

# THE PRESENCE OF PATHOGENIC BACTERIA IN TRADITIONAL CHEESE SOLD IN LOCAL MARKET IN HATAY PROVINCE, TURKEY

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**Abstract.** Contamination of cheese by foodborne pathogens may pose a risk for human health. Especially, microbiological quality of cheese sold in local market and produced on a small scale is generally lower than industrial production. The aim of the present research was to investigate the presence of pathogenic bacteria such as coagulase positive staphylococci, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. in twenty white cheese samples from local market on a small scale in the province of Hatay, Turkey. In this study, none of cheese samples contained *L. monocytogenes* and *Salmonella* spp. while *E. coli* and coagulase positive staphylococci were detected in cheese samples. The higher prevalence of coagulase positive staphylococci (100%) than *E. coli* (65%) were detected in cheese samples, but contamination levels of *E. coli* (mean 4.05 log cfu/g) were found higher than coagulase positive staphylococci (mean 2.47 log cfu/g) in terms of viable cell counts of pathogenic bacteria in cheese samples. The present results indicated that traditional cheeses manufactured in Hatay province were generally satisfactory microbiological safety according to Turkish Food Safety Criteria. As a result, the presence of pathogenic bacteria in traditional cheese from Hatay region could cause outbreaks affecting public health and reduce positive impression on traditional cheeses which have potential for geographical indication.

**Keywords:** *small scale food producers, Turkish food codex, microbiological criteria, dairy products, contamination*

## Introduction

Cheese is fermented dairy product known as a safe and nutritious food with high protein and dietary calcium contents. Cheese is often consumed due to these beneficial impacts on human health, favorable sensorial and nutritional properties (Mikulec and Jovanovic, 2005; Kurşun et al., 2008; Choi et al., 2016). In Turkey, cheese is produced by both traditional methods (manual handling and unmechanized) and industrial methods (production based on mechanization). The production and consumption of domestic natural or traditional cheese in the world is rising year by year. Traditional cheeses which have distinctive taste and odor are produced on a small scale by using raw milk in mostly east, southeast region of Turkey (Hayaloğlu et al., 2002; Ozer et al., 2004; Jorgensen et al., 2005). East, south east and south of Turkey are famous for quite high summer temperatures, for example Hatay. Hatay is one of the cities where food products are frequently sold in open market places or local market on a small scale due to traditional habits (Cokal et al., 2012).

Turkish traditional white cheese is usually produced under unmechanized or artisanal conditions and is handled at various stages of manufacture. Thus, various types of microorganisms may enter the cheese during manufacture and subsequent handling (Hayaloglu et al., 2002). As a result of climate and improper hygiene conditions, white cheese producer on a small scale of Turkey often encounter with contamination risk of pathogens. In particular traditional cheese sold local market have low hygienic quality

(Ozer et al., 2004; Başkaya et al., 2006; Uraz et al., 2008). Four bacterial pathogens including *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and pathogenic *Escherichia coli* are the predominant microorganisms that caused outbreaks as result of contaminated traditional cheese (Motarjemi et al., 2002; Hudson et al., 2003). *Salmonella* spp. is gram negative gastroenteritis-inducing pathogens and the majority of *Salmonella* cases are foodborne (Donnelly et al., 2018). The primary source of *Salmonella* spp. is humans and animals as carrier (mostly their feces). Animal products are among the most common foodstuffs of *Salmonella*. The main foods as source of *Salmonella* are meat, milk and eggs and their products and also inadequately heat treated food products (Akkaya and Alişarlı, 2006). In many reports, the outbreaks of *Salmonella* were associated with raw milk cheese (Donnelly, 2018). *Staphylococcus aureus* as coagulase positive staphylococci plays an important role on human health due to the production of thermo-stable enterotoxins that causes foodborne illness. Particularly, in the dairy industry *S. aureus* is mostly pathogen as it can induce foodborne intoxications through dairy products including cheeses (Donnelly, 2018). *S. aureus* is usually transmitted to food by personnel (hands, coughing, sneezing, etc.). Milk and milk products are the main foods that cause poisoning from *S. aureus* (Pacheco and Galindo, 2010). *L. monocytogenes* is gram positive facultative anaerobic foodborne pathogen and one of the most dangerous pathogens in food industry. It is usually found in soil, surface waters, sewage, plants, faeces and animal feed. Diseases from *L. monocytogenes* are more rare than others, but their effect is more severe and even fatal (Chavant et al., 2002; Pacheco and Galindo, 2010). Human listeriosis outbreaks are often associated with ready-to-eat food products including cheese. Several outbreaks and sporadic cases of disease associated with the consumption of pasteurized milk, cheeses made from unpasteurized milk, and other dairy products in USA and Europe in the past decade (Esho et al., 2013). *L. monocytogenes* contamination was mostly found in raw milk (Esho et al., 2013). *Escherichia coli* is gram negative, *Enterobacteriaceae* family of *Escherichia* genus dependent, facultative anaerob, mostly moving, non-spore, rod-shaped bacteria. *E. coli* infection may cause foodborne diseases such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. According to epidemiological findings, *E. coli* epidemic is generally seen as a result of cucumber, carrot and lettuce consumption (Almosoud et al., 2015). *E. coli* are known as indicator organisms of fecal contamination during cheese processing (IDF, 2016).

Today, foodborne