

# Food-Borne Pathogens Associated with Natural Cheese Related Outbreaks: A Review

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## Abstract

Natural cheeses are made from pasteurized or raw milk and commonly consumed around the world. Due to their varying practices of production and/or processing, natural cheeses can be susceptible for contamination with food-borne pathogens becoming potential source of health risk for consumers. Food-borne pathogens are concerns not only for consumers but also for industries involved in production of dairy products. As in the case of all processed foods, cheese making require superb production and processing hygiene in production lines over the value chain to prevent occurrence and adverse effects of pathogens. Among others, pathogenic *E. coli*, *Salmonella* spp., *S. aureus* and *L. monocytogenes* are pathogens of the concern implicated with natural cheese related outbreaks worldwide. Therefore, this review tries to show the general characteristics of these pathogens, their brief epidemiology, source and routes of transmission, clinical significance, isolation and identification, and their prevention and control. This is aspired to serve readers and concerned audiences as important updates to contribute their parts for prevention and control of these pathogens in cheese making process.

**Keywords:** Natural cheese, *E. coli*, *Salmonella*, *S. aureus*, *L. monocytogenes*

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## Introduction

Cheese is one of the most popular manufactured food products of dairy origin. Natural cheese is the concentrated form of milk containing nutrients that can supply dietary requirement of humans and support the growth and survival of microorganisms. Cheese may also comprise natural microflora such as lactic acid bacteria, manually added starter and non-starter cultures, and possibly contaminants including foodborne pathogens. Among others, *Listeria monocytogenes*, *Salmonella*, pathogenic *Escherichia coli* and *Staphylococcus aureus* are pathogens more often associated with cheese related foodborne disease outbreaks worldwide (FSANZ, 2009; Williams and Withers, 2010). This review, briefly describes these four major foodborne pathogens based on their characters, epidemiology, source and route of transmission, clinical significance, isolation and identification, and prevention and control.

## Listeria monocytogenes

### Characteristics

*Listeria* is short (0.5 to 2  $\mu\text{m}$  x 0.5  $\mu\text{m}$ ), gram positive, rod shaped, and psychrotrophic bacteria with rounded ends. The cells can be found singly or in short chains and sometimes in long filaments. *Listeria* is also motile at the temperatures between 20°C and 25°C with peritrichous flagella showing characteristic tumbling movement. However, it is not motile at other growth temperature conditions including optimum temperature of 37°C. *Listeria* spp. are catalase positive, oxidase negative, and they are facultative anaerobes (Liu, 2006; Liu, 2013). *L. monocytogenes* is a zoonotic pathogen that causes different infections including mild gastroenteritis up to fatal septicemia, encephalitis, abortion or stillbirth. There are six species of *Listeria* known that include *L. monocytogenes*, *L. ivanovii*, *L. welshimeri*, *L. grayi*, *L. innocua* and *L. seligeri*. Among these, *L. monocytogenes* is pathogenic both for humans and animals (Liu, 2006; Liu, 2013). *L. ivanovi*, however, is pathogenic for animals and rarely associated with human infection while other species are mostly nonpathogenic. In addition to the previously known six species, four novel species of *Listeria* are reported recently. These include *L. rocourtiae*, *L. marthii*, *L. fleischmannii*, and *L. weihenstephanensis* making the number of *Listeria* species be ten (Graves, et al., 2010; Liu, 2013).

### Epidemiology

*Listeria monocytogenes* widely distributed in nature, and it is a zoonotic pathogen both for animals and human. Human listeriosis has high fatality rate of up to 30% in the risk group such as pregnant women, infant, elderly and immunocompromised adults. According to CDC report, *Listeria monocytogenes* one of the major pathogens that result in an estimated 1591 illnesses, 1455 hospitalizations, and 255 deaths in the US per year (Scallan, et al., 2011). A surveillance result from France also showed that an annual incidence of 3.4 listeriosis cases occurred per 1000000 inhabitants during 2001 – 2003 (Goulet, et al., 2006). Another study from Germany indicated that an estimated 0.62 cases per 100000 inhabitants occur per year, and that report also showed increasing

trend of listeriosis than the previous year/s (Koch, et al., 2006). The national survey result of Japan also showed an estimated incidence of listeriosis as 0.65 cases per million inhabitants that are lower than the cases in other developed countries (Okutani, et al., 2004). Other reports from different regions of the world also show the distribution of *L. monocytogenes* all over the world (Chenal-Francois, 2011; Blum-Menezes, 2013).

