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Participation of Microorganisms in Milk and Milk-products Contamination and Safety

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Abstract

Milk and milk-products represent the main basic nutritional healthy food in the human diet; however, milk is also a favorable source of microbial infection for human health when milk and milk products are consumed without applying hygiene milk practices methods such as pasteurization and other effective methods to avoid contamination risk. The presence of microorganisms in milk could result in spoilage and severe diseases to humans. Several recent preservation systems such as heating, refrigeration, and the addition of safe antimicrobial compounds can be used to reduce the risk of outbreaks of dairy product poisoning. Proper food control programs must be implemented in all countries around the world to ensure the safety of food and dairy products. Investigators reported the importance of applying effective hygiene practices during milking and handling of raw milk to reduce the risk of contamination on the farm and in the milk processing plant in the industry.

1. Introduction

The control and disposal of contaminated and undesirable microorganisms in dairy and veterinary industries are very important in determining the quality of their final products **(Pal, 2014).** In dairy products some beneficial species of microorganisms are required for the production process of many dairy products such as Yogurt and cheese making **(Settanni and Moschetti, 2010; Singh *et al.*, 2016).** These include the conversion of milk constituents by enzymes of various species to attain the desirable flavor, taste, aroma, ripening and texture of these dairy products **(McSweeney *et al.*, 1997Ayad *et al.*, 2001)**. Buttermilk and Yogurt are examples of fermented milk products depending mainly on characteristic microflora that is responsible for their flavor and texture **(Ali *et al.*, 1995).** On the other hand microbes are undesirable in milk and their products causing disease. To ensure the safety of dairy products to consumers scientists should checked for decolorization, rancidity, ropiness, putrefaction, gassiness and many other defects that caused by different harmful microorganisms **(*Garcha, 2018).*** In this connection, many hygienic milk practices such as pasteurization, storage, handling, transport and distribution before consumption have greatly decreased the threat of milk-born diseases **(Yogesh *et al.*, 2012)**. Examples of the main discovered bacterial pathogens in milk and milk products are *Escherichia coli, Salmonella sp., Bacillus cereus, Campylobacter jejeni, Yersinia enterocolitica* **(Lubote *et al.*, 2014).** Some other genera of filamentous fungi producing mycotoxins are able to grow on milk and milk products (such as *Penicillium, Aspergillus and Fusarium*) that can be a fatal hazard to the consumers. Although some molds are responsible for ripening of many types of cheese (such as Roquefort and Camembert) and their enzymes such as amylase in making bread or citric acid used in soft drinks, some of them are mainly responsible for food spoilage at room temperature up to 30^0C and low pH, and have minimum moisture requirement. Yeasts capable to ferment sugars to ethanol and CO_2 such as *Saccharomyces cerevisiae* (or Backers' yeast) and *Sach. carlsbergensis* are used mainly in the process of making bread and fermentation of most beers respectively and the fungus *Agaricus bisporus* is one of the most used mushroom as a food source. Psychotrophs are mainly involved in milk spoilage and mainly destroyed by pasteurization, however many bacterial species such as *Pseudomonas fragi* and *Ps. flurescens* have the ability to produce heat resistant extracellular proteolytic and lipolytic enzymes capable of causing spoilage (Table 1). This review sheds light on some microbes found in milk and milk products and the appropriate methods to get rid of them using many recent effective methods.

2. Microbial contamination of milk and Milk products

The microbial contamination of milk and milk products takes places usually during processing, storage, transport and distribution before consumption. Dairy-borne infections have been identified as an important economic problem and public health in all countries around the world **(Pal et al., 2014).** For this reason microbial dairy safety represents a significant global issue for the consumer and industry. Microbial contamination is one the leading causes of milk and milk-products spoilage. Spoilage of milk and milk-products involve any change, which renders them unacceptable for human consumption. This is

