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Digestive Pathologies Caused by *E. coli* Bacteria in Non-Treated Dairy Products

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REVIEW

Abstract

The purpose of this review is to summarize current possibility of contamination of milk and dairy products and human pathologies as well, that may occur if these products are not processed properly. Scientific literature was consulted to examine the methods of contamination of milk and dairy products. In raw or pasteurized milk stored at 5 °C was not reported that it can grow *E. coli*, however can grow slowly at higher temperatures. *E. coli* is considered a high-risk potential pathogen in cheese, due to its unusual ability to tolerate low pH values for long periods and it's linked to unpasteurized raw milk.

Keywords: contamination, dairy products, microorganism, milk, pathologies.

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INTRODUCTION

The milk contains most of the vitamins, significantly increasing its nutritional and biological value. These vitamins include A, D, E, K and B1, B2, B6, B12, H , PP, C, pantothenic acid, folic acid, para-aminobenzoic acid, choline, water-soluble. Many of these vitamins act as growth factors for microorganisms that contaminate or are added to milk for technological purposes (Barzoi, 1985). In developed countries, the raw milk at the dairy farms, is usually immediately cooled and stored in refrigerated tanks at <7 °C before collection. The tanker truck, collects the milk every other day or, sometimes, less frequently, therefore some of the raw milk provided by the dairy farmers may be up to 48 hours old at the time of collection. Temperature control is essential to minimize microbial growth, and tank drivers are usually permitted to reject milk stored at a temperature too high with an abnormal appearance or odor. Therefore, milk storage temperature as well as transportation duration are important factors. Upon arrival at the processing site, the milk is transferred to bulk storage tanks or silos before processing. Milk can be stored in silos for 2-3 days, and during this period, psychotropics bacteria may continue to grow. The degree of growth depends on the initial microbial load, storage time, and temperature (Ternstrom et al., 1993). Although the milk produced from the mammary glands of healthy animals is initially sterile, microorganisms can enter the udder through the teat canal (McKinnon et al., 1990). Gram-positive cocci, streptococci, staphylococci, and micrococci; lactic acid bacteria, *Pseudomonas spp*. (Boor, 1997) and yeast are most commonly found in aseptically aspirated udder milk; corynebacteria are also common. If the mammary tissue becomes infected and inflamed, a condition known as mastitis, a large number of microorganisms and somatic cells are usually introduced into the milk. Mastitis is a very common disease (Bramley & Dodd, 1984) in dairy cows and may be present in a subclinical form, which can

only be diagnosed by examining the milk for an increased somatic cell count. Many bacterial species can cause mastitis, but the most common are *Staphylococcus aureus* (Sears et al., 1990), *Streptococcus agalactiae*, *Streptococcus uberis* (Vasavada, 1988) and *Escherichia coli* (Van et al. 1997; Waage et al., 1999; Sargeant et al., 1998; Bramley & McKinnon, 1990). Although organisms involved in mastitis (Barkemaet al., 1998) are usually unable to grow in refrigerated milk, they are prone to survival and growth, therefore their presence can be a health concern. Sick cows (Steglich, 2006) can also accumulate other human pathogens in their milk, including *Mycobacterium bovis*, *Brucella abortus*, *Coxiella burnetii*, *Listeria monocytogenes*, and *Salmonella* (Wahniyathi, 2013; Mahsa et al., 2021; Oliver et al., 2005). The exterior surface of the udder is also a major source of microbial contamination of the milk. The surface unfortunately, can be contaminated with a variety of materials, including soil, bedding, feces, and silo as well as other feed residues (Garber et al., 1999). Consequently, animal hygiene and efficient udder cleaning and disinfection before milking are important for minimizing or avoiding contamination (Fernandez, 2008; Pankey, 1989).

Untreated raw milk (Constance et al., 1964) may contain pathogens derived from cows (or other dairy animals), such as *Campylobacter spp*., *Salmonella spp*., *Cryptosporidium*, *E. coli*, *S. aureus* and *L. monocytogenes* (Kumar & Sinha 1989; Ntuli et al., 2016; Abd El Rahaman et al., 2016) which will be subsequently destroyed by the pasteurization process (Great Britain Regulation No. 2383, 1989).