### Source and route of transmission

*L. monocytogenes* is a ubiquitous microorganism that exists in a wide range of environments including soil, water, plants, decaying vegetation, slaughter house wastes, sewage, food processing environments, etc. (Sauders, et al., 2007). This allows it potential access to foods of different origin including dairy products, meat products, vegetables, bakery products, sea foods and ready to eat foods from various sources. Because of its ubiquity, therefore, human listeriosis is believed to transmit mainly through consumption of contaminated foods (Sauders, et al., 2007; Buchholz, et al., 2001). Cheese contamination can be contracted at various stages of cheese-making process. A study conducted by Brito and colleagues showed that storage coolers might have been the principal source of contamination for cheese produced from pasteurized milk (Brito, et al., 2008). Milk as raw material and cheese making environment can also be the major sources of cheese contamination leading to transmission of the pathogen and consequent foodborne listeriosis incidences in the absence of proper hygienic measures.

### Clinical significance

Foodborne listeriosis is a disease that affects people known as risk groups that have weakened immunity. These include elderly people, newborn, pregnant women or immunocompromised people due to other diseases. Listeriosis has the clinical signs that involve febrile gastrointestinal disease with mild and flu-like symptoms. The disease may also develop into more invasive forms of listeriosis including meningitis, meningoencephalitis and septicemia in the risk groups (Buchholz, et al., 2001; Swaminathan and Gerner-Smidt, 2007; Allerberger, et al., 2010). The pathogen requires a long incubation period of about 30 days where the range varies between 3 and 70 days (Swaminathan and Gerner-Smidt, 2007; Allerberger, et al., 2010). Healthy individuals with normal immunocompetence may not develop symptoms, and if they develop, it appears in a mild flu-like symptoms or mild enteric form of illness. On the risk groups, the disease is manifested in more systemic form, and it has a high fatality rate of about 30% in the risk groups (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* grows in many foods including those treated with salt or food grade acids. As the organism is cold tolerant, it can also grow in those foods stored at refrigerated temperature conditions and poses a risk to the consumers. Thus, its ability to endure harsh conditions allows the pathogen to grow and reach a high number of cells in the foods as storage time extends.

### Isolation and identification

Several methods have been developed and used to isolate and identify *L. monocytogenes*, in different specimens including food samples. Conventional bacteriological methods of ISO, FDA, and USDA-FSIS, are used as standard methods for detection of the pathogen (Donnelly and Nyachuba, 2007). These methods involve two steps that include pre-enrichment and enrichment processes in selective culture media followed by biochemical identification of suspected *L. monocytogenes* colonies. Since *Listeria* spp. is capable of hydrolyzing esculin into esculetin, the culture methods comprise esculin and ferric ion as an important component to indicate the presence of esculin hydrolyzing bacteria. Then esculin reacts with ferric ion resulting in the formation of blackening of the media, and this indicates the occurrence of esculin hydrolyzing bacteria in the target sample.

The enrichment methods also contain selective agents that support the growth of *L. monocytogenes* from a given sample while inhibiting other microorganisms. Among these, lithium chloride is used for amplification of the pathogen in the presence of gram-negative bacteria while nalidixic acid is inhibitory for gram-negative through interference with DNA gyrase. Moreover, acriflavin, polymyxin B, and ceftazidime are used for inhibition of gram-positive cocci and to prevent the growth of gram-negative rods that are background microflora of food and environmental samples (Donnelly and Nyachuba, 2007).

Since the start of cold enrichment techniques, a variety of detection methods have been used for isolation and identification of *L. monocytogenes*. However, none of the methods are best for the selective isolation and identification of the pathogen. Thus, efforts for improving the existing methods are part of the continuous hard work of scientific community. Among the existing enrichment methods, UVM broth is recommended method by USDA-FSIS for selective isolation of *L. monocytogenes* from meat, egg, and poultry samples. This media contains per liter of proteose peptone (5 g), tryptone (5 g), lab-lamco powder (5 g), yeast-extract (5 g), sodium chloride (20 g), disodium phosphate 2-hydrate (12 g), potassium phosphate mono-basic (1.35 g), esculin (1 g), nalidixic acid (20 mg) and acriflavine HCl (12 mg). The medium is also called *Listeria enrichment* broth I (LEB I) and similar media with modification of its nalidixic acid content to (25 mg/L) is known as LEB II. Frazer broth is another enrichment media commonly used for isolation of *L. monocytogenes*. This broth is a modification of LEB II, and it contains 3 g/L of lithium chloride in addition to the components in the LEB II (Donnelly and Nyachuba, 2007).

In addition to the enrichment broth, selective agar media are used for differential identification of the pathogen.

Among these PALCAM and Oxford-agar are most commonly used solid media. In more recent times, chromogenic agar media have also been developed that take advantage of the specific enzyme activity that exist in *L. monocytogenes* and *L. ivanovii*, but not in other *Listeria* spp. (Gasnov, et al., 2005). Even though the culture methods are efficient to discriminate *Listeria monocytogenes* selectively, food-processing companies increasingly prefer to use more rapid quality control tests. These rapid methods include those PCR-based methods, and they are known to deliver results within a short time allowing batches of foods for quick release following completion of the tests.