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mainly due to the presence of highly nutritious components in milk and dairy products make them especially good media for the growth of microorganisms **(Ledenbach and Marshall, 2009).** The infectious undesired microorganisms present in contaminated milk and milk-products include for example *Escherichia coli*, *Mycobacterium bovis*, *Mycobacterium tuberculosis, Yersinia enterocolitica*, *Salmonella* and *Listeria*. These microbes can cause serious disease for immunity compromised individuals, children and pregnant women **(Pal, 2007; FAO, 2013)**. It is worthy to mention that the process of pasteurization cannot destroy all pathogenic microorganisms in milk, as many investigators reported the presence of *E. coli*, *Yersinia enterocolitica, Salmonella* spp.and *Staphylococcus aureus*, **(Pal *et al*., 2012)**. In order to maintain the safety of milk and milk-products molecular, immunological and microbiological techniques should be implemented to detect the presence of these pathogens. The main cause of failure of processing and packaging systems is the development of resistant bacterial biofilms to chemical sanitizers on equipment surfaces **(Ledenbach and Marshall, 2009; Pal *et al*., 2013). Sarkar (2015)**, reported that the poor quality of raw milk is due to microbial contamination, improper temperature control and inadequate packaging system **(Sarkar, 2015).** Fluid dairy products get easily contaminated with microorganisms than the dried products such as *Streptoccocus, Staphylococcus, Pseudomonas* spp. **(Fernandes, 2008).**

3. Cheese

Cheese is a dairy product rich in protein, calcium, phosphorus and vitamins produced by casein coagulation and entrapment of milk in the coagulum **(Fernandes, 2008)**. Different sources including environment, handling and packaging are responsible for microbial contamination of cheese **(Pal *et al*., 2014)**. **Vrdoljak *et al*., (2016)** reported that *Listeria monocytogenes* is the most food-borne pathogen in cheese in the processing phase. Other investigators indicated that certain strains such as *Streptococcus thermophilus* and *Lactobacillus helveticus* are capable of producing carbon dioxide gas resulting to the presence of cracks in cheeses **(Ledenbach and Marshall, 2009).** Although cheese is considered as a safe food due to the presence of antagonistic properties of lactic acid bacteria in the process of cheese making **(Kousta *et al.,* 2010),** however food borne outbreaks were found to be a result of contamination with *Staphylococcus aureus* the main cause of mastitis in cows (Rabello *et al.,* 2007). *S. aureus infections in cheese is due to* improper handling or to the use of unpasteurized milk because this bacterium produces heat-resistant enterotoxin **(Delbes *et al.,* 2006; CDC, 2010)**. **Ryser, (2001)** reported that all various *Salmonella* serotypes have been involved in cheese-borne outbreaks and all *Salmonella* strains are gastroenteritis-inducing pathogens. In this connection, microbial risk assessment is the proper scientific method for preventing, regulating and understanding the risk caused by hazardous microorganisms in cheese **(EPA, 2012).**

4. Yogurt

Yogurt is a unique fermented type of dairy product containing many nutritional components including protein, vitamins, calcium, phosphorus and magnesium **(Pal *et al.,* 2015).** The addition of fruits and flavor compounds to yogurt improve required conditions for the growth of molds and yeasts, but after a while they die out due to the acidic medium and the antagonistic effect exerted by lactic acid bacteria. The composition of the pleasant flavor of mature yogurt is mainly due to the presence of about equal proportions of starter cultures namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the former adds flavor and aroma to yogurt, however the latter is mainly responsible for acid production **(Yamani and Ibrahim, 2007; Goel *et al.,* 1971)**. Yogurts have been found to be contaminated with both spoilage and pathogenic microorganisms due to unhygienic production processes which give the yogurt unsatisfactory sensory quality **(Mbaeyi-Nwaoha and Egbuche, 2012; Makwin *et al.,* 2014)**. Pathogenic bacteria that can cause spoilage of yogurt include Gram-negative psychrotrophs and coliforms **(Willey *et al.,* 2008; Oyeleke, 2009; Yabaya and Idris, 2012)**.