Most types of cheese (Quinto et al., 1997) are made by fermenting milk (Yusuf et al., 2018). The finished product typically contains a large number of lactic acid-producing bacteria that have been used to produce fermentation along with the molds and bacteria used to impart traditional flavors (Chandan & Shahani, 1995). Fresh cheese often has a small number of bacteria of about 10^3 organisms/g due to the destruction of lactic acid bacteria by heat during cheese production (Boor et al., 1998; Chang et al., 2000; Cian et al., 2015).

Pasteurization is the process (Chintescu and Patrascu, 1918) of destroying the vegetative forms of milk microorganisms. During pasteurization, some spores present in the milk are also destroyed.

Sterilization by UHT (ultra-high temperature processing), consists of rapidly heating the milk to very high temperatures of 145 degrees Celsius for a few seconds or 150 degrees Celsius (¾ of a second) followed by sudden cooling.

Milk sterilized by this process tastes close to that of fresh milk and does not undergo changes in nutritional value and has a shelf life of several weeks - months or unlimited time (Apostu, 2006).

Cheese can be made from raw milk (Asmahan et al., 2011), pasteurized milk or milk that has undergone a sub-pasteurization treatment. Pasteurization destroys vegetative cells of pathogens, as well as many decaying organisms or some of the enzymes naturally present in milk. It is claimed that pasteurization affects the maturation and flavoring of cheese and that only raw milk cheeses develop a full, mature flavor. (Nicol & Robinson, 1999).

However, a recent study suggested that when using high-quality milk, the pasteurization produces differences in texture, but flavor and other characteristics were little affected (Dunkley & Stevenson, 1987). A process of sub-pasteurization (thermization) (typically 65-70 °C for 15-20 seconds) can be used to destroy many vegetative cells, but without inactivating some enzymes involved in flavor's development.

The main disadvantage of raw milk is the possible presence of pathogens such as *Staphylococcus, Listeria, Salmonella* and *Escherichia coli* (Adesiyun, 1994; Cousin, 1982) that have caused outbreaks of infection associated with unpasteurized cheeses. Ideally, from a safety point of view, only pasteurized milk can be used to produce cheese. Despite this, there is a constant demand for unpasteurized cheese, which can be perceived as a superior product with higher quality. The manufacture of unpasteurized cheese must be very well managed, with effective control measures in place (Dineen, 1998). Pasteurized milk for cheese production has a bacterial flora made up of thermomod organisms that have survived pasteurization, such as corynebacteria, micrococci, enterococci, *Bacillus* and *Clostridium* spores, and post pasteurizing contaminants including coliforms and psychotropic gram-negative organisms (Hooi et al., 2004).

The reports indicating that cheese is safe for consumption (Johnson et al., 1990) are relatively good, considering the very large quantities are consumed around the world. However, there have been a number of serious outbreaks of foodborne illness associated with cheese, and these are well documented. The most serious outbreaks were caused by enteropathogenic *Listeria monocytogenes*, *Salmonellae* and *Escherichia coli* (Marrier et al., 1973; Neil, 2012; Upton & Cola, 1994). In recent years, a number of cheese-related outbreaks of *E. coli* (Jun et al., 2017; Soomro et al., 2002) have been recorded (Knappstein et al., 2001).

Cheese made from unpasteurized milk are particularly at risk because they can become contaminated by pathogens originally present in milk (Stephane et al., 2014; Reitsema et al., 1996). Pathogens can also enter during processing (Doyle et al., 2012), if hygiene and process control are inadequate. The characteristics of individual cheese varieties significantly influence the potential presence and survival of pathogens. The processing and storage temperature, acid production by starter crops, and the addition of salt are all important. In general, soft and semi-soft cheeses with water activity present fewer obstacles to pathogen survival and growth than hard cheeses (Fernandes, 2008).

MATERIALS AND METHODS

Methods

Study design

This study was made on the basis of articles, books and reviews found in the field of study of the processing and obtaining technology dairy products.

Population

No direct participation of individual participants is need in the current work.

Inclusion and exclusion criteria

Inclusion criteria

Studies conducted under this review were different regions where there are local milk processing factories, heat treatment of milk and dairy food.

Exclusion criteria

Unpublished reports, letters, were excluded.

Search strategy and resources

The review of this domain was made through deep search of relevant articles on the topic from the essential data base. The relevant articles were searched from web of science, PubMed, Research gate, Science direct, National Library of Medicine and other articles obtained from local factories.