### Prevention and control

Since *L. monocytogenes* is a ubiquitous microbe in the environment including food processing setups, it is difficult to prevent contamination totally. However, implementation of appropriate hygiene and sanitation steps with good manufacturing practices and HACCP can serve for prevention and control of this pathogen. Moreover, as *L. monocytogenes* is susceptible to cooking temperature, heat treatment along with prevention of recontamination is useful options to prevent the risk of listeriosis in foods (Huss, et al., 2000). In addition, adjusting acidification in the early stages of the process is a key factor to prevent the development of *L. monocytogenes* effectively with low-level contamination in raw milk cheese (Millet, et al., 2006).

In addition, different countries set the standard limit as control option so that a given food can contain the pathogen below that limit and be considered as safe for public consumption. The limits are based on the specific conditions of each country and vary from country to country. However, all the existing regulations require either absence or lower than 100 cfu/g of *L. monocytogenes* in cheese and other ready-to-eat food samples (Dixon, 2000; Jay, et al., 2005). For instance, USA has a strict rule of zero tolerance policy where detection of *L. monocytogenes* in cheese or any food sample may make the food subject to seizure. According to the regulation of European Union, however, soft cheese should be free from *L. monocytogenes* per 25g of the sample while less than 100 cfu/g of the other cheese sample is acceptable (Dixon, 2000). Implementation of these regulations might have contributed to the reduction of outbreaks related to *L. monocytogenes* in the US (Jay, et al., 2005).

### Salmonella

#### Characteristics

Salmonellae are facultative anaerobic, gram-negative, small rod-shaped bacteria (0.7 – 1.5 x 2.0 – 5.0 µm). They are member of the Enterobacteriaceae family. With the exception of *S. Pullorum* and *S. Gallinarum*, Salmonellae are motile with peritrichous flagella. They are catalase positive, oxidase negative and reduce nitrates to nitrites. *Salmonella* produces hydrogen sulfide, decarboxylate lysine, and they are negative to indole and urease (Bell and Kyriakides, 2002). Salmonellae are mesophilic bacteria that have a wide range of growth temperature extending between 5°C and 46°C where optimum growth temperature is around 37°C. However, they are killed by pasteurization temperature and time. *Salmonella* also have a wide range of pH between four and nine while optimum pH being around neutrality. *Salmonella* do not multiply at an  $A_w$  of 0.94, especially in combination with a pH of 5.5 and below but the cells survive in frozen and dried states for a long time. They can also proliferate in many foods without affecting the acceptance qualities (James, et al., 2005; Ray, 2004).

The genus *Salmonella* has two species namely *S. enterica* and *S. bongori*. *S. enterica* is also subdivided into six subspecies based on their specific biochemical and genomic characteristics. As differentiation tool, Roman numbers and specific names associated with geographic location are used to designate these six subspecies. Accordingly *S. enterica* subsp. *enterica* is designated by the Roman number (I); likewise *S. enterica* subsp. *salamae* (II); *S. enterica* subsp. *arizonae* (IIIa); *S. enterica* subsp. *diarizonae* (IIIb); *S. enterica* subsp. *houtenae* (IV) and *S. enterica* subsp. *indica* is designated by the Roman number (VI) (James, et al., 2005). Among these, *S. enterica* subsp. *enterica* is responsible for the majority of foodborne diseases.

#### Epidemiology

*Salmonella* causes bacterial foodborne disease around the world. According to the estimates, it causes 93.8 million cases of foodborne illnesses worldwide leading to 155,000 deaths each year (Majowicz, et al., 2010). Another estimate also showed that *Salmonella* causes about 1 million illnesses per year in the United States which result in 19336 hospitalizations and 378 deaths (Scallan, et al., 2011). A food poisoning statistics report of Ministry of Health Labor and Welfare of Japan also showed that *Salmonella* caused an estimated 1518 illnesses in 67 incidents occurred in various parts of the country in 2009 (MHLW, 2009). The annual burden report of different regions of the world showed that *Salmonella* is estimated to cause about 56300 illnesses in North Africa and Middle East. Moreover, it causes 2.5 million illnesses in Africa, 53.6 million in Asia-Pacific and Oceania, 29.8 million in South and South East Asia, 5.1 million in Europe and 2.2 million in the Americas (Majowicz, et al., 2010).