5. Protection of dairy products from spoilage microorganisms

To give dairy products the desirable shelf-life it is of importance to implement protection from spoilage during their preparation, storage and distribution. Several preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of dairy products poisoning. Nowadays, the food industry investigates more modern preservation techniques to replace the traditional food preservation including milk products in order to be accepted by consumer demand for nutritious, tasty, natural and easy-to-handle food products. In this connection, sorbic acid, lactic acid, benzoic acid and acetic acid are the most common classical preservative agents that inhibit the growth of bacterial and fungal cells **(Arneborg *et al.,* 2000).** Sorbic acid was found to inhibit the outgrowth of bacterial spores. The process of freezing prevents microbial growth and their enzymes, therefore the ingredients should be added prior freezing to ensure safety of food-products **(Rawat, 2015)**. The microbiological standard methods used for indicator microorganisms as a predictor of the safety and quality of milk and dairy products differ from country to country, each have their specific tests, regulations or guidelines for the contaminated microorganisms. The most used methods include Standard plate count (SPC) [100,000/ml max individual bulk tank]; Somatic cell count (SCC) [750,000/ml max individual bulk tank]; Aerobic plate count (APC) [100,000/ml max.]; Test for Coliforms, *E.coli* and Psychrotrophs.

6. Microorganisms in dairy products

6.1 Psychrotrophic microbes

This type of microorganisms prefers to grow on cold temperature especially in raw milk at 3-7^{0}C. They can easily hydrolyze proteins and lipids for their growth. The proper salt concentration in the cottage cheese content insufficient to limit the growth of these contaminating bacteria. For this reason psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese **(Ledenbach and Marshall, 2009).** Investigators reported that the presence and subsequent replication of populations of psychrotrophs may lead to the spoilage of milk **(Pinto *et al.*, 2006; Nörnberg *et al.*, 2010).** Development of molecular biology for bacterial identification has revealed the presence of psychrotrophic bacteria not previously detected by the traditional methods **(Raats *et al.*, 2011; Almeida and Araujo, 2013). Mcphee and Griffiths, (2011)** reported that the reduction in cheese yield mainly due to the enzymes secreted by psychrotrophs that affect rennet coagulation times and altered starter activity and growth rate. The main cause of cheese reduction is mainly due to the loss of soluble casein degradation products into the whey instead of forming a part of the curd **(Mcphee and Griffiths, 2011; Mankai *et al.*, 2012)**. **Beales, (2004)** reported that refrigeration of milk and dairy products alone or in combination with other methods including the addition of preservatives is the most proper means of preservation. Generally the psychrotrophic bacteria in milk and milk products represented predominantly by Gram -ve genera including *Pseudomonas*, *Achromobacter*, *Serratia*, *Alcaligenes,* *Aeromonas*, *Chromobacterium* and *Flavobacterium* spp. and in much lower numbers by Gram +ve genera including *Streptococcus, Lactobacillus*, *Clostridium*, *Corynebacterium*, and *Microbacterium* spp **(Sørhaug and Stepaniak, 1997; Mcphee and Griffiths, 2011)**.

6.2 Coliforms

These microorganisms are Gram negative, facultative anaerobic, rod shaped bacteria capable of fermenting lactose to produce gas and acid, belong to the family *Enterobacteriacea* such as *E.coli*, *klebsiella, Enterobacter aerogenes*, **(Pal and Mahendra, 2015)**. The slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria. In case of soft ripened cheeses production, the increase in pH during the process of ripening reflects directly to the increase of coliform bacterial growth **(Ledenbach and Marshall, 2009)**. Application of strictest sanitary measures during milking process in the farm and milk storage and transportation to the dairy industry is the best way to prevent coliforms contamination. Recently the major challenge for dairy producers is to prevent post-pasteurization contamination (PPC) with spoilage microorganisms including coliforms **(Ranieri and Boor, 2009; Martin *et al.,* 2011).** The detection of coliforms in dairy products and pasteurized milk play a major role as a hygiene indicator tool for contamination. Their growth at refrigerated storage temperatures are of concern for dairy industry which can result in degradation of the product in addition to unacceptable sensory characteristics due to the formation of proteolytic and lipolytic enzymes **(Nörnberg *et al.,* 2010).**

6.3 Listeria monocytogenes

L. monocytogenes is a Gram-positive, rod-shaped, non-spore-forming, and facultative anaerobe bacterium causing public health problems. Both normal and diseased animals are the main source of the food-borne human pathogen *Listeria monocytogenes* in milk and dairy products. The pasteurization of milk does not *eliminate the milk and dairy products contamination by this bacterium* **(Sukhadeo and Trinad, 2009; Gould *et al.,* 2013)**. Investigators described non-thermal technologies high hydrostatic pressure (HHP) and pulsed electric fields (PEFs) as new preservation methods to control and prevent the growth of food-borne pathogens including *L. monocytogenes* **(Norton and Sun, 2008; Tomasula *et al.,* 2014).** These methods are mainly used to avoid undesirable changes in the nutritional bioactive compounds such as vitamins, and pigments in addition to sensory properties such as texture, taste, and flavor and consequently reducing their acceptability by consumers **(Cebrian *et al.,* 2016; Barba *et al.,* 2017)**.