RESULTS AND DISCUSSIONS

Recent studies have shown that on a dairy farm the degree of microbial loading of milking milk from 200 healthy cows of the same farm was followed and found that 2.5% of cows gave sterile milk, 15% of cows gave milk with 1-100 germs / ml, 42% of cows gave milk with 1000 - 5000 germs / ml, 8.5% of cows gave with 5000-1000 germs / ml, 4% of cows gave milk with> 10000 germs / ml. (Davies & Wilkinson, 1973)

The current state of research shows that in 2016 between May 16 and December 30, from 1484 samples of milk, and cheese, taken from different parts of the country, in 69 samples was discovered the *E. coli* bacterium (Davidson et al., 2004; Voetsch et al., 2007).

A study conducted in the Africa, showed that the process of obtaining milk and dairy products is affected by the application of improper processes during milking and processing. Contamination levels of raw milk samples evaluated during 6 weeks varied widely; about half of the samples complied with threshold values for TBC (<6.3 Log CFU/ml), while high coliforms count (>5.0 Log CFU/ml) were detected in some samples. In pasteurized milk, high residual counts were observed, indicating an insufficient efficacy of thermal treatment applied at the small-scale unit; residual values >3 Log CFU/ml were detected for TBC, in some cases associated with high counts for the other parameters.

Among dairy products, mozzarella and soft cheese were mostly contaminated by coliforms (mean value ≥ 2.7 Log CFU/g), while low contamination levels were detected for ricotta and yoghurt. Some samples of mozzarella also harboured high counts (>3 Log CFU/g) of *E. coli* (Belli et al., 2013).

In the region of Posada de Valdeon, the Valdeon cheese is produced on an industrial scale from raw pasteurized cow and/or goat milk. They evaluate the impact of pasteurization on the microbiological, physicochemical and sensory characteristics of cheese.

In general, microbial counts obtained in raw milk cheeses were higher than those obtained in batches made from pasteurized milk, except for moulds and yeasts counts, which were significantly lower in cheeses made from raw milk ($P < 0.05$).

Similarities can be observed in the presumptive *Leuconostoc* and *Lactococcus* counts. However, *Lactobacillus* counts were approximately 2 log units higher in raw milk cheeses than in pasteurized milk cheeses. *Enterobacteriaceae* counts were not detected in pasteurized milk cheeses due to heat treatment; however, counts were elevated in raw milk cheeses. *Enterococcus* counts were very low or even undetected in cheeses made from pasteurized milk (Diezhandino et al., 2022).

The production of heat-treated drinking milk shall include all necessary measures, in particular checks by random sampling, to determinate the total plate count to ensure that raw milk, if not processed within 36 hours of procurement, does not exceed, prior of heat treatment, a total plate count of 300 000/ml, in the case of cow's milk. Or for milk which has undergone previous pasteurization has, prior of the second heat treatment, a total plate count of germs at 30 °C not exceeding 100 000/ml. Heat-treated drinking milk will be subjected to periodic checks (Christen, 1993) to determinate the presence of excess water by checking the freezing point.

To this end, a control system shall be established under the supervision of the competent veterinary authority. When excess water is detected, the competent veterinary authority will take appropriate measures.

In the case of products manufactured from milk, the operator or manager of the processor must take all necessary measures to ensure that the milk is heat-treated or raw milk is used as soon as possible after being received, if it has not been cooled, and not later than 36 hours after receipt, if the milk is stored at a temperature not exceeding + 6 °C and no later than 48 hours after reception, if the milk is stored at a temperature not exceeding +4 °C. Heat-treated milk must meet certain requirements.

In the case of cow's milk, it must be obtained from raw milk with a total plate count of germs at a temperature of 30 °C right before thermisation and not exceeding 300 000/ml (Serap, 2007), if the milk in question is not heat-treated within 36 hours of its reception in the establishment.

If it's used to produce pasteurized milk, UHT milk or sterilized milk, it must meet the following standards before treatment: total plate count at 30 °C ≤ 100 000/ml.

Pasteurized milk must be obtained through a process of heat treatment involving a high temperature for a short time, at least 71,7 °C for 15 seconds, or equivalent combinations, or by a pasteurization process using combinations of different time and temperature to obtain an equivalent effect.

UHT milk must be obtained by heat treatment of raw milk at a continuous heat flow involving the application of a high temperature for a short time, not less than +135 °C for at least one second, the ultimate goal of this treatment being the destruction of any microorganisms and their spores with minimal chemical changes, physical and organoleptic of milk.