#### Source and route of transmission

*Salmonella* is transmitted through fecal-oral route mainly through ingestion of contaminated food. Consumption

of contaminated non-animal food products, contaminated water, foods of animal origin or contact with reservoir animals are possible routes of *Salmonella* transmission (Majowicz, et al., 2010). In addition to this, mass production and distribution of food products disseminate pathogens rapidly to communities and occurrence of antibiotic resistance in *Salmonella* spp. making it a challenge to control and prevent *Salmonella* infections. Farm animals are the major reservoir for non-typhoid *Salmonella* spp. They are naturally prevalent in chickens, ducklings, sheep, goats, pigs, reptiles, amphibians, birds, pet rodents, dogs, cats, and in a variety of wild animals. Thus, consumption of foods contaminated with gastrointestinal fluid or fecal matter of these animals could result in transmission of *Salmonella* spp. Moreover, infections of *Salmonella* spp. associated with pet transmission could affect infants, and result in invasive disease and severe complications (Sánchez-Vargas, et al., 2011). Thus, though the majority of human *Salmonella* infections are foodborne, salmonellosis can also be acquired through contaminated drinking water, contact with infected animals and direct human-to-human transmission. Therefore, contact with a variety of animal species that are infected with *Salmonella*, such as mammals, birds, and reptiles or consumption of contaminated food, could be a route of transmission for human infection (Majowicz, et al., 2010; Switt, et al., 2009).

### **Clinical significance**

*Salmonella* spp. causes foodborne salmonellosis and result in infections that vary from mild and self-limiting gastroenteritis to sometimes systemic infections such as bacteremia. It has the incubation period of 7 to 72hr after ingestion of the contaminated food. The symptoms include fever, nausea, abdominal cramping, and non-bloody diarrhea that may tend to decrease within few hr or days. In some cases, septicemia could result as a complication of gastroenteritis leading to fatal cases in immunocompromised patients. Prolonged septicemic infections may also result in localized tissue and organ infections, especially in those previously damaged or diseased individuals. The severity and duration of symptoms may depend not only on the type and amount of pathogens ingested but also on the susceptibility of the host. The symptoms in immunocompetent hosts may typically last up to 2 to 7 days (Talbot, et al., 2006; Callaway, et al., 2008; Parry, et al., 2013; Dhanoa and Fatt, 2009).

### **Isolation and Identification**

Regardless of the contamination level, presence of *Salmonella* in a given food is considered as a significant risk due to its low infective dose. Isolation and identification of *Salmonella*, conducted routinely following conventional culture techniques based on the distinct features of *Salmonella* spp. (Roberts and Greenwood, 2003; Jeníková, et al., 2000). Since, food samples might have passed through different treatment methods such as cold storage or heat treatment, the cells of the pathogen could appear in the injured state. This condition may also result in reduced recovery rate of the cell by culture method and could lead to false negative results that in its turn could pose the potential health risk to the consumers. For this reason, isolation method usually involves pre-enrichment step to allow the recovery of injured cells. The pre-enrichment step of food sample uses non-selective media such as buffered peptone water. However, a selective RV broth is used for the enrichment step that helps the selective isolation of the *Salmonella* spp. Then the culture from RV broth is streaked on the selective differential agar media. RV broth contains malachite green and magnesium chloride that inhibits other bacteria and allows the growth of *Salmonella* spp. Among others, Desoxycholate agar, *Salmonella-Shigella* agar, modified brilliant green agar, xylose lysine Desoxycholate agar and brilliant green MacConkey agar represent selective agar media used for isolation of *Salmonella*. Moreover, Bismuth sulfite agar, Mannitol lysine crystal violet brilliant green agar, chromogenic agar, etc. can be used for selective isolation of *Salmonella* (Ray, 2004).

*Salmonella* spp. can be further identified by using biochemical tests. Among others, gram stains and responses to various sugar fermentation tests are commonly used methods for identification of these pathogens. In addition, ELISA and PCR-based methods are also available that has high efficiency for discrimination and get the result with shorter time than the culture methods (Ray, 2004; Roberts and Greenwood, 2003).

### **Prevention and control**

Keeping proper sanitation measures, safe and hygienic food handling practice, and enhancing public awareness through appropriate education are some of the most important steps for the prevention of foodborne salmonellosis. *Salmonella* is widely present in the gastrointestinal tract of food animals and it can be shed through feces that can be the source of food contamination. Hence, prevention measures for such cases should be focused on implementing proper hygienic measures to avoid fecal contamination of foods. Among others, foods of animal origin such as eggs, poultry, and beef should be cooked properly to prevent *Salmonella* infection. Milk and dairy products should also be heat treated properly including pasteurization and appropriate cooking. Moreover, it is important to keep hygiene of food making environment such as kitchen surfaces and utensils used to prevent cross contamination of other food items due to faulty practice of hygiene during household food making. Furthermore, it is important to avoid contact with gastrointestinal fluid with carcass in the slaughter houses. Avoiding the contact of infants to pet animals is also recommended as these animals are reservoirs for *Salmonella*. However, in the case

of adult, it is recommended to wash hands properly following the contacts of pets (Majowicz, et al., 2010; Sánchez-Vargas, et al., 2011).