6.4 Spore-Forming Bacteria

The most resistant life forms known in milk and milk-products are the pathogenic and spoilage associated species belonging to Bacilli and Clostridia classes. Clostridium species are well known contaminants in milk due to their ubiquitous nature and can enter the milk chain from different sources and their biofilms are highly resistant to heat and disinfectants. Many species such as *Clostridium botulinum* and *Clostridium perfringens* produce toxins causing dairy products poisoning. In this connection many cephalosporins antibiotics such as cephalexin and the cephamycin cefoxitin, have been found to inhibit effectively sporulation **(Miyamoto *et al.,* 1997; Hao and Kendrick 1998; Doyle *et al.,* 2015; Gopal *et al.,* 2015; Kumari and Sarker, 2016).** Bacillus species such as *Bacillus licheniformis* and *Bacillus pumilus* have been reported as the most commonly identified species in raw milk **(Miller *et al.,* 2015).**

6.5 Fungi

Fungi have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Some spoilage molds are toxigenic while others are not **(Pitt and Hocking, 1997).** They grow at a pH range of 3 to 8 and attack a wide variety of foods including milk products, their spores can tolerate unsuitable environmental conditions but most of them are sensitive to heat treatment. Different fungal species have different optimal growth temperature; however some few others can grow on cold conditions. Spoilage fungi can be categorized into the following groups: a) Zygomycetes: These fungi have the ability to grow on simple carbon sources and require high water activity for growth. Examples of this group are *Rhizopus* and *Mucor* species. b) *Penicillium*: They are distinguished than other spoilage microorganisms by their reproductive structures that produce chains of conidia. They are able to produce antibiotics and other dairy products such as blue cheese. Some species of this genus can produce mycotoxins and others can attack refrigerated milk and milk products. In this connection, a related genus namely *Byssochlamys* is the most serious causing spoilage fungi due to its high heat resistance of its spores. c) *Aspergillus*: These fungi are generally resistant to high degree of temperature and low water activity as previously described in case of Penicillium genus. They prefer warmer climate for growth. Many of them produce ochratoxin, aflatoxins and mycotoxins and can affect many food sources such as grains, peanuts and some spices. c) Others: These types of fungi are belonging to several genera and have been isolated and characterized from spoiled milk and food; they are able to produce mycotoxins such as *Fusarium* species **(Pal, 2014).**

4. Conclusion

The contamination of milk and milk products by pathogenic bacteria and spoilage producing microbes result in great financial loss to the dairy sector. Bacterial contamination occurs either by direct transfer from the blood due to systemic infection (endogenous contamination) or by contamination by faeces, skin, utensils and environment during and after milking (exogenous contamination). The main undesirable contaminants of milk in the refrigerated dairy food chain are psychrotrophic bacteria. From the previously mentioned contamination sources measures should be taken to avoid and prevent the spread of zoonotic diseases among animals, improving their hygiene, controlling the infection from feed and fodder, safe waste management and easy access to veterinary service. Investigators reported that most pathogenic as well as spoilage microorganisms can be unable to grow in milk and milk-products when the pH of the environment is 4.5 or lower. These microbiologically safe products would contribute towards the nutrition of susceptible infants to diarrheal diseases. The dairy-borne diseases in public health programs are of importance in the surveillance of milk food borne diseases by monitoring microbial contamination and milk borne pathogens in milk products. Proper governmental policy for the assurance the quality of milk and dairy products should be implemented to reduce the public health risks towards these products. More studies should be conducted concerning the inactivation kinetic determination to establish how the process conditions for microbiological safety should be done.

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Declaration of interest

Author declares that he does not have any conflict of interest.

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