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Review

# Microbial benefits and risks of raw milk cheese

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ABSTRACT

Consumer preference for raw milk cheese is continually growing, owing to its more intense and varied flavor than pasteurized milk cheese. Flavor development in raw milk cheese is mainly governed by its naturally existing microbial community, which also contributes to the inhibition of food-borne pathogenic bacterial growth. Lactic acid bacteria, the dominant indigenous microorganisms of raw milk cheese, produce pathogen-inhibiting substances such as bacteriocin, organic acids, and hydrogen peroxide, and it is possible to manufacture cheese with desirable microbiological qualities. Nonetheless, outbreaks of food-borne illnesses have been linked to the consumption of raw milk cheese, and concerns have been raised regarding the microbiological safety of cheese manufactured from raw milk. Consequently, efficient and accurate methods for detecting contaminated bacterial pathogens in raw milk cheese have been promptly developed, including conventional plating, PCR-based technology, and immunoassay-integrated methods. The microbiological risk of the cheese can be reduced by proper ripening processing. However, additionally, hygiene in the environments for milk production and cheesemaking and the post-manufacturing stage needs to be constantly microbiologically monitored.



**Contents**



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## 1. Introduction

Humans have made cheese for a long time to concentrate and preserve milk, and cheese is one of the oldest known types of manufactured foods (Nelson, 1984). In particular, raw milk cheeses have been produced because of their intense and stronger flavor compared with that of pasteurized milk cheeses (Casalta, Sorba, Aigle, & Ogier, 2009; Masoud et al., 2012). In the United States, 65 food-borne outbreaks related to dairy products were reported during 1993–2006. Of those outbreaks, 27 (45%) were linked to raw milk cheese and 38 (58.5%) were linked to pasteurized milk cheese. Therefore, in recent decades, raw-milk cheeses have often been considered risky foods (West, 2008). In fact, there have been reported global outbreaks of food-borne disease attributed to consumption of raw milk cheese. Especially, soft-ripened cheeses such as camembert cheese, which has recently become popular, are considered to be at risk for harboring foodborne pathogens (Brooks et al., 2012). Thus, some consumers believe that raw milk cheese is not safe compared with pasteurized milk cheese. However, several studies have reported that pasteurized milk cheese caused outbreaks of food-borne illness, in some cases at a higher incidence rate than that for raw milk cheese (Koch et al., 2010). In addition, an extremely low or zero percentage of raw milk cheeses has been contaminated with major pathogens including *Listeria monocytogenes* (Little et al., 2008; Ryser, 2007). Since microbiota, a natural microbial community in raw milk, prevents the growth of contaminating food-borne pathogens during cheesemaking, raw milk cheese can be evaluated as being a rather microbiologically safe food in this respect (Masoud et al., 2012). Taking the reports together, the microbiological safety of raw milk cheese is still a highly controversial topic. Therefore, the objective of this communication was to review published literature regarding the microbial safety concern of raw milk cheese.

## 2. Microbiological benefits of raw milk cheese

### 2.1. Sensory diversity

Raw milk cheeses have been extolled as having a more intense and stronger flavor than that of pasteurized milk cheeses, which has been attributed to a number of indigenous microbiota, such as *Lactococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Enterococcus* spp. (Casalta et al., 2009; Masoud et al., 2012; Verdier-Metz, Michel, Delbès, & Montelm, 2009). Thus, pasteurization of milk causes adverse effects, such as the inactivation of enzymes such as proteases or lipases and the natural microbiota present in raw milk, both of which play significant roles in enhancing the sensory quality of cheeses (Grappin & Beuvier, 1997). Raw milk cheeses contain higher amounts of volatile compounds such as carboxylic acids, esters, and alcohols as a result of fermentation of milk components by natural microbial communities compared to pasteurized milk cheeses (Ocak, Javidipour, & Tuncturk, 2015). Besides flavor, the texture of raw milk cheese can be diversified depending on raw milk microbiota composition, and processing and seasonal conditions of cheesemaking (Beuvier et al., 2004; Tunick, Hekken, Call, MolinaCorral, & Gardes, 2007). In this regard, microbial diversity of raw milk contributes to the manufacture of cheese variety with different sensory characteristics such as flavor and texture, that is absent in pasteurized milk cheese.

### 2.2. Microbial safety improvement

Pasteurization of milk is regarded as one of the most effective measures for preventing microbial contamination and thus improving milk hygiene. However, spore-forming bacteria such as *Clostridium* spp. and *Bacillus* spp. and heat-resistant microorganisms can withstand pasteurization and survive (Rasooly & Do, 2010). Indeed, the heat treatment causes a reduction in the numbers of indigenous antagonistic microbiota that contribute to the inactivation of pathogenic bacteria including *L. monocytogenes* and *Staphylococcus aureus* (Samelis et al., 2009).

Naturally existing microbial communities of raw milk cheeses have been investigated using PCR, denaturing gradient gel electrophoresis, and pyrosequencing of the 16S rRNA gene (Masoud et al., 2012). The microbiota obstructed the growth of *L. monocytogenes*, *Listeria innocua*, and *S. aureus* in raw milk cheeses, but the bacteria responsible for growth inhibition and its mechanism have not been found yet (Masoud et al., 2012; Millet, Saubusse, Didienne, Tessier, & Montel, 2006). *Lactobacillus plantarum* is predominantly present in Mexican Oaxaca raw milk cheese and exhibits antimicrobial activity against certain pathogenic species including enterotoxin-producing *S. aureus* and *L. innocua* (Caro et al., 2013). Similarly, important foodborne pathogens including *L. monocytogenes*, *Salmonella* spp., and *S. aureus* were scarcely found in raw milk and soft cheese owing to antagonistic activity of indigenous lactic acid bacteria (Ortolani, Yamazi, Moraes, Viçosa, & Nero, 2010b).

Nevertheless, it has been widely reported that raw milk cheeses are microbiologically unsafe because no thermal treatment is applied to destroy pathogenic bacteria in raw milk. However, a surveillance analysis found that all raw milk cheeses tested were negative for major pathogens such as *Campylobacter*, *Escherichia coli* O157:H7, *L. monocytogenes*, and *Salmonella* (Brooks et al., 2012). In addition, farmstead cheeses made from the raw milk of cow, sheep, and goat display a low incidence of bacterial pathogens such as *S. aureus*, *Salmonella*, and *E. coli* O157:H7, suggesting that raw milk cheese is microbiologically safe (D'Amico, Groves, & Donnelly, 2008). Consistently, large-scale investigations of aged raw milk cheeses demonstrated that only one sample out of 181 different cheeses was contaminated with *L. monocytogenes*, and none of 1819 samples in Europe and the United Kingdom were positive for *Salmonella* (Little et al., 2008; Ryser, 2007). More importantly, pasteurized milk cheeses caused a high number of *Listeria*-associated outbreaks in Germany during 2006–2007 (Koch et al., 2010). Thus, it cannot be asserted that cheese made from pasteurized milk is more microbiologically safe than raw milk cheese.

#### 2.2.1. Bacteriocin-producing bacteria

Bacteriocins, which comprise ribosomally synthesized peptides or proteins, exhibit antimicrobial activity against other microorganisms (Klaenhammer, 1993). Based on their structures and modes of action, bacteriocins can be categorized into three groups, class I (lantibiotics), class II (non-lanthionine-containing

bacteriocins, IIa, IIb, IIc, and IId), and class III (bacteriolycin). Class I bacteriocins include nisins A, Z, and Q, which are isolated from *Lactococcus lactis* (Reviewed in Perez, Zendo, & Sonomoto, 2014). Nisins were found to be effective inhibitors of the growth and biofilm formation of *S. aureus* strains isolated from raw milk and cheeses (Sudagidan & Yemenicioğlu, 2012). Similarly, another research group isolated an indigenous nisin A-producing *L. lactis* strain from raw milk, and found that addition of the microbial adjunct culture impeded the growth of coagulase-positive *S. aureus* in traditional Greek graviera cheese (Samelis et al., 2014). In addition, the use of nisin A-producing *L. lactis* subsp. *cremoris* as an additive to thermized milk was found to completely inhibit the growth of *L. monocytogenes* (Lianou & Samelis, 2014).