## **Pathogenic *Escherichia coli***

### **Characteristics**

*E. coli* is Gram-negative, facultative anaerobic, rod-shaped bacteria belonging to the Enterobacteriaceae family. It is commensal bacteria that exist in the lower intestinal tract of human hosts and other animals in mutualistic beneficial relationship. As components of normal microflora in human intestinal tract and other warm-blooded animals, *E. coli* can be detected in big number from the fecal samples. It is considered as harmless commensal and commonly used as indicator of fecal contamination. In addition, if its number is high, it may suggest possible presence of enteric pathogens in foods. Though many strains of *E. coli* are harmless inhabitants of the gastrointestinal system, some of them cause disease in humans. The pathogenic *E. coli* are characterized by their serogroup, virulence genes, and production of toxins and associated disease symptoms. Accordingly, there are classes of *E. coli* pathotypes known to cause foodborne gastrointestinal disease in humans. These include enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC). In addition, enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), and adherent invasive *E. coli* (AIEC) are among the pathotypes of pathogenic *E. coli* (Croxen, et al., 2013; Farrokh, et al., 2013).

### **Epidemiology**

Among pathogenic *E. coli*, more focus of research is given for Shiga toxin-producing *E. coli* specifically enterohemorrhagic *E. coli* (EHEC) and more data is available for this group than other pathotypes. Diseases related to the pathogenic *E. coli* are widely distributed all over the world. A report of CDC shows an estimated 205781 illnesses, 2429 hospitalizations and 20 deaths in the US per year due to pathogenic *E. coli*. The pathotypes include STEC O157, non-O157 STEC, ETEC and diarrheagenic *E. coli* other than STEC and ETEC (Scallan, et al., 2011). A review of Croxen and colleagues (2013) also showed that the prevalence of EPEC that goes beyond developed countries. Though it is more associated with infant diarrhea in developing countries, the problem remains in developed countries too. In the US, however, the rate related to this group of pathogens is reducing. Among all pathogenic *E. coli*, EHEC continues as the major concern of foodborne public health issue worldwide (Croxen, et al., 2013; Farrokh, et al., 2013). In addition to STEC O157, other groups such as O26, O45, O103, O111, O121, and O145 can cause hemolytic colitis and hemolytic uremic syndrome. The symptoms are apparent in infants, elderly and immunocompromised people. EHEC alone result in an estimated 112572 illness and 271 hospitalizations in the US annually (Scallan, et al., 2011). The CDC estimate also shows that the rate of EHEC incidence between 2005 and 2010 was 0.97 per 100000 inhabitants for O157 while the estimate for non-O157 was 1.10 per 100000 people. The hospitalization rate for the same period, however, was 43% for O157 while it was 18% for non-O157 groups. The fatality rate also was twice high in the case of O157 while it was relatively low in the case of non-O157 *E. coli*. The incidence of O157, however, dropped by 42% in 2011 than the earlier years in the US. In Canada, Australia, and Europe the incidence reports show higher figures than those reported by CDC in the US (Croxen, et al., 2013).

### **Source and route of transmission**

Ruminants are the major reservoir of STEC, and consumption of the food contaminated with cattle manure is the major source of the pathogen (Croxen, et al., 2013; Farrokh, et al., 2013). The main route of transmission is fecal-oral route through consumption of food contaminated with the pathogen. Food contamination with cattle manure can occur through different routes and lead to the incidence of foodborne illnesses on the consumers. Direct contact with reservoir animals may also lead to human infection in the absence of appropriate hygiene. Moreover, human infection might also occur by direct contact with infected individuals especially in the places such as care centers (Croxen, et al., 2013). Thus washing hand after the contact with animals should be a regular practice before handling foods.

### **Clinical significance**

Pathogenic *E. coli* cause various range of clinical syndromes that involve bloody or watery diarrhea based on the pathotypes. Among these, infection of STEC can range from mild watery diarrhea to bloody diarrhea (hemolytic colitis) and may result in the development of hemolytic uremic syndrome (HUS) (Croxen, et al., 2013; Farrokh, et al., 2013). The incubation period before the onset of the symptoms could be about three days. Despite the variation in severity, both O157 and non-O157 STEC can cause similar symptoms including bloody diarrhea and HUS on children, elderly or immunocompromised people. EPEC, on the other hand, causes infectious diarrhea accompanied by fever, vomiting and dehydration in children under two years of age. It has a rapid onset of three-hour incubation time on human volunteers after ingesting wild-type bacteria. Other clinical features of this group include the

intolerance for the cow's milk and failure to respond oral rehydration therapy. Clinical manifestation of EIEC is similar with that of *Shigella* and it is presented with bacillary dysentery and bloody diarrhea (Croxen, et al., 2013).

