at least to some extent, on the application of polymerase chain reaction (PCR) technology, can be used to confirm the results generated through traditional tests.

Prevention and control

Proper cleaning and disinfections of equipment after each milking is important for reduction of contamination of milk from the equipment, and with rinsing about 10% of the number of bacteria found in milk can be reduced (Murphy, 1996) [21]. The important pathogenic organisms are destroyed by pasteurization of milk. The typical milk pasteurization treatment is a 'high-temperature short-time' (HTST) approach involving heating to 72 °C for 15 s. This can help to further reduce bacterial counts (Fromm & Boor, 2004) [12] and to eliminate microorganisms of concern including *Mycobacterium avium ssp. paratuberculosis* (Grant *et al*., 2002) [15] and *L. monocytogenes* (Doyle *et al*., 1987) [9]. In well-developed farms, tuberculin tests followed by segregation, examination, treatment and sacrificing of sick and infected animals according to their stage of disease. The shed must be washed every day and cleaned twice a day. All employees except administrative staff should be permanent and selected after a medical inspection. They should wash and scrub with nail brush their hands thoroughly with soap and water and put on their working clothes which should be clean and suitable for duties. All manure should be removed from the depot. It is a potent source of fly breeding and pollution. Storage at refrigeration temperatures can reduce the growth of most bacteria, with the exception of psychrotolerant microorganisms that can proliferate under these conditions and become a major cause of milk spoilage (De Jonghe *et al*., 2011) [8]

