Adventitious Microbes Can Affect the Safety and Quality of Cheese

Microbial contaminants in milk or introduced later during production can lower cheese yields, lead to defects, and raise food safety concerns

Dennis J. D’Amico

The microbiological quality and safety of cheese begin with milk. Its abundance of nutrients, high moisture, and near-neutral pH make it an excellent growth medium not only for the microorganisms that are used for making cheese but for adventitious microorganisms, including those that spoil milk and cheeses and those that cause illness in humans, as well.

Milk, essentially sterile when secreted into the alveoli of the udder, soon becomes contaminated with microorganisms originating from infection, farm environment and feedstuffs, and milking and processing equipment. Contamination is of great concern to cheese makers because milk of high microbiological quality is important for optimizing yield, quality, and safety. In addition, contaminants can access the cheese during manufacture and aging, leading to additional defects and food safety concerns.

Contaminant Microbes Can Affect Cheese Quality

The microflora associated with the mucosal membrane of the teat canal and sphincter consist mainly of streptococci, staphylococci, and micrococci and, to a lesser extent, corynebacteria and coliforms. Some of these organisms, in addition to lactococci, pseudomonads, and yeasts, also inhabit the teat skin and easily enter milk during harvest. Before modern refrigeration, gram-positive bacteria, particularly lactic acid bacteria (LAB) such as Streptococcus, Enterococcus, Lactobacillus, Leuconostoc, Lactococcus, and Pediococcus, dominated what grew in milk.

LAB were, and still are in some areas, responsible for producing cultured dairy products such as cheese. Currently, many cheeses are manufactured mainly with select pure cultures; however, wild-type LAB that fail to produce significant amounts of acid during manufacturing are referred to as nonstarter lactic acid bacteria (NSLAB). Because of their diverse metabolic and enzymatic activities, NSLAB are apt to impart both positive attributes to the finished cheeses as well as defects, including high acidity, off flavors, and excessive gas.

The amounts of LAB and other organisms in raw milk that benefit cheese making are lower than they were before collection and handling practices were made more hygienic, and rapid cooling and refrigerated storage vessels were made more widely available. Although reduced temperatures hinder the outgrowth of mesophilic contaminants of milk, including most pathogens, they favor psychrotrophic organisms that grow below 7°C. Gram-negative bacteria are the main psychrotrophic flora of raw milk, most of which derive from soil, water, and vegetation. Although pasteurization eliminates most psychrotrophic bacteria, they can access milk from improperly cleaned equipment. Additionally,
psychrotrophs secrete heat-stable proteases and lipases into milk and milk products that may persist during refrigerated storage and pasteurization.

The presence and growth of psychrotrophs in milk and cheese and their production of heat-stable enzymes can lead to numerous defects in cheese, including surface discoloration, off odors, bitterness, and rancidity. *Pseudomonas* species in water can gain access to milk during the cleaning and rinsing of equipment. These oxygen-requiring *Pseudomonas* spp. tend to predominate on the surfaces of cheeses, where they release volatile compounds and can turn the surface rinds yellow to brown. One water-soluble pigment, pyoverdin, fluoresces under ultraviolet light (Fig. 1). Their presence also is associated with poor development of ripening flora. In addition to producing off flavors, enzymatic activity from these microbes may produce cheeses with higher-than-normal moisture, which can turn the cheese into a runny paste.

Another major group of microorganisms associated with water and improperly cleaned equipment consists of the coliforms such as *Citrobacter*, *Escherichia*, *Enterobacter*, and *Klebsiella*. These bacteria tend to make cheese taste yeasty, putrid, gassy, or “unclean.” Their presence also leads to excessive release of carbon dioxide and hydrogen gases that, in turn, are linked to “early blowing” defects (Fig. 2). Coliforms also serve as an indicator of fecal contamination in milk, cheese, and the surrounding environment.