### Isolation and identification

Pathogenic *E. coli* comprises several pathotypes and big number of serotypes. STEC is one of the pathotypes that have more than 400 serotypes while only a few of them are pathogenic for humans (Croxen, et al., 2013; Farrokh, et al., 2013). Isolation methods of the pathogenic *E. coli* are also variable with serotypes that include culture techniques and immunological or molecular based identification methods (Vimont, et al., 2006). Since food samples might initially contain low numbers of STEC together with a high level of competing microflora, selective enrichment step is required for the isolation of STEC (Pinto, et al., 2005). Such enrichment step from inspection of processed foods could allow the recovery of injured or stressed bacterial cells due to harsh manufacturing processes (Croxen, et al., 2013; Gyles, 2007). Different enrichment protocols use varying basal broths, and diversity of selective agents are added and used with different incubation times and temperature combinations. For instance, tryptic soy broth (TSB) and *E. coli* (EC) broth with novobiocin are used to identify *E. coli* O157H7 and non-O157 STEC from food samples (Croxen, et al., 2013; Gyles, 2007).

### Prevention and control

Sanitizing and keeping good hygienic measure is the best way for preventing contamination of food with pathogenic *E. coli*. Since *E. coli* are highly sensitive to heat, proper temperature treatment including pasteurization or cooking foods can greatly help to prevent infection by these pathogens. In addition to heat treatment, proper refrigeration, and prevention of cross-contamination is a useful measure to control *E. coli* O157H7 in ready-to-eat food including cheese. Therefore keeping appropriate hygienic condition of food making environment is a useful step in preventing the contamination. Moreover, personal hygiene and proper temperature treatment of foods as well as refrigeration and prevention of cross-contamination is an essential measure to prevent and control pathogenic *E. coli* in foods.

### Staphylococcus aureus

#### Characteristics

*Staphylococcus aureus* is gram-positive cocci that have characteristic cell arrangement of the grape-like structure. The cells appear singly, in paired cocci, or in clustered structures. It is a non-motile and non-spore forming bacteria that is facultative anaerobic (Le Loir, et al., 2003). *S. aureus* ferment mannitol, produce coagulase and catalase, and also produce thermostable nuclease (TNase). It grows in a wide temperature range that vary between 7 and 46°C while it optimally grows in the mesophilic temperatures between 30°C and 37°C. *S. aureus* also grow in relatively low  $A_w$  (0.86), low pH (4.8). Moreover, it can also grow in high salt and sugar concentrations of up to 15%, and in anaerobic conditions (Le Loir, et al., 2003; Kadariya, et al., 2014; Ray, 2004). Therefore, due to their ability to grow under adverse conditions, *S. aureus* can multiply in many foods including natural cheeses. However, they are poor competitors to the natural flora that found in foods, and the presence of active starter culture inhibits their growth (Ray, 2004). *S. aureus* exists in the skin, hair, and mucosal surfaces of healthy humans and animals. In addition, anterior nares of healthy people are commonly inhabited by *S. aureus* on healthy carriers (Le Loir, et al., 2003; Argudín, et al., 2010). Most of the staphylococcal foodborne diseases, therefore, are thought to result from consumption of contaminated foods and due to unhygienic practices by the food handlers. Once *S. aureus* gets access to foods, it can grow and produce staphylococcal enterotoxins that are responsible for food poisoning.

#### Epidemiology

*S. aureus* causes bacterial foodborne intoxication worldwide. The data from Japanese ministry of health labor and welfare shows that *S. aureus* is one of the top most pathogens causing bacterial foodborne diseases in Japan (MHLW, 2009). *S. aureus* caused a total of 2525 staphylococcal food poisoning outbreaks that resulted in 59,964 illnesses, and three deaths during 20 years period of 1980 – 1999 in Japan (Shimizu, et al., 2000). Asao and colleagues (Asao, et al., 2003) also reported an extensive outbreak of the staphylococcal foodborne outbreak in Kansai area of Japan caused by the consumption of contaminated dairy products and affected 13420 people in the year 2001. *S. aureus* is also the second most important foodborne pathogen in France next to *Salmonella*. Moreover, according to another report, 86 confirmed and 173 suspected foodborne outbreaks are caused in France due to *S. aureus* among a total of 1787 foodborne illnesses reported during 2001 – 2003 (Kérouanton, et al., 2007). *S. aureus* is among the major causative agents of foodborne diseases in the US where according to CDC estimate, it result in 241,148 illness, 1,064 hospitalizations and 6 deaths each year (Scallan, et al., 2011).

#### Source and route of transmission

*S. aureus* is a commensal bacterium that inhabits skin and anterior nares of humans. Among healthy humans, 20 –

30% of the population is considered as permanent carriers. *S. aureus* also exists in the skin, hair and feathers of domestic animals and birds (Le Loir, et al., 2003; Argudín, et al., 2010). Hence, food handlers are considered as the major source of food contamination by *S. aureus*. Especially, for the heat treated foods, contamination due to the faulty handling is the main route for the transmission of the pathogen to foods. Though it is poorly competent, *S. aureus* can grow in wide varieties of foods if it gets access. Among others, milk and its products, cream filled pastries and cheeses are some of the commonly incriminated foods for staphylococcal food poisoning. Moreover, meat and meat products, poultry and egg products, salads, and bakery products, cooked meals, sandwich fillings are associated with staphylococcal food poisoning (Le Loir, et al., 2003; Ray, 2004; Argudín, et al., 2010).