Prepackaging and packaging must be carried out hygienically in premises specially designated for that purpose. Without prejudice to existing national legislation, prepackaging and packaging must satisfy hygiene rules and the materials used must be sufficiently strong to effectively protect the products covered by this Directive.

Bottling, filling containers with heat-treated milk and liquid milk-based products, sealing containers and packaging must be carried out mechanically.

In the case of limited dairy production, the competent authorities may authorize sealing methods which are not automatic, provided that such methods offer the same standards regarding hygiene.

Materials used for prepackaging and packaging may not be reused for products covered by health rules, with the exception of certain types of containers which can be reused only after they have been thoroughly sanitized and disinfected.

The sealing must be carried out in the establishment where the last heat treatment of drinking milk and/or liquid milk-based products was applied, immediately after filling the containers, using sealing devices which ensure the preservation of characteristics of the product.

In order to ensure the validity of products covered by the veterinary standard, which cannot be stored at ambient temperature, they shall be stored at the temperatures set by the manufacturer and specified on the product label. Pasteurized milk can be stored before shipping and during transportation at a maximum temperature of +6 °C.

When storage takes place in cold conditions, storage temperatures must be recorded and cooling must be carried out so that the products reach the required temperature as quickly as possible.

Tanks, or any other containers used for transporting pasteurized milk must comply with all hygiene conditions and, in particular, their internal surfaces and any other part likely to come into contact with milk must be made of smooth material which is easy to clean, sanitize and disinfect, and cannot be corroded and does not transfer any substances to milk to endanger public health or to impart abnormal organoleptic characteristics to the milk. The containers must be designed in such a way that milk drainage is complete. If fitted with taps, they must be easy to dismantle, clean, sanitize and disinfect and must be cleaned, sanitized and disinfected immediately after each use and, if necessary, before reuse.

The equipment of transport, receptacles and containers used for transporting pasteurized milk must be designed and equipped in such a way that the required temperature is maintained throughout transport.

Transport equipment used for transporting heat-treated drinking milk and milk in small containers must be well maintained and comply with hygiene standards. They may not be used for the transport of other products as they can alter or deteriorate the normal characteristics of milk.

The interior of vehicles intended for the transport of heat-treated milk must comply with all hygiene rules. Vehicles intended for the transport of heat-treated milk in small or small containers must be designed in such a way as to adequately protect the product against any contamination and external actions and may not be used for the transport of animals.

In order to ensure hygiene requirements, the competent veterinary authority shall periodically check the means of transport and the loading and unloading conditions.

Products covered by this Directive shall be unloaded in such a way as to protect them from possible contamination or external action.

During transport, the temperature of pasteurized milk transported in tanks or packaged in small containers and in the few must not exceed +6 °C.

Additional conditions for the storage and transport of milk and specific milk-based products may be drawn up in accordance with the procedure laid down by the central veterinary authority.

The establishments referred to in the health standard shall be subject to supervision by the competent veterinary authority, which must ensure that the requirements of the veterinary standard are complied with, and in particular to verify the hygiene of premises (Clegg at el., 1967), equipment and staff and the effectiveness of self-checks carried out by the establishment and the microbiological and hygienic conditions of milk and milk-based products; the effectiveness of heat treatments applied to heat-treated drinking milk and milk-based products, hermetically sealed containers and containers by random sampling, the appropriate health mark applied to milk and milk-based products, storage and transport conditions, to take any samples for laboratory testing whenever necessary, to carry out any control necessary to ensure compliance with the provisions of the sanitary norm (Ministry of Agriculture, 2002).

A study conducted in dairy farms to extend HACCP certification on raw milk quality (Tatiane et al., 2014) showed that data obtained on bacterial and somatic cell counts in HACCP-certified farms was statistically ($P < 0.05$) higher compared with the milk in noncertified farms. Similarly, milk fat and milk protein were statistically ($P < 0.05$) superior in HACCP-certified farms. The study finds that the practical barriers to implementing HACCP include inadequate financial assistance, incentive programmes, HACCP consulting and education, consumer awareness and knowledge about HACCP-certified products (Chon et al., 2021).

CONCLUSIONS

Cultivation on culture media is one of the main analyses that can be done in the laboratory to detect the contamination of milk and dairy products following the technological process of processing, treatment and packaging. In order to avoid contamination in raw milk or dairy products, rigorous monitoring of the entire technological process and well-established control and quality systems are needed. *E. coli* is one of the most common bacteria in both milk and dairy products but can be destroyed if subjected to proper technological processes or proper handling.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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