**Heat Treatments Overcome Several Problems**

Although many cheeses are made from raw milk, others are made from heat-treated milks. In some countries, such as Canada, significant amounts of cheese are made from milk that is held at 63°C for 10–15 seconds, which preserves enzymes and NSLAB that can help to develop flavors. This or similar heat treatments that are milder than pasteurization are effective in inactivating large proportions of spoilage bacteria, including most psychrotrophs, and also reduce pathogen populations. Pasteurization eliminates a larger proportion of spoilage bacteria and all vegetative pathogenic bacteria but does not
completely inactivate non-spore-forming thermoduric bacteria such as *S. thermophilus* or bacterial endospores. However, these nonsporulating thermoduric bacteria are not commonly associated with major cheese defects.

Some anaerobic spore-forming *Clostridium* species, including *C. sporogenes*, *C. butyricum*, and, most importantly, *C. tyrobutyricum*, are responsible for the "late blowing" defect in brine-salted hard and semi-hard cheeses such as Grana, Gouda, and Emmental (Fig. 3). These cheeses are particularly susceptible because the relatively high initial pH and low salt, resulting from slow diffusion after brining, allows *Clostridium* to grow. The defect, which typically appears after 1–4 months of aging, is characterized by rancid off flavors and excessive gas from fermentation of lactic acid into butyric acid, acetic acid, carbon dioxide, and hydrogen.

Spore-forming *Bacillus* spp. are aerobic, and many are capable of psychrotrophic growth in milk. As a spoilage organism, proteolytic and lipolytic *B. cereus* limits the shelf life of pasteurized milk and gives rise to defects such as sweet curdling and off flavors. *Bacillus* spp. also secrete heat-resistant proteinases, lipases, and phospholipases that can decrease yield or give rise to texture, body defects, bitterness, and other off flavors, including rancidity.

Although yeasts are found in freshly drawn milk, yeast and mold spoilage of dairy products usually arises from postpasteurization contamination. Their growth in milk leads to musty and fruity odors, respectively. In cheese, they can me-
tabolize lactic acid and hydrolyze protein, releasing ammonia and amino acids, while increasing pH and changing texture. Although some airborne yeasts such as *Debaryomyces hansenii*, *Candida* spp., and *Kluyveromyces marxianus* var. *lactis* improve the flavor of certain cheeses, characteristic heterofermentative metabolic spoilage by yeast can produce ethanol, leading to fermented off flavors and gas-related defects from carbon dioxide.

Yeast proteolysis also can produce odors reminiscent of rotten eggs, while lipolytic activity can lead to rancidity by releasing fatty acids. Molds, which require oxygen for growth, grow on most cheese surfaces exposed to air, including gaps in packaging and exposed surfaces in brined cheese. In addition to visual defects, unwanted mold growth on cheese is associated with musty and bitter flavors. Some highly proteolytic molds produce ammonia and cause spoilage. Meanwhile, some fungi, including *Geotrichum candidum*, *Penicillium camemberti*, and *Penicillium roqueforti*, are used for making blue and surface-mold-ripened cheeses.

**Microbes and Cheese Safety**

Poor sanitation and improper milk handling practices as well as animal health issues can lead to sporadic cases of foodborne diseases among humans along with more substantial outbreaks of dairy-related diseases. Because pasteurization eliminates pathogens from raw milk, the risk of foodborne illness from cheese often comes from the use of raw milk or inadequate or faulty pasteurization or processing that fails to inactivate pathogens. Their presence and persistence in dairy processing plants also lead to the risk of postprocessing contamination. Although other microorganisms, including mycotoxin-producing molds, sometimes are recovered from dairy products, the main source for foodborne illness from cheeses is adventitious pathogenic bacteria.

In general, cheese is an unlikely vehicle of foodborne illness from several pathogens, including parasites, viruses, and bacteria from the genera *Clostridium*, *Bacillus*, *Yersinia*, and *Campylobacter*. However, changes that trace to the 1970s in national and global agricultural and food pro-
cessing practices led to changes in patterns of illnesses attributable to dairy-associated foodborne pathogens in the United States. The pathogens include *Listeria monocytogenes*, multidrug-resistant (MDR) *Salmonella* species, and Shiga-toxin producing strains of *E. coli* (STEC). *Staphylococcus aureus* continues to pose challenges as well.