### **Clinical significance**

Consumption of one or more staphylococcal enterotoxins pre-produced in the food results in the staphylococcal food poisoning. Staphylococcal foodborne illnesses have rapid onset that normally develop within 30min. to 8hrs. following ingestion of the contaminated food. The symptoms include nausea, vomiting, and abdominal cramps and with or without diarrhea (Le Loir, et al., 2003; Argudín, et al., 2010). The symptoms may also include headaches, cold sweats and rapid pulse, transient changes in blood pressure, prostration and dehydration depending on the amount of toxin ingested. Moreover, the amount of enterotoxin that causes the illness can vary depending on the susceptibility and immune condition of the individuals. However, 100 – 200ng toxin is thought to be enough to cause symptoms (Ray, 2004). A report of major Japanese outbreak estimated that about 20 – 100 ng of the enterotoxin was responsible for causing symptoms on the consumers (Asao, et al., 2003). Despite its high incidence and economic pressure on the patients, staphylococcal foodborne illness is self-limiting, and recovery from it takes one to two days. However, in rare cases it may result in complication or hospitalization (Kadariya, et al., 2014). Though it is one of the top most causes of bacterial foodborne illnesses in Japan, the death rate related to staphylococcal food poisoning is low or, no death recorded from it in recent reports (MHLW, 2009).

### **Isolation and Identification**

*S. aureus* is isolated from food samples and other specimens following one of the standard methods such as ISO method and FDA methods among others. Enrichment in selective agar media is used for isolation of the pathogen and coagulase tests, thermo nuclease test, and other biochemical tests are used for identification (Brown, et al., 2005; Kateete, et al., 2010; Pinto, et al., 2010). Standard methods are based on the specific growth requirement of the bacteria and Baird Parker Agar and Mannitol Salt Agar with egg yolk are most commonly used agar media for isolation of *S. aureus*. Baird Parker Agar contains Tryptone and Beef Extract as major carbon and nitrogen source. Yeast Extract is another component used as essential nutrient supplement and as a main source of vitamins and growth stimulant for *S. aureus*. In addition to Yeast extract, the growth of the bacteria is stimulated by the presence of glycine and sodium pyruvate. Moreover, glycine, lithium chloride and potassium tellurite act as a selective agent, and egg yolk is the substrate used to detect lecithinase or lipase activity produced by *S. aureus*. Mannitol Salt Agar is another media used for selective isolation of *S. aureus*. In this medium, most other bacteria are selectively inhibited by the presence of high sodium chloride concentration. In addition to sodium chloride, mannitol is a component used as a selective agent. *S. aureus* ferments mannitol and formation of acid due to this turns the media into yellow. Other coagulase-negative staphylococci do not ferment it and grow as red colonies. Phenol red is the pH indicator that is converted to yellow when the fermentation of mannitol occurs reducing the pH of the medium to lower than 6.8. In the absence of mannitol fermentation, however, the medium remains red. Mannitol Salt Agar also contains peptone and beef extract that supply the nutrient requirement of the bacteria including carbon, nitrogen and other essential growth factors such as trace nutrients.

Following the isolation of *S. aureus* on selective media, identification is achieved through respective biochemical analysis specific to this pathogen that includes colony morphology and gram staining. Moreover, coagulase test, latex agglutination test, DNase and thermo nuclease test, and tests based on commercial biochemical kits are used for identification. Furthermore, PCR-based tests of molecular methods targeting specific genetic markers of *S. aureus* can be used for identification (Brown, et al., 2005). Among others, coagulase test is most commonly used test for identification of *S. aureus* in routine laboratory activities (Kateete, et al., 2010). There are two types of coagulase tests used for screening and identifying *S. aureus*. These include slide coagulase test (SCT) and tube coagulase test (TCT). Bound coagulase produced by the *S. aureus* and also known as clumping factor can be identified by using slide coagulase test. This test crosslinks  $\alpha$  and  $\beta$  chains of fibrinogen in rabbit plasma to produce fibrin clots that deposit on the cell wall of *S. aureus*. As a result, every cell of *S. aureus* sticks to each other and show the resultant clumping of cells (Kateete, et al., 2010; Katz, 2013). Tube coagulase test, on the other hand, is used to identify the presence of free extracellular coagulase. This clots rabbit plasma in the absence of calcium and the test is the standard method for routine identification of *S. aureus*.