Class IIa bacteriocins are known to display a growth-inhibiting effect against *L. monocytogenes* with high specificity; thus, they are also called anti-*Listeria* bacteriocins (Perez et al., 2014). For instance, *Enterococcus* spp. are lactic acid-producing bacteria that are considered to be a part of the natural flora in mammal intestines and have also been isolated from various foods, including cheeses (Alvarado, García-Almendárez, Martin, & Regalado, 2005; Templer & Baumgartner, 2007). Certain *Enterococcus* species, such as *Enterococcus faecium*, contribute to the development of organoleptic properties during cheesemaking (Renye, Somkuti, Vallejo-Cordoba, VanHekken, & Gonzalez-Cordova, 2008). Enterocin-producing *E. faecium* and *Enterococcus faecalis* have been isolated from raw milk, which might be used for manufacturing raw milk cheese. These isolates were found to strongly hinder the growth of *Listeria* spp. including *L. monocytogenes* (Chanos & Williams, 2011; Jaouani et al., 2014). Further, *Enterococcus* species such as *E. faecium* and *Enterococcus durans* isolated from Hispanic-style cheeses efficiently obstructed the growth of *Listeria* spp. by secreting anti-listerial bacteriocins (Renye, Somkuti, Paul, & Van Hekken, 2009). Besides these, there have been more reports regarding naturally occurring bacteriocinogenic lactic acid bacteria in raw milk and cheese, which also contribute to the control of bacterial pathogens (Alegria et al., 2009, 2010; Moraes et al., 2012; Ortolani et al., 2010a; Perin, Moraes, Silva, & Nero, 2012). However, in contrast to the desirable traits of bacteriocin-producing *Enterococcus* isolates, most of these lactic acid bacteria carry genes for virulence factors such as gelatinase, lipase, DNase, and hemolysin, and it is difficult to draw any firm conclusion about the microbiological safety of the isolates present in raw milk cheese (Moraes et al., 2012).

#### 2.2.2. Pathogen-inhibitory bacterial antagonists

Raw milk cheese contains a multitude of microbial communities, among which a representative subgroup of species are lactic acid bacteria. As aforementioned, lactic acid bacteria including lactococci, lactobacilli, and enterococci play a leading role in inhibiting the growth of food-borne pathogens within raw milk cheese, mainly by a bacteriocin-mediated mechanism. In addition to bacteriocin, lactic acid bacteria produce other antimicrobial substances such as organic acids and hydrogen peroxide that are effective in inhibiting the growth of other pathogenic microorganisms. For example, *L. plantarum* appeared to obstruct the growth of *Salmonella* Typhimurium due to its acid production during ripening of raw milk Montasio cheese (Stecchini, Sarais, & de Bertoldi, 1991).

*Lactococcus garvieae* is frequently found in raw milk and raw milk cheese as a member of the natural microbiota. Although *L. garvieae* is known to cause several diseases in hosts such as marine and freshwater fish species, cows, and humans, human infection with *L. garvieae* caused by consumption of raw milk cheese was never reported until recently (Evans, Klesius, & Shoemaker, 2009; Kim et al., 2013; Teixeira et al., 1996). On the contrary, *L. garvieae* strains were found to produce bacteriocins active against food-related pathogens (Florez et al., 2012; Fortina et al., 2007). In addition, this species secretes the oxidative stress-causing molecule hydrogen peroxide, which inhibits the growth of *S. aureus*, and further exerts an inhibitory effect on other pathogenic bacteria, probably resulting in changes in the microbial balance during the cheese aging period (Delbès-Paus, Dorchies, Chaabna, Callon, & Montel, 2010). In other words, this bacterium contributes to improving the microbiological quality of cheese without having an adverse sensory impact.

*Enterococcus* spp. are important microorganisms in the microbial communities within various raw milk cheeses (reviewed in Psoni et al., 2006). Although these bacterial species have also been considered as causative agents of human infection, and multidrug-resistant enterococci have increasingly emerged, it is well known that they play beneficial roles in enhancing the microbiological safety and organoleptic quality of raw milk cheese during ripening (Centeno, Menendez, Hermida, & Rodríguez-Otero, 1999; Lebreton et al., 2013; Psoni et al., 2006). Among the isolates of enterococci from raw milk cheese, *E. durans* was predominantly found and displayed an effective antimicrobial activity against several food-borne pathogens such as *Bacillus cereus*, *S. aureus*, *E. coli*, *L. monocytogenes*, and *E. faecalis* (Psoni, Tzanetakis, & Litopoulou-Tzanetaki, 2003, 2006).

A gram-negative enterobacterial genus, *Hafnia*, is frequently found in raw milk (Ercolini, Russo, Ferrocino & Villani, 2009; Kagkli, Vancanneyt, Vandamme, Hill, & Cogan, 2007; Viana, Campos, Ponce, Mantovani, & Vanetti, 2009; Vithanage, Yeager, Jadhav, Palombo, & Datta, 2014) and raw milk cheeses (Abriouel, Martín-Platero, Maqueda, Valdivia, & Martínez-Bueno, 2008; Fuka et al., 2013; Morales, Fernández-García, & Nuñez, 2003). Although most gram-negative bacteria in cheese are known to cause spoilage and adverse flavor, *Hafnia* species such as *Hafnia alvei* and *Hafnia paralvei* can improve cheese flavor by producing sulfur aromatic compounds (Irlinger et al., 2012). In addition, *H. alvei* strains are suggested to be ripening bacterial agents and are commercially available (Alonso-Calleja, Carballo, Capita, Bernardo, & García-López, 2002). According to Delbès-Paus et al. (2013), these bacteria diminish the levels of *E. faecalis* but boost the growth of *L. plantarum* present in cheese. A survey revealed that over 90% of raw milk French cheeses contained enterococci, and *E. faecalis* appeared to be the predominant antibiotic-resistant species, but no major nosocomial multi-drug resistant enterococci were isolated (Jamet et al., 2012). Thus, competitors such as *Hafnia* species are able to control the growth of antibiotic-resistant *E. faecalis*. In addition, inoculation of *H. alvei* at 6 log CFU/mL to cheese hinders the growth of *E. coli* O26:H11 (Delbès-Paus et al., 2013).

## 3. Microbiological risks of raw milk cheese

### 3.1. Raw milk cheese and food-borne illness

Although there have been substantial numbers of reports regarding the microbiological safety of raw milk cheese, this issue is still controversial. Unpasteurized milk cheeses have been generally considered to be microbiologically unsafe because it is possible for pathogenic bacteria to contaminate the milk products either at the dairy farm or post-cheesemaking (Masoud et al., 2012; Rudol & Scherer, 2001). Many studies have conducted microbiological comparative analyses of raw and pasteurized milk cheeses (Gould, Mungai, & Behravesh, 2014; Little et al., 2008; Renye et al., 2008). In addition, global outbreaks ascribed to cheeses made from both types of milk have been compared by aetiological analysis during the period 1973–2006 (Food Standards Australia New Zealand, 2009). According to the survey, cheese is associated with various foodborne bacteria including *Brucella melitensis*, *Campylobacter*

spp., *Coxiella burnetii*, *E. coli* O157:H7, *L. monocytogenes*, *Mycobacterium bovis*, *Salmonella*, *S. aureus*, and *Streptococcus* spp. (Food Standards Australia New Zealand, 2009). Among them, *E. coli*, *B. melitensis*, *Campylobacter* spp., *C. burnetii*, *M. bovis*, and *Streptococcus* spp. were found only in raw milk cheese, whereas *Clostridium* spp. were found only in pasteurized milk cheese. Although vegetative cells of *Clostridium* spp. in milk are eradicated by pasteurization-based heat treatment, heat-resistant endospores remain dormant in pasteurized milk and germinate into vegetative bacterial cells (Rasooly & Do, 2010). Remarkably, *B. melitensis*, *E. coli*, *L. monocytogenes*, *Salmonella* spp., and *S. aureus* were the major contaminants of raw milk cheese (Food Standards Australia New Zealand, 2009). Similarly, De Buyser et al. described enterohaemorrhagic *E. coli*, *L. monocytogenes*, *S. aureus*, and *Salmonella spp.* as being the most prevalent pathogenic bacteria in raw milk cheeses (De Buyser, Dufour, Maire, & Lafarge, 2001). According to a survey, fresh and short-term ripened raw milk cheeses manufactured on-farm in Sweden were microbiologically safe in general, whereas *E. coli* and enterotoxin-producing *S. aureus* were detected at relatively high proportions (Rosengren, Fabricius, Guss, Sylvén, & Lindqvist, 2010). Moreover, a surveillance study of over 100 Norwegian raw milk cheese samples showed that they were contaminated frequently with *S. aureus* (Jakobsen, Heggebø, Sunde, & Skjervheim, 2011). Mexican fresh cheeses such as panela and adobera, which are mostly made from unpasteurized milk, were contaminated with *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* at a high incidence rate, and indeed, their consumption caused a number of illness outbreaks (CDC, 2008; Cody et al., 1999; Gould et al., 2014, MacDonald et al., 2005; Torres-Vitela et al., 2012). Global outbreaks of food-borne diseases ascribed to raw milk cheese and pasteurized milk cheese are listed in Table 1 and Table 3, respectively. Surprisingly, there have been reported many outbreaks attributed to the consumption of Queso fresco cheese at a relatively high incidence rate (Table 2). The main reason may be that traditional Queso fresco, a high-moisture Hispanic-style cheese, does not meet the 60-day ripening requirement in the US. Moreover, much Queso fresco is illegally imported from Mexico or produced by Hispanic communities in the US.

Among several cheese types, soft cheeses such as gorgonzola, emmentaler, brie, camembert, and cottage have a property of relatively high water activity in the range of 0.97–0.99 and low acidity. In addition, they are often produced at high ripening temperatures (Cogan, 2003). Thus, pathogenic bacteria are easily able to grow during the ripening process of soft cheeses. The minimum water activity required for the growth of bacterial pathogens varies from 0.86 (*S. aureus*) to 0.99 (*Campylobacter* spp.) (ICMSF, 1996). As the water activity becomes higher, the lag phase and generation time of bacterial growth are shortened, finally resulting in increased numbers of bacterial cells. Thus, it is not surprising that 30.9% of raw milk soft cheeses produced by small dairy farms in Brazil were found to have been contaminated with coagulase-positive staphylococci (Moraes, Viçosa, Yamazi, Ortolani, & Nero, 2009). Indeed, most outbreaks of food-borne illness have been attributed to soft cheese (Table 1).

Therefore, there still exists a risk of pathogenic bacterial contamination in raw milk cheese. The association of each significant pathogen with raw milk cheese is discussed below.

### 3.2. Raw milk cheese and spoilage

Unpasteurized milk is frequently stored at refrigerated temperatures until it is used for cheesemaking. Low temperature storage of raw milk inhibits the growth of mesophilic bacteria. However, psychrotrophic bacteria such as *Pseudomonas* spp., *Acinetobacter* spp., and Enterobacteriaceae, mostly gram negative, which are indigenously found in raw milk, are able to actively proliferate at refrigerated temperatures to relatively high bacterial counts (Ercolini, Russo, Ferrocino, & Villani, 2009; Hantsis-Zacharov & Halpern, 2007; Martins, Pinto, Rocha, de Araújo, & Vanetti, 2006). Thus, these psychrotrophic microorganisms become predominant in the raw milk microbiota during periods of cold storage (Corrêa, Daroit, Velho, & Brandelli, 2011). Among the psychrotrophs, the genus *Pseudomonas* was reported to be dominant among microbial groups in raw milk in cold storage (Corrêa et al., 2011; Decimo, Morandi, Silvetti, & Brasca, 2014). In fact, although psychrotrophic bacteria were not superior in numbers in raw milk, an increase in the number of psychrotrophic bacterial cells occurred during milk creaming for manufacturing a grana trentino cheese, an Italian variety of raw cow's milk cheese (Franciosi, De Sabbata, Gardini, Cavazza, & Poznanski, 2011). The practice of milk creaming at 15–18 °C is performed to eliminate initial microbial contaminants in raw milk, but this process enables psychrotrophs to thrive owing to the poor growth of other microorganisms at lowered temperatures (Franciosi et al., 2011). These psychrotrophic microorganisms in raw milk produce hydrolytic enzymes such as proteases and lipases, finally resulting in spoilage (Hantsis-Zacharov & Halpern, 2007). More importantly, even if psychrotrophs are removed during raw milk cheesemaking, enzymes already released by these bacteria may remain and cause a deterioration of raw milk cheese throughout the manufacturing and storage periods. In addition, the dominant growth of psychrotrophic bacteria during periods of refrigeration changes the microbial balance of raw milk (Fricker, Skånseng, Rudi, Stessl, & Ehling-Schulz, 2011; Raats, Offek, Minz, & Halpern, 2011; Rasolofo, St-Gelais, LaPointe, & Roy, 2010). Furthermore, these psychrotrophic bacteria may outcompete other bacteria, including beneficial bacteria, throughout the cheesemaking process, in the case for Camembert where the curd is formed at 25–30 °C. Thus, the initial removal of psychrotrophic contaminants in raw milk might be a prerequisite for manufacturing cheese with satisfactory microbiological and organoleptic qualities.

### 3.3. Bacterial pathogens related to raw milk cheese

#### 3.3.1. Enterohaemorrhagic E. coli

*E. coli* is known to be an indicator of contamination with animal feces and potential presence of other pathogenic microorganisms of fecal origin in foods (Ghafir, China, Dierick, De Zutter, & Daube, 2008). Raw milk can be contaminated with Shiga toxin-producing *E. coli* (STEC) from animal feces, which are discharged from animals harboring the pathogen in their gastrointestinal tract, often without any appearance of illness (Miszczycha et al., 2013; Sarimhmetoglu et al., 2009). Thus, the prevalence of the STEC strains in raw milk cheeses is ascribed to raw milk contaminated with animal feces. It has been reported that food-borne illness outbreaks related to raw milk cheeses were attributed to STEC strains (Baylis, 2009; Espié et al., 2006; Honish et al., 2005). In addition, almost all STEC-associated illnesses after cheese consumption have been related to raw milk cheese, rather than pasteurized milk cheese (Table 1). For example, *E. coli* O26:H11-related outbreaks occurred in France in 2005 and were attributed to the consumption of raw cow's milk cheese (Espié et al., 2008). In Canada, an outbreak of *E. coli* O157:H7 haemorrhagic colitis in late 2002 was attributed to the consumption of gouda cheese, which is a raw milk hard cheese (Honish et al., 2005).

Various STEC serogroups have been isolated from raw milk cheeses (Bonyadian, Moshtaghi, & Akhavan Taheri, 2014; Caro & García-Armesto, 2007; Elhadidy & Mohammed, 2013; Farrokh et al., 2013; McCollum et al., 2010; Mora et al., 2007; Stephan et al., 2008; Vernozy-Rozand, Montet, Berardin, Bavai, & Beutin,

**Table 1**
Global outbreaks of food-borne illness attributed to consumption of raw milk cheese.

| Causative pathogen | Number of patients (Deaths) | Country of incidence | Year of incidence | Cheese name | Cheese type | Ripening period (days) | pH | Ref. |
|---|---|---|---|---|---|---|---|---|
| *Brucella melitensis* serovar 3 | 11 | Spain | 2002 | Raw goat milk cheese | NA | NA | NA | Méndez Martínez et al., 2003 |
| Enterotoxigenic *Escherichia coli* | 45 | USA | 1983 | Brie | soft | 28–42 | 5.8 | MacDonald et al., 1985 |
| *Escherichia coli* O26, O80 (*vtx2*, *eae*) | 6 | France | 2005 | Brie | soft | NA | NA | INVS, 2007 |
| *Escherichia coli* O26:H11 | NA | France | 2005 | NA | NA | NA | NA | Espié et al., 2008 |
| *Escherichia coli* O103 | 4 | France | 1994 | Farm fresh goat milk cheese | NA | NA | NA | Decludt, 1995 |
| *Escherichia coli* O110:H- | 3 | Germany | 1994 | NA | NA | NA | NA | Bockemühl & Karch, 1996 |
| *Escherichia coli* O119:B14 (*vtx2*) | 4 (1) | France | 1992–1993 | Farm fresh cow and goat milk cheese | NA | NA | NA | Casenave et al., 1993 |
| *Escherichia coli* O157 (PT28, *vtx2*) | 22 | Scotland | 1994 | Farm raw milk cheese | NA | NA | NA | Ammon, 1997 |
| *Escherichia coli* O157 (PT2, *vtx2*) | 10 | England | 1998 | NA | NA | NA | NA | CDSC, 1998b |
| *Escherichia coli* O157 (PT21, PT28) | 27 | Scotland | 1999 | Homemade raw goat milk cheese | NA | NA | NA | Curnow, 1999 |
| *Escherichia coli* O157 (*vtx2*, *eae*) | 3 | France | 2004 | Farm fresh goat milk cheese | NA | NA | NA | Espié et al., 2006 |
| *Escherichia coli* O157 | 4 | Scotland | 1998 | Farm raw milk cheese | NA | NA | NA | Strachan et al., 2006 |
| *Escherichia coli* O157:H7 | 63 | USA | 1998 | Cheddar curds | NA | NA | NA | CDC, 2000 |
| | 38 | USA | 2000–2010 | Gouda | semi-soft to hard | 120 | 5.8 | William, 2011 |
| | 13 | Canada | 2002 | Gouda | semi-soft to hard | 120 | 5.8 | Honish et al., 2005 |
| | 2 | UK | 1997 | Lancashire-type cheese | semi-hard | 30–720 | 5.35 | Anon, 1997a |
| | 3 | Canada | 2004 | NA | NA | NA | NA | MAPAQ, 2004 |
| | 8 | USA | 2010 | Sally Jackson cheese | soft | NA | NA | The Marler Clark Network, 2015 |
| | 3 | France | 2006 | Raw goat milk cheese | NA | NA | NA | Espié et al., 2006 |
| | 16 | Canada | 2008–2009 | NA | NA | NA | NA | Gaulin et al., 2012 |
| *Escherichia coli* O157:H7 *Escherichia coli* O157:NM(H-) | 15 | USA | 2010 | NA | NA | NA | NA | CDC, 2014 |
| *Escherichia coli* O157:H7 *Listeria monocytogenes* | 38 | USA | 2010 | Gouda | Semi-soft to hard | NA | NA | CDC, 2014 |
| *Mycobacterium bovis* | NA | USA | NA | NA (imported from Mexico) | Soft | NA | NA | Harris et al., 2007 |
| *Salmonella* Berta | 35 | Canada | 1994 | Farm soft raw milk cheese | Soft | NA | NA | Ellis et al., 1998 |
| *Salmonella* Dublin | 42 | England Wales | 1989 | Stilton | Hard | 63–84 | 5.2 | Maguire et al., 1992 |
| | 25 (5) | France | 1995 | Mont d'Or | Soft | 35–49 | 5.1 | Vaillant et al., 1996 |
| | 14 (1) | France | 1996 | Mont d'Or | Soft | 35–49 | 5.1 | Infuso, Vaillant, & Desenclos, 1997 |
| *Salmonella* Enteritidis phage type 8 | 215 | France | 2001 | Cantal | Semi-hard to hard | 30–180 | 5.0–6.6 | Haeghebaert et al., 2003 |
| *Salmonella* Meleagridis, A multidrug-resistant strain of *Salmonella* Newport | 96 | USA | 2006 | Cotija | Hard | 90 | approx. 5 | The Marler Clark Network, 2015 |
| *Salmonella* Montevideo | NA | France | 2006 | NA | Soft | NA | NA | Dominguez et al., 2009 |
| *Salmonella* Muenster | NA | Canada | 1982 | Cheddar | Hard | 150–180 | 5.4 | D'Aoust, 1985 |
| | 25 | France | 2008 | Raw goat milk cheese | NA | NA | NA | van Cauteren et al., 2009 |
| *Salmonella* Newport | 85 | USA | 2000–2010 | Cotija | Hard | 30–90 | approx. 5 | William, 2011 |
| | 27 | USA | 2001 | NA | NA | NA | NA | CDC, 2014 |
| | 85 | USA | 2006–2007 | Mexican-style aged cheese | NA | NA | NA | CDC, 2008 |
| *Salmonella* Paratyphi B | 273 (1) | France | 1993 | Raw goat milk cheese | NA | NA | NA | Desenclos et al., 1996 |
| | 277 | France | 1990 | Raw goat milk cheese | NA | NA | NA | Desenclos et al., 1996 |
| *Salmonella* Typhimurium | 215 | Switzerland | 1984–1985 | Vacherin Mont d'Or | Soft | 35–49 | 5.1 | Sadik et al., 1986 |
| | >40 | France | 1985 | Vacherin Mont d'Or | Soft | 35–49 | 5.1 | Sadik et al., 1986 |
| | 29 | USA | 2007 | NA | NA | NA | NA | CDC, 2007 |
| *Salmonella* Typhimurium PT12 | 113 | France | 1997 | Morbier | Semi-soft | 45–90 | NA | De Valk et al., 2000 |
| *Salmonella* Typhimurium var Copenhagen phage DT104 | 54 | USA | 1997 | Mexican-style cheese | Soft | NA | NA | Villar et al., 1999 |

(*continued on next page*)

**Table 1** (*continued*)

| Causative pathogen | Number of patients (Deaths) | Country of incidence | Year of incidence | Cheese name | Cheese type | Ripening period (days) | pH | Ref. |
|---|---|---|---|---|---|---|---|---|
| | 31 | USA | 1997 | Mexican-style cheese | Soft | NA | NA | Cody et al., 1999 |
| *Salmonella* Typhimurium non-var Copenhagen phage DT104b | 79 | USA | 1997 | Mexican-style cheese | Soft | NA | NA | Cody et al., 1999 |
| *Salmonella* Typhymurium phage type 561 | NA | Netherlands | 2006 | NA | Hard | NA | NA | Van Duynhoven et al., 2009 |
| *Listeria monocytogenes* | 14 | France | 1997 | Livarot, Pont-L'eveque | Soft | 120–180 | 7.2 | Jacquet, Saint-Cloment, Brouille, Cartimel, & Rocourt, 1998 |
| | 3 | USA | 2000 | Mexican-style cheese | Soft | NA | NA | CDC, 2001 |
| | 10 (5) | Switzerland | 2005 | Tomme | Soft | 42–56 | NA | Bille et al., 2006 |
| | 122 (34) | Switzerland | 1983–1987 | Vacherin Mont d'Or | Soft | 35–49 | 5.1 | Bille, 1990; Bula, Bille, & Glauser, 1995 |
| *Staphylococcus aureus* | 14 | Switzerland | 2014 | Tomme | Soft | NA | NA | Johler et al., 2015 |
| | 50 | Brazil | 1999 | Minas | Semi-soft | 4–10 | 5.5 | do Carmo et al., 2002 |
| *Staphylococcus* sp. (Suggestive) | 155 | UK | 1988 | Stilton | Hard | 63–84 | 5.2 | Maguire et al., 1991 |
| *Mycobacterium bovis* | NA | USA | NA | NA (imported from Mexico) | Soft | NA | NA | Harris et al., 2007 |

NA, not available.