The behavior and survival of these pathogens depends on extrinsic factors such as curd cooking and intrinsic parameters such as moisture, acidity, salt content, and competitive flora. Various combinations of these factors affect pathogen growth and toxin formation. For example, *S. aureus*, a major cause of mastitis in dairy cows, is frequently isolated from raw milk. However, food poisoning arises not from ingesting the organism but from heat-stable staphylococcal enterotoxins (SE) that some strains produce when they reach levels greater than 100,000 CFU/ml. SEs are resistant to subsequent heat treatments and low pH conditions that inactivate *S. aureus*. Further, with 30–50% of the human population carrying *S. aureus* in their nostrils and on skin and hair, milk and milk products can also become contaminated during processing and handling.

Many SE outbreaks linked to the use of raw milk trace to mastitis in dairy cows. However, despite its presence in cheeses, notable cheese-related outbreaks of *S. aureus* are rare because it is such a poor competitor to starter-culture microorganisms. Indeed, the substantial decrease of dairy-related illnesses from staphylococcal poisoning in the U.S. during the past 40 years is attributed to increased monitoring for mastitis in dairy cattle, improved sanitation and cheese-making process controls, and pasteurization.

**Other Pathogens Present Other Risks**

Despite the vast number of serotypes, all *Salmonella* spp. in cheeses are limited, documented outbreaks of nontyphoidal salmonellosis are linked to raw and pasteurized milk and milk products. Outbreaks of cheese-borne salmonellosis generally result from practices such as the use of raw or inadequately pasteurized milk coupled with failure to follow good manufacturing practices.

*E. coli*, commonly found in soil and water, are part of the normal intestinal flora of humans and other warm-blooded animals. While many strains are innocuous, some cause disease. Of particular concern to the food industry are Shiga-toxin producing *E. coli* (STEC), including the subset of enterohemorrhagic *E. coli* (EHEC) with increased virulence. Among these pathogens, *E. coli* O157:H7 is one of the most notorious; its infectious dose is thought to be fewer than 100 organisms. Several *E. coli* O157:H7 outbreaks are attributed to dairy products, including consumption of unpasteurized fluid milk. Of the outbreaks attributed to cheese, almost all are associated with cheeses manufactured from unpasteurized milk—despite the organism not being isolated from samples of incriminated milk or the processing environment.

*Listeria monocytogenes*, widely disseminated bacteria that are pathogenic to both humans and animals, account for only an estimated 0.02% of foodborne human illnesses per year. However, infections from these bacteria yield a high rate of mortality, in part because this pathogen typically infects hosts with suppressed immune functions. *L. monocytogenes* is of particular concern in high-moisture, low-acid cheeses. The increase in pH to near neutral during ripening of surface-ripened cheese is of particular concern because these conditions favor this pathogen’s growth. Unlike other pathogens, *L. monocytogenes* can grow during refrigerated storage.

*L. monocytogenes*, found widely in soil, manure, and silage, can readily contaminate raw milk and cheese processing environments in the absence of proper controls. Several outbreaks of listeriosis trace to cheese produced from raw milk, including from unlicensed facilities or through imports of Mexican-style cheeses such as queso fresco. Although heat readily inactivates this pathogen, most outbreaks and sporadic cases of listeriosis linked to pasteurized milk and milk products trace to postprocessing contamination. Some persistent strains of *L. monocytogenes* contaminate food processing facilities, presenting a particular risk in cheese plants due to increased exposure during various production steps, including hooping, cutting, and packaging. Small-scale cheese production involving extensive hand contact also raises the risk of contamination.
Because cheese production relies in part on the outgrowth of microorganisms, it also enables unwanted contaminants to replicate. Thus, poor sanitation, improper handling of milk, and animal health issues can decrease yields and lead to poor-quality products while also leading to sporadic cases and outbreaks of dairy-related diseases. Nonetheless, cheese has an excellent food safety record, causing only a relatively few outbreaks of foodborne illnesses considering the huge amounts produced and consumed worldwide. As its production and consumption continue to grow, the dairy industry must remain steadfast in its efforts to make safe and high-quality cheeses.

Dennis J. D’Amico is Assistant Professor in the Department of Animal Science, University of Connecticut, Storrs.

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