### **Virulence factors**

*S. aureus* has diverse virulence factors that include adhesions and extracellular proteins; and that enable them to

colonize and cause various kind of illnesses (Pereira, et al., 2009). Among others, staphylococcal enterotoxins are the most important virulence factors related to the food poisoning illnesses. Classically five staphylococcal enterotoxins (SEA, SEB, SEC, SED, and SEE) are known for their involvement in staphylococcal food poisoning incidences. However, at present there are about 22 staphylococcal enterotoxins identified among which some of them are known to be emetic and recognized for their involvement in foodborne outbreaks (Argudín, et al., 2010; Hennekinne, et al., 2010). Therefore, those having emetic activities are designated as staphylococcal enterotoxins. However, the other group either lack emetic activity or not yet examined for such activity and they are designated as staphylococcal enterotoxin like super-antigens (Lina, et al., 2004).

### Prevention and control

Since *S. aureus* can contaminate foods mainly due to faulty hygienic practice, improving overall production hygiene is the most important way to prevent contamination. In addition, since *S. aureus* is highly susceptible to heat treatment appropriate temperature treatment including pasteurization or cooking is highly important preventive measure. Moreover, personal hygiene that involves washing hands and equipment properly, wearing masks, wearing hair nets, using separate equipment among others are useful for prevention of contamination (Kadariya, 2014). Furthermore, as *S. aureus* can't grow at that temperature, storing natural cheese at the temperature lower than 5°C may help to prevent and control the pathogen and possible toxin production (Kadariya, 2014; Ray, 2004).

### Conclusion

Natural cheese is the concentrated form of milk containing nutrients that can supply dietary requirement of humans. Among others, *Listeria monocytogenes*, *Salmonella*, pathogenic *Escherichia coli* and *Staphylococcus aureus* are pathogens often associated with cheese related foodborne disease outbreaks worldwide. *L. monocytogenes* is widely distributed in nature, and it is a zoonotic pathogen. Human listeriosis has high fatality rate of up to 30% in pregnant women, infant, elderly and immunocompromised adults. Listeriosis has the clinical signs that involve febrile gastrointestinal disease with mild and flue like symptoms. The disease may also develop into more invasive forms of listeriosis including meningitis, meningoencephalitis and septicemia in the risk groups. Implementation of appropriate hygiene and sanitation steps with good manufacturing practices and HACCP can serve for prevention and control of this pathogen.

*Salmonella* is facultative anaerobic, gram-negative, small rod-shaped bacteria of Enterobacteriaceae family. It is transmitted through fecal-oral route mainly when contaminated food products are ingested. *Salmonella* causes foodborne salmonellosis and result in infections that vary from mild and self-limiting gastroenteritis to sometimes systemic infections such as bacteremia. The symptoms include fever; nausea, abdominal cramping, and non-bloody diarrhea that may tend to decrease within few hr or days. Regardless of the contamination level, presence of *Salmonella* in a given food is considered as a significant risk due to its low infective dose. Keeping proper sanitation measures, safe and hygienic food handling practice, and enhancing public awareness through appropriate education are some of the most important steps for the prevention of foodborne salmonellosis.

*E. coli* is Gram-negative, facultative anaerobic, rod-shaped bacteria belonging to the Enterobacteriaceae family. It is commensal bacteria that exist in the lower intestinal tract of human hosts and other animals. *E. coli* can be detected in big number from the fecal samples. Diseases related to the pathogenic *E. coli* are widely distributed all over the world. Ruminants are the major reservoir of STEC, and consumption of the food contaminated with cattle manure is the major source of the pathogen as it mainly transmits through fecal-oral route. Pathogenic *E. coli* cause various range of clinical syndromes that involve bloody or watery diarrhea based on the pathotypes. Sanitizing and keeping good hygienic measure is the best way for preventing contamination of food with pathogenic *E. coli*. Since *E. coli* are highly sensitive to heat, proper temperature treatment including pasteurization or cooking foods can greatly help to prevent infection by these pathogens.

*S. aureus* is gram-positive cocci that have characteristic cell arrangement of the grape-like structure. It is a non-motile and non-spore forming, facultative anaerobic bacteria. *S. aureus* causes bacterial foodborne intoxication worldwide. *S. aureus* is a commensal bacterium that inhabits skin and anterior nares of humans. Among healthy humans, 20 – 30% of the population is considered as permanent carriers. It also exists in the skin, hair and feathers of domestic animals and birds. Consumption of one or more staphylococcal enterotoxins pre-produced in the food results in the staphylococcal food poisoning. Staphylococcal foodborne illnesses have rapid onset that normally develop within 30 min to 8 hrs. The symptoms include nausea, vomiting, and abdominal cramps and with or without diarrhea. Since *S. aureus* can contaminate foods mainly due to faulty hygienic practice, improving overall production hygiene is the most important way to prevent contamination. In addition, as *S. aureus* is highly susceptible to heat treatment appropriate temperature treatment including pasteurization or cooking is highly important preventive measure.

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