**Table 2**
Outbreaks of food-borne illness in USA attributed to consumption of Queso fresco made from raw milk cheese.

| Causative pathogen | Number of patients (deaths) | Year of incidence | Ref. |
|---|---|---|---|
| *Brucella melitensis* | 3 | 1973 | Altekruse et al., 1998 |
| *Brucella melitensis* | 9 | 1985 | Boor & Zadoks, 2003 |
| Brucella spp. | 2 | 2005 | The Marler Clark Network, 2015 |
| Brucella spp. | 3 | 2007 | The Marler Clark Network, 2015 |
| Campylobacter spp. | 1 | 2000–2010 | William, 2011 |
| Campylobacter spp. | 1 | 2010 | The Marler Clark Network, 2015 |
| Campylobacter spp. | 10 | 2008 | The Marler Clark Network, 2015 |
| *Campylobacter jejuni* | 10 | 2009 | The Marler Clark Network, 2015 |
| *Escherichia coli* O157:H7 | 3 | 2004 | The Marler Clark Network, 2015 |
| *Listeria monocytogenes* | 12 | 2003 | The Marler Clark Network, 2015 |
| *Listeria monocytogenes* | 9 | 2005 | The Marler Clark Network, 2015 |
| *Listeria monocytogenes* | 12 | 2005 | The Marler Clark Network, 2015 |
| *Listeria monocytogenes* | 1 | 2010 | The Marler Clark Network, 2015 |
| *Listeria monocytogenes* | 1 | 2012 | The Marler Clark Network, 2015 |
| *Mycobacterium bovis* | 35 (1) | 2001 | CDC, 2005 |
| *Salmonella* Typhimurium DT104 | 54 | 1997 | Villar et al., 1999 |
| *Salmonella* Typhimurium | 39 | 2000–2010 | William, 2011 |
| *Salmonella* Typhimurium | 20 | 2006 | The Marler Clark Network, 2015 |
| *Salmonella* Typhimurium | 29 | 2007 | The Marler Clark Network, 2015 |
| *Salmonella* Newport | 2100 | 2009 | The Marler Clark Network, 2015 |
| Salmonella spp. | 25 | 2013 | The Marler Clark Network, 2015 |
| *Streptococcus equi* *Streptococcus zooepidemicus* | 16 | 1983 | Altekruse et al., 1998 |
| NA | 17 | 1975 | Altekruse et al., 1998 |

NA, not available.

2005). Some enterotoxigenic isolates of *E. coli* exhibited multidrug resistance to antibiotics used commonly for pharmaceutical treatment (Bonyadian et al., 2014). Four hundred raw milk cheeses collected from French retail stores were investigated for the five major pathogenic serotypes (O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7) of STEC by performing multiplex real-time PCR using genetic markers such as Shiga toxin-encoding gene (*stx*), intimin-encoding gene (*eae*), and O groups. Based on real-time PCR analysis, it was found that combinations of these genes were detected in 6.5% of the cheeses, indicating that raw milk cheeses contain STEC at a relatively high ratio (Madic et al., 2011). Also, Miszczycha et al. assessed the growth and survival of different STEC serogroups, such as O26:H11, O103:H2, O145:H28, and O157:H7, throughout all cheesemaking stages. Based on this study, it was determined that rapid acidification and long ripening were able to eliminate STEC growth, and O157:H7 displayed weaker growth and was less well retained than other serotypes (Miszczycha et al., 2013). Among a variety of STEC serotypes, O26:H11 and O157:H7 have most often been associated with *E. coli* outbreaks involving unpasteurized milk cheeses (Baylis, 2009; Espié et al., 2006; Farrokh et al., 2013). Although both serotypes can survive in raw milk cheeses from the initial cheesemaking stage

**Table 3**
Global outbreaks of food-borne illness attributed to consumption of pasteurized milk cheese.

| Causative pathogen | Number of patients (deaths) | Country of incidence | Year of incidence | Cheese name | Cheese type | Ref. |
|---|---|---|---|---|---|---|
| *Campylobacter jejuni* | 3 | USA | 2010 | Whole milk cheese | NA | CDC NORS, 2012 |
| *Clostridium botulinum* type A | 8 | Italy | 1996 | Mascapone | soft | Aureli et al., 2000 |
| *Clostridium perfringens* | 9 | USA | 1995 | NA | NA | CDC, 2002 |
| *Escherichia coli* O157:H7 | 28 (1) | Canada | 2013 | Gort's Gouda cheese | hard | The Marler Clark Network, 2015 |
| *Listeria spp.* | 57 (16) | Switzerland | 1995 | Soft cheese | soft | Bula et al., 1995 |
| *Listeria monocytogenes* | 152 (48–52) | USA | 1985 | Queso fresco | soft | Altekruse et al., 1998; Linnan et al., 1988 |
| | 8 (4) | USA | 2008 | Queso fresco (Mexican-style) | soft | CDC NORS, 2012 |
| | 2 (2) | USA | 2009 | NA | NA | CDC NORS, 2012 |
| | 18 | USA | 2009 | Mexican-style cheese | soft | CDC, 2013 |
| | 8 | USA | 2009 | Mexican-style cheese (indistinguishable) | soft | CDC, 2013 |
| | 5 (1) | USA | 2010 | Mexican-style cheese | soft | CDC NORS, 2012 |
| | 5 | USA | 2010 | Queso fresco (Mexican-style) | soft | FDA, 2010 |
| | 6 | USA | 2010 | Mexican-style cheese (indistinguishable) | soft | CDC, 2013 |
| | 5 | USA | 2010 | Queseria Bendita fresh cheese | soft | The Marler Clark Network, 2015 |
| | 2 | USA | 2011 | Chive and ackawi cheese | soft | CDC, 2013 |
| | 2 | USA | 2011 | Mexican-style cheese | soft | CDC, 2013 |
| | 22 (4) | USA | 2012 | Ricotta | soft to semi-soft | The Marler Clark Network, 2015 |
| | 1 | USA | 2012 | NA | NA | The Marler Clark Network, 2015 |
| | 22 (4) | USA | 2012 | Sheep milk cheese | NA | Real Raw Milk Facts, 2014 |
| | 5 (1) | USA | 2013 | Les Freres cheese | semi-soft | The Marler Clark Network, 2015 |
| | 5 (1) | USA | 2013–2014 | Oasis Brands cheese (Hispanic-style cheese) | soft | The Marler Clark Network, 2015 |
| | 8 (1) | USA | 2014 | Cheese (Roos foods) | Soft to semi-soft | The Marler Clark Network, 2015 |
| | 3 (1) | USA | 2015 | Queso fresco (Queseria Bendita Latin-style cheese) | soft | The Marler Clark Network, 2015 |
| *Salmonella* Enteritidis | 5 | USA | 1994 | Goat milk cheese | NA | CDC, 2002 |
| *Salmonella* Enteritidis | ~700 | Canada | 1998 | Cheddar | hard | CCDR, 1999 |
| *Salmonella* Goldcoast | 84 | UK | 1996 | Cheddar | hard | Anon, 1997b |
| *Salmonella* Heidelberg | 339 | USA | 1986 | Cheddar | hard | Altekruse et al., 1998 |
| *Salmonella* Heidelberg | 28,000–36,000 | USA | 1976 | Cheddar | hard | Fontaine, Cohen, Martin, & Vernon, 1980 |
| *Salmonella* Infantis | 45 | France | 2001 | Brie | soft | Simon, Emmanuelle, & Vaillant, 2002 |
| *Salmonella* Java | 70 | USA | 2008 | Cheddar | Hard | CDC NORS 2012 |
| *Salmonella* Javiana | 164 | USA | 1989 | Mozzarella | semi-soft | Altekruse et al., 1998; Hedberg et al., 1992 |
| *Salmonella* Oranienberg | | | | | | |
| *Salmonella* Montevideo | 20 (9) | USA | 2007 | Shredded cheese | NA | CDC NORS 2012 |
| *Salmonella* Newport | 4 (1) | USA | 2001 | NA | NA | CDC NORS 2012 |
| *Salmonella* Typhimurium | 321 (2) | USA | 1981 | Mozzarella | semi-soft | Altekruse et al., 1998 |
| *Salmonella* Typhimurium PT10 | >2700 | Canada | 1984 | Cheddar | hard | D'Aoust et al., 1985 |
| *Shigella sonnei* | >200 | Spain | 1995–1996 | Fresh pasteurized milk cheese | NA | Garcia-Fulgueiras et al., 2001 |
| *Staphylococcus aureus* | 2 | UK | 1983 | NA | NA | Barrett, 1986 |
| *Staphylococcus aureus* | 3 | USA | 2001 | NA | NA | CDC NORS 2012 |

NA, not available.

to the end of gastrointestinal transit in humans, the survival of O26:H11 strain was 13-fold greater than that of O157:H7 strain at the final stage of simulated human digestion of the cheeses, implying that O26:H11 strain has an ability to survive under extreme acidic environments like the stomach (Miszczycha et al., 2013). A speculative explanation for this result is that the O157:H7 serotype lacks the normal GAD system activity that is involved in acid resistance, which is present in O26:H11 strain (Jung & Kim, 2003; Miszczycha et al., 2013).