References

1. Abrahamsen RK, Borg GI, Harstad OM, Haug A, Wetlessen A. Milk Quality-Future Approach from a Researcher's Point of View. Norwegian food research institute (Matforsk), Osloveien, Norway. J. Animal and Feed Sci. 2007; 16(1):209-226.
2. Ashenafi, Byene. Microbial load, micro flora, and keeping quality of raw and pasteurized milk from dairy farm. Bulletin of animal health production in Africa. 1994: 42:55-59.
3. Bidawid S, Farber JM, Sattar SA, Hayward S. Heat inactivation of hepatitis A virus in dairy foods. J Food Prot. 2000; 63(4):522-8.
4. Bille PG, Vovor MN, Goreseb, J, Keya EL. Evaluating the Possibility of adding value to Goat's Milk by Producing Yoghurt using Low Cost Technology Method for Rural Namibia. J Food Tech Africa. 2000; 5:130-144.
5. Champagne CP, Laing RR, Roy D, Mafu AA, Griffiths MW, White C. Psychrotrophs in dairy products: their effects and their control. Critical Reviews in Food Science & Nutrition, 1994; 34(1):1-30.
6. Cisak E, Wojcik-Fatla A, Zajac V, Sroka J, Buczek A, Dutkiewicz J. Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and sheep in eastern Poland. Ann Agric Environ Med. 2010; 17(2):283-6.
7. Cook JG, Holliman A. Mastitis due to *Nocardia asteroides* in a UK dairy herd following restocking after FMD. Vet Rec. 2004; 154(9):267-8.
8. Jonghe DV, Coorevits A, Hoorde K, Messens W, Landschoot VA, Vos DP *et al*. Influence of storage conditions on the growth of Pseudomonas species in refrigerated raw milk. Appl Environ Microbiol. 2011; 77:460–470.
9. Doyle MP, Glass K, Beery J, Garcia G, Pollard D, Schultz R. Survival of Listeria monocytogenes in milk during high-temperature, short-time pasteurization. Appl Environ Microbiol. 1987; 53:1433–1438.
10. Drobeniuc J, Favorov MO, Shapiro CN, Bell BP, Mast EE, Dadu A, *et al*. Hepatitis E virus antibody prevalence among persons who work with swine. J Infect Dis. 2001; 184(12):1594-7.
11. Fadaei A. Bacteriological quality of raw cow milk in Shahrekord, Iran. Veterinary World. 2014; 7(4):240-243.
12. Fromm HI, Boor K. Characterization of pasteurized fluid milk shelf-life attributes. J Food Sci. 2004, 69.
13. Gebra-Emanuael T. Food Hygiene Principles and Methods of Food Borne Disease Control with Special Reference to Ethiopia. 1sted. Faculty of Medicine, Department of Community Health, Addis Ababa University. 1997, 73-87.
14. Godefay B, Molla B. Bacteriological Quality of Raw Milk from Four Dairy Farms and Milk Collection Center in and around Addis Ababa. Munich. Tierarzil wschr. 2000; 113:1-3.
15. Grant IR, Hitchings EI, McCartney A, Ferguson F, Rowe MT. Effect of commercial-scale high-temperature, short-time pasteurization on the viability of Mycobacterium paratuberculosis in naturally infected cows' milk. *Appl Environ Microbiol*. 2002; 68:602–607.
16. Hagstad HV, Hubbert WT. Food Quality Control, Foods of Animal Origin. 1sted. the IWA State University Press/Ames. 1986; 52:87-88.
17. Hayes S. Dairy Microbiology. National Dairy Council, London. 1981, 309-382p
18. Homan EJ, Michael AW. Technical Dairy Guide. 2nded. The Babcock Institute for International Dairy Research and Development, International Agricultural Programs, University of Wisconsin, Madison, USA. 1996, 15-23.
19. Kurwijila RL, Hansen KK, Macha IE, Abdallah K and Kadigi HJS. The Bacteriological Quality of Milk from Hand and Machine Milked Dairy Herds in Morongo, Tanzania, J. Afr. Livestock Res. 1992; 2:59-67.
20. Michel V, Hauwuy A, Chamba J. Raw milk microbial composition: differences in links with microbial practices. *Rencontres Rech Rumin*. 2006; 13:309-312.
21. Murphy SC. Sources and Causes of High Bacteria Count in Raw Milk. Cornell University, New York. 1996, 32-34.
22. O'Mahony F. Rural Dairy Technology, Experiences in Ethiopia. ILCA Manual No 4. Dairy Technology Unit. ILCA, Addis Ababa, Ethiopia. 1988, 64.
23. Oliver SP, Boor KJ, Murphy SC and Murinda SE. Food safety hazards associated with consumption of raw milk. Foodborne pathogens and disease. 2009; 6(7):793-806.
24. Ombui JN, Arimi SM, Mcdermott JJ, Mbugua SK, Githua AA, Muthoni J. Quality of Raw Milk Collected and Marketed by Dairy Cooperative Societies in Kiambu District, Kenya. Bull. Anim. Hlth. Prod. Afr. 1995; 43:277-284.
25. Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF and Cotter PD. Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. International journal of food microbiology. 2011; 150(2):81-94.
26. Quigley L, O'sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF *et al*. The complex microbiota of raw milk. FEMS microbiology reviews. 2013; 37(5): 664-

698.
27. Ruegg PL. Practical Food Safety Interventions for Dairy Production. J. Dairy Sci. 2003, 86.
28. Seker E. Identification of Candida species isolated from bovine mastitic milk and their in vitro hemolytic activity in Western Turkey. *Mycopathologia*. 2010; 169(4):303-8.
29. Wouters JT, Ayad EH, Hugenholtz J, Smit G. Microbes from raw milk for fermented dairy products. International Dairy Journal. 2002; 12(2):91-109.
30. Yazdankhah SP, Hoiby EA, Roberrtson L Nrvhus J, Blom H, Fossum K *et al.* Qualitative Assessment of the Risks of Transmission of Microorganisms to Humans from Consumption of Raw Milk. Norwegian scientific committee for food safety, Panel on Biological Hazards, Oslo, Norway. 2004.
31. Zaragoza CS, Olivares RA, Watty AE, Moctezuma Ade L, Tanaca LV. Yeasts isolation from bovine mammary glands under different mastitis status in the Mexican High Plateu. Rev Iberoam Micol. 2011; 28(2):79-82.
32. Zhao T, Zhang R, Wang M. Prediction of candidate small non-coding RNAs in *Agrobacterium* by computational analysis. J Biomed Res. 2010; 24(1):33-42.