In summary, various serotypes of STEC strains are very closely associated with raw milk cheese and they can be considered to be among the major pathogenic agents causing raw milk cheese-related illness.

### 3.3.2. *Salmonella enterica*

*S. enterica*, one of the major foodborne pathogens, is responsible for causing large outbreaks of human salmonellosis worldwide (Majowicz et al., 2010). It has been reported that the prevalence of *Salmonella* in cheese resulted from insufficient pasteurization of milk used for cheese manufacturing (D'Aoust, Warburton, & Sewell, 1985). In general, *Salmonella* spp. can grow even under unfavorable conditions such as low temperature and moisture and high salinity (El-Gazzar & Marth, 1992; Modi, Hirvi, Hill, & Griffiths, 2001). Therefore, the pathogens can persist in relatively harsh food environments such as hard cheddar cheese made from raw milk (Modi et al., 2001). Also, it was reported that even low levels of the contaminated bacteria in hard cheese can cause salmonellosis, suggesting the significance of complete removal of *Salmonella* from

cheese (Ratnam & March, 1986). More significantly, a surveillance investigation revealed that the incidence of outbreaks associated with consumption of raw milk cheese in the United States during 1998–2011 was attributed to *Salmonella* (34%), whereas the incidence percentage of *Salmonella*-related outbreaks decreased to 18%, and instead, that of outbreaks caused by *L. monocytogenes* increased to 24% in the case of pasteurized milk cheese (Gould et al., 2014). Among the outbreaks in the United States, one death due to salmonellosis following the consumption of raw milk cheese occurred and was caused by *Salmonella* Typhimurium (Gould et al., 2014). Besides, there have been other reports of outbreaks related to raw milk cheeses occurring in several countries, which were caused by *Salmonella* infection after the consumption of raw milk cheese (Altekruse, Timbo, Mowbray, Bean, & Potter, 1998; CDC, 2007, CDC, 2008, Cody et al., 1999; Desenclos et al., 1996; De Valk et al., 2000; Dominguez et al., 2009; Haeghebaert et al., 2003; Maguire et al., 1992; Vaillant et al., 1996; Van Duynhoven et al., 2009). Etiological surveys revealed that many outbreaks of salmonellosis have been caused by *Salmonella enterica* serotypes such as *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Dublin, and *Salmonella* Montevideo, and these outbreaks were linked to the consumption of soft or hard cheese manufactured from unpasteurized milk.

### 3.3.3. *Listeria monocytogenes*

*L. monocytogenes* operates elaborate stress adaptation machinery under various hostile environments (e.g., cheese) such as acidic pH, refrigerated temperatures, and high salinity (Badaoui Najjar, Chikindas & Montville, 2007; Yoon, Lee, Lee, Kim, & Choi, 2015). The storage temperature of raw milk cheese affects the growth of *L. monocytogenes*. This bacterial pathogen can proliferate even at refrigerated temperatures, while lactic acid bacteria, one of the primary indigenous microbiota in raw milk, grow at non-refrigerated temperatures (Valero et al., 2014). Thus, a decrease in the levels of *L. monocytogenes* occurred during storage at ambient temperatures (22 °C) due to diminished humidity and outgrowth by lactic acid bacteria (Valero et al., 2014).

It was suggested that *L. monocytogenes* contamination in raw milk and cheese can occur in two ways, firstly by transmission from livestock feces or from surroundings with improper hygiene, and secondly by immediate contamination from animals suffering from diseases such as listeriosis and mastitis (Schoder et al., 2011). Until recently, *L. monocytogenes* outbreaks attributed to cheese had been reported in many countries including France, Japan, Switzerland, USA, Canada, and Austria (Bille et al., 2006; De Buyser et al., 2001; Gaulin, Ramsay, & Bekal, 2012; Jackson et al., 2011; Makino et al., 2005; Schoder, Rossmanith, Glaser, & Wagner, 2012). However, it is also known that raw milk and traditional cheeses made from raw milk exhibit antimicrobial activity against *L. monocytogenes* due to the existence of an autochthonous bacterial community composed of a variety of lactic acid bacteria including streptococci and lactococci (Alvarado et al., 2005; Donnelly, 2001; Ortolani et al., 2010b). Thus, the prevalence of *L. monocytogenes* in raw milk appears lower than that in thermized milk (Brouillaud-Delattre, Maire, Collette, Mattei, & Lahellec, 1997). Indeed, there are also many incidences of outbreaks by the infection of *L. monocytogenes* through the consumption of pasteurized milk (Table 3). Nevertheless, raw milk is still considered to be a vector for *L. monocytogenes*, which can be transferred to milk products like cheese during the cheesemaking processing and storage (Almeida et al., 2013; Kousta, Mataragas, Skandamis, & Eleftherios, 2010). Indeed, it was reported that 50% of tested samples of raw sheep milk used for lighvan cheesemaking were contaminated with *L. monocytogenes*, which can be a potential risk factor for cheese consumers (Moosavy, Esmaeili, Mostafavi, & Bagheri Amiri, 2014). However, since *L. monocytogenes* can also contaminate cheese after the cheesemaking process, the risk of *L. monocytogenes* contamination may be highly similar in both raw milk cheese and pasteurized milk cheese (Rudol & Scherer, 2001).

### 3.3.4. *Campylobacter jejuni and Campylobacter coli*

*Campylobacter* spp. are the leading causative agents of human gastroenteritis worldwide (Nichols, Richardson, Sheppard, Lane, & Sarran, 2012). The most prevalent species of *Campylobacter* are *Campylobacter jejuni* and *C. coli* (Medeiros, Sattar, Farber, & Carrillo, 2008). These bacterial pathogens are associated with the ingestion of contaminated raw and ready-to-eat foods, including raw milk and its products (Hussain, Shahid Mahmood, Akhtar, & Khan, 2007; Lacerc et al., 2002, Wegmüller, Lüthy, & Candrian, 1993). A surveillance study in Pakistan revealed that about 10% of raw milk samples were contaminated with *Campylobacter* spp. (Hussain et al., 2007). However, pathogenic species could not survive during cheesemaking stages such as the ripening process for Swiss hard and semi-hard cheeses made from raw milk (Bachmann & Spahr, 1995). In addition, 41 different samples of raw milk cheeses in the US have been assessed to be free from *Campylobacter* species (Brooks et al., 2012). Also, none of the 34 raw milk cheeses including brie and camembert manufactured in Canada were positive for *Campylobacter* spp. (Medeiros et al., 2008). Although human campylobacteriosis has been recorded as the second-highest incidence of all food-borne pathogens, *Campylobacter* strains have been scarcely recovered and isolated from foods, since they lack the ability to adapt to hostile environmental conditions such as low temperature, high NaCl, and presence of oxygen, thus causing poor proliferation (Medeiros et al., 2008; Yoon et al., 2015).

### 3.3.5. *Staphylococcus aureus*

*S. aureus* often resides on the mucosal membrane of the noses and vaginas of dairy animals, especially goats, and then is dispersed into dairy farms. The pathogen can be further transmitted into raw milk and raw milk cheeses (Mørk, Kvitle, Mathisen, & Jørgensen, 2010). Thus, raw milk products are often suspected of contamination with *S. aureus*, which has indeed been found in a high percentage of raw milk cheese samples (Cremonesi et al., 2007; Rosengren et al., 2010; Tham, Hajdu, & Danielsson-Tham, 1990). Surprisingly, the presence of *S. aureus* in Norwegian raw milk samples from bovine and caprine sources was extremely high and maximum levels of *S. aureus* were reached in samples taken 5–6 h after the first pressing during the cheese manufacturing process (Jakobsen et al., 2011).

However, this species in raw milk cheeses is in a microbiological safety concern only when it is present at higher than a level of $10^4$ cfu/g and produces enterotoxins. Staphylococcal enterotoxin (SE)-encoding genes are clustered in the *egc* (enterotoxin gene cluster) genomic region and equipped with mobile genetic elements (Le Loir, Baron, & Gautier, 2003; Viçosa, Le Loir, Le Loir, de Carvalho, & Nero, 2013). Enterotoxigenic *S. aureus* isolates from raw milk soft cheese were investigated for the presence of *egc* loci, and 40% of the isolates harbored a complete set of individual *egc* genes, but only 4 isolates appeared to be carriers of *egc* loci, implying the existence of *egc* genes outside of the *egc* loci or the presence of new *egc* types in these isolates (Viçosa et al., 2013). Some *S. aureus* strains are able to form four different serotypes of staphylococcal enterotoxins (SEs), SEA, SEB, SEC, and SED, which are produced in foods by contaminated by *S. aureus* (Pexara, Solomakos, Sergelidis, & Govaris, 2012). Among the toxins, only SED was found in *S. aureus* isolates from feta and galotyri cheeses made from raw ovine milk at the end of storage when a high inoculum was added (Pexara et al., 2012). According to a risk assessment analysis by Lindqvist et al., enterotoxigenic *S. aureus*

can be present at high levels in fresh and short-ripened raw milk cheeses at the consumption stage (Lindqvist, Sylvén, & Vågsholm, 2002). The enterotoxigenic *S. aureus* strains have frequently been isolated from raw milk cheeses and the levels of these strains were found to be considerably decreased when starter cultures were added (Rosengren et al., 2010). Over 30% of those *S. aureus* isolates displayed a diminished susceptibility to penicillin (Rosengren et al., 2010). In addition, Swiss raw milk cheese is the most contaminated with enterotoxin-producing *S. aureus* genotype B, which is considered to be highly pathogenic and contagious, and is frequently transmitted to milk from Swiss dairy herds with mastitis (Hummerjohann, Naskova, Baumgartner, & Graber, 2014).

Besides enterotoxin production, antibiotic resistance is another important pathogenic phenotype for *S. aureus*, because antibiotic resistance-related genes are easily transferred to other strains of the same or different species through the exchange of mobile genetic elements harboring the genes. In addition, *S. aureus* strains carrying antibiotic resistance genes are found in livestock such as cows and could be transmitted into foods containing animal products. Research surveys have found that methicillin-resistant *S. aureus* (MRSA) was present in milk from cows with mastitis and also in cow milk cheeses, indicating the possibility that MRSA was transferred from cows into dairy products (Normanno et al., 2007; Vanderhaeghen et al., 2010). Over 40 strains of *S. aureus* isolated from raw sheep milk cheeses were investigated for the presence of antibiotic resistance genes, and approximately 30% of the strains appeared to carry resistance genes against ampicillin, penicillin, and ciprofloxacin (Spanu et al., 2014).

In addition, coagulase-negative *Staphylococcus* (CNS) strains are frequently found as part of the indigenous microflora in fermented foods such as raw milk cheeses (Coton et al., 2010; Soares et al., 2011). Although the presence of CNS facilitates flavor development due to its lytic enzyme activities that break down proteins and lipids, some strains display characteristics associated with pathogenicity, such as enterotoxin production (Aquilanti et al., 2007; Iacumin, Comi, Cantoni, & Cocolin, 2006; Rall et al., 2010). However, CNS strains isolated from raw milk and cheese have been found to carry pathogenicity-associated genes at low levels of incidence (Ruaro, Andrighetto, Torriani, & Lombardi, 2013).

The growth of *S. aureus* during ripening or the end of manufacturing of raw milk cheeses, especially feta and galotyri, was hampered due to the combined impact of several antimicrobial factors, including low levels of pH and water activity, with high levels of NaCl, and the presence of lactic acid bacteria also having been implicated in reducing the growth of *S. aureus* (Pexara et al., 2012). Lactic acid bacteria such as *L. lactis* have been shown to obstruct the growth of *S. aureus* via their metabolites such as bacteriocins, diacetyl, and organic acids (Charlier, Cretenet, Even, & Le Loir, 2009). Actually, it was elucidated that a *L. lactis* strain efficiently inhibited the growth of *S. aureus* present in cheeses at the ripening stage (Hamama, El Hankouri, & El Ayadi, 2002; Pereira, Graça, Ogando, Gomes, & Malcata, 2009).

#### 3.3.6. Other important bacterial pathogens

Cheese made from raw milk is an important transmission vector for the *Brucella*-caused disease, human brucellosis (Lusk, Strain, & Kase, 2013). Survival of the pathogen *B. melitensis* was obstructed during the ripening stage of the manufacture of raw goat's milk cheese, which was conducted at a relatively high temperature (24 °C), low pH (5.0), and low water activity (0.89) (Méndez-González et al., 2011). An epidemiological investigation revealed that a *B. melitensis*-associated outbreak in Spain was attributed to the consumption of raw goat's milk cheese (Méndez Martínez et al., 2003).

*C. burnetii*, a causative agent of Q fever, can be shed in milk from infected livestock, and accordingly, raw milk products can be contaminated by this pathogen (Guatteo, Beaudeau, Joly, & Seegers, 2007; Loftis, Priestley, & Massung, 2010). A very small proportion of Q fever cases result from the consumption of raw milk or, less frequently, its products, including cheese (Gale, Kelly, Mearns, Duggan, & Snary, 2015). Nevertheless, it cannot be ignored that the consumption of raw milk products can sometimes cause *C. burnetii* infection.

In addition, cows suffering from tuberculosis shed *M. bovis* cells into their milk, or their feces may contaminate milk at a higher rate than that of the shedding route (Rowe & Donaghy, 2008). Indeed, an outbreak of human tuberculosis in the United States has been linked to the consumption of Mexican raw milk soft cheese contaminated with *M. bovis* (Harris et al., 2007). Several real-time PCR-based tests showed that although no viable *M. bovis* cells were detected due to their slow growth, 4–25% of raw milk cheeses were positive for *Mycobacterium* species, hence cautioning possible infection with these pathogens through the consumption of contaminated cheese (Botsaris et al., 2010; Pereira-Suárez et al., 2014; Stephan, Schumacher, Tasara, & Grant, 2007).

*Yersinia enterocolitica*, a psychrotrophic bacterium, causes food-related gastrointestinal illness (Lambertz, Nilsson, Hallanvuo, & Lindblad, 2008). Virulent *Y. enterocolitica* was prevalent in raw milk cheeses collected from the north-west of Iran (Hanifian & Khani, 2012). About 10% of the raw milk cheese samples were found to be positive for the presence of the attachment invasion locus (*ail*) gene, which is specific to *Y. enterocolitica* (Hanifian & Khani, 2012).

## 4. Methods for detecting microbial contamination in raw milk cheese

The prevalence of outbreaks associated with consumption of raw milk cheese has spurred the development of new microbial detection methods. Traditionally, pathogenic bacteria have been detected and isolated by plate-based technology, which usually requires enrichment in the levels of pathogenic bacteria, especially for pathogens with a low detection limit. Thus, this kind of investigation fails to detect the presence of pathogens quantitatively and is not appropriate for the detection of non-cultivable or difficult to culture bacteria. Various analytical methods have been suggested to overcome these limitations.

Generally, the presence of pathogenic bacteria in foods including raw milk cheese is analyzed by PCR-based technology, which is a more rapid and efficient method for microbial detection than are conventional culture-based plating methods (Frece, Markov, Cvek, Kolarec, & Delas, 2010). The PCR-based molecular method has been used for the detection of pathogens such as *L. monocytogenes* and *S. aureus* in raw milk cheeses (Cremonesi et al., 2007; Ercolini, Blaiotta, Fusco, & Coppola, 2004; Frece et al., 2010). Although the pathogenicity of STEC serotypes appears to be different according to the serotypes, general PCR analysis is not able to distinguish between STEC serotypes. Thus, multiplex real-time PCR analysis has been used for direct differential detection of the main pathogenic STEC serotypes including O26:H1, O103:H2, O104:H4, O111:H8, O145:H28, and O157:H7 in raw milk cheeses (Alteba & Marumo, 2014; Madic et al., 2011). According to the ISO13136 reference method published in 2012, five STEC serotypes except for O104 are distinguishably identified by detecting the presence of the following genetic markers: 1) the main virulence genes, including Shiga toxin-encoding gene (*stx*) and intimin-encoding gene (*eae*), and 2) the O group genes ($wzx_{O26}$, $wzx_{O103}$, $wbdI_{O111}$, $ihp1_{O145}$, and $rfbE_{O157}$) and the H group genes ($flic_{H11}$, $flic_{H2}$, $flic_{H8}$, $flic_{H28}$, and $flic_{H7}$) related with the serotypes O26, O103, O 111, O145, and O157, respectively. The O104 serotype, a new isolate in

Germany, is identified by detecting $wzx_{O104}$ as a O group specific for this serotype as suggested by Alteba and Marumo (2014). Along with genetic approaches, immunoassay-based approaches have been used to detect certain major bacterial pathogens such as *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, and staphylococcal enterotoxin in milk and milk products including unpasteurized milk cheeses (Brooks et al., 2012; Henning, Flowers, Reiser, & Ryser, 2004; Torres-Vitela et al., 2012). Recently, it was reported that the detection of *E. coli* O157:H7 using multiplex PCR can be improved by combination of this technique with immunomagnetic separation methods using antibody-conjugated beads for selective bacterial isolation in raw milk cheese, thus increasing the sensitivity and efficiency of detection for virulence factors (Ertas et al., 2013). In another recent report, a novel immunomagnetic-based technology combined with surface-enhanced Raman scattering-equipped nanoparticles was developed to monitor pathogenic bacteria such as *E. coli*, *Salmonella*, or *Listeria* in a variety of foods including raw milk cheese and manufacturing environments simultaneously with enrichment in real time (Weidemaier et al., 2015).

Different from direct detection of food-borne pathogens, it has been suggested as an indirect detection method that the presence of certain microorganisms can act as an indicator for the presence of environmental contaminants (e.g. feces) and thus of microbial pathogens. Since the genus *Bifidobacterium* grows well only in the mammalian gut, these organisms can be considered as a valuable indicator of fecal contamination (Resnick & Levin, 1981). Therefore, *Bifidobacterium* species have been suggested as more time-effective and sensitive indicators of animal fecal pollution in raw milk and raw milk cheeses than *E. coli* (Beerens, Hass Brac de la Perriere, & Gavini, 2000; Delcenserie, Gavini, China, & Daube, 2011; Jian & Dong, 2002). Delcenserie et al. demonstrated that among *Bifidobacterium* species, *Bifidobacterium pseudolongum* could be used for detection of animal feces to evaluate the microbiological quality in raw milk cheeses, because the bacterial strain is predominantly found in animal feces but is absent in human feces (Delcenserie et al., 2011).

## 5. 60-day aging for improving the microbiological quality of raw milk cheese

Aging is a process of cheesemaking that is also called ripening or maturation. The aging process triggers biochemical modifications such as proteolysis and lipolysis, leading to a cheese with varied texture (Arenas, González, Sacristán, Tornadijo, & Fresno, 2015). Higher temperatures can shorten the time for ripening by beneficial microorganisms, but can also enable the growth of harmful bacteria. Thus, the ripening temperature is an important factor, and a carefully chosen temperature can both prevent the growth of unwanted bacteria causing spoilage and foodborne disease and facilitate the ripening process (Leclercq-Perlat et al., 2015; Méndez-González et al., 2011).

The US Food and Drug Administration stipulates that 60-day aging should be conducted for improving the microbiological quality of unpasteurized milk cheeses (D'Amico, Druart, & Donnelly, 2010). Several studies have demonstrated that 60-day aging of cheese has a growth-inhibiting effect against pathogenic microorganisms in raw milk cheeses. Brooks et al. evaluated forty-one raw milk cheeses for the antimicrobial effectiveness of 60-day aging, and revealed that all the aged cheeses contained very low levels of foodborne pathogens including *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, and *Campylobacter*, suggesting that 60-day aging of raw milk is able to ensure the production of microbiologically safe cheeses (Brooks et al., 2012). Likewise, after 60-day ripening of raw ewe's milk cheese, there were no important food-borne pathogens including *E. coli*, *Clostridium*, *Salmonella* spp. or *L. monocytogenes* detected in the cheese products (Gil et al., 2007). Moreover, the counts of *Salmonella* spp. were considerably decreased after 60-day aging of gouda cheese made from raw milk to less than 1 cfu/g (D'Amico et al., 2014). Similarly, ripening for 60 days of a traditional Brazilian cheese (minas cheese) improved the microbiological hygienic quality such that the levels of pathogenic bacteria were high at the beginning of the ripening time, whereas *Salmonella* spp. were not found and coagulase-positive staphylococci were hardly detectable by the end of the aging period (Cardoso et al., 2013).

However, several other studies have revealed that 60-day aging does not guarantee complete control of the growth of existing *E. coli* O157:H7 in cheeses manufactured from unpasteurized milk contaminated with low population levels of this bacteria (D'Amico et al., 2010; Schlesser et al., 2006). According to these studies, the number of pathogenic *E. coli* cells decreased by less than 1 and 2 log after 60- and 120-days ripening periods, respectively, and the cells remained at detectable levels even after aging for longer than 270 days (D'Amico et al., 2010; Schlesser et al., 2006). In addition, viable cells of *Mycobacterium* spp. remained in raw milk cheese after a long period of ripening, even after aging for 3–4 months, especially in certain types of raw milk cheese (Lafont & Lafont, 1981; Spahr & Schafroth, 2001). Surely, the outcome of microbial reduction through a 60-day aging depends on several factors, such as cheese type, microbial species, and initial numbers of contaminants. Thus, 60-day aging can reduce microbial counts to a certain degree in some cases, like cheese made from milk contaminated with high numbers of pathogens, but cannot be regarded as a complete solution for the inactivation of contaminating pathogenic microbes in raw milk cheese.

## 6. Conclusion

This review investigated the published literature to consider whether cheese made from raw milk is microbiologically safe and how cheese contamination by food-borne pathogens can be controlled. Although not many countries currently permit the distribution or import of raw milk cheeses due to the prejudice against their microbiological safety, consumer interest in such products shows an increasing trend and the market continuously expands. Accordingly, the microbiological safety of cheese made from unpasteurized milk is predicted to be of increased concern in the future.

Raw milk cheese contains a wide variety of microbiota including beneficial bacteria, especially lactic acid bacteria, which control the proliferation of many contaminating bacterial pathogens and thus protect the product from microbiological risk. Hence, cheese manufactured from unpasteurized milk may seem to be superior to cheese made from pasteurized milk in its microbiological safety. However, although the growth of several pathogenic bacteria is impeded by antagonistic microorganisms belonging to the microbial community in raw milk cheese, not all potential contaminating pathogens in cheese can be completely removed by their antagonistic activities, and some pathogens remain at a detectable level. In addition, since the composition of the raw milk microbiota appears to vary according to the region and livestock species from which it is produced, there is no consistency in the antagonistic effect of microbiota of raw milk against pathogenic bacteria. Nevertheless, even if its effect of microbial reduction varies depending on initial amounts and type of microbial pathogen, the microbiological safety on raw milk cheese can be assured through appropriate 60-day ripening processing or further period of ripening if it is necessary. Although pasteurization eradicates most beneficial and pathogenic bacteria from raw milk, pasteurized milk cheese may contain viable

pathogenic bacteria such as *Clostridium* sp.; hence, it is difficult to assert whether raw or pasteurized milk cheeses have greater microbiological safety. Therefore, regardless of whether milk has been pasteurized before cheesemaking, it should be considered that the microbial hazard of cheese also depends on other factors such as the hygiene of the environments and workers for milk production and cheese manufacturing, and the possibility of post-processing contamination. Thus, cheesemaking processes of raw milk cheese should be accompanied by appropriate ripening period and continuous microbiological monitoring until cheese consumption.

## Author contributions

Y. Yoon and K.-H. Choi designed the study and both wrote the paper. S. Lee conducted the collection, analysis, and interpretation of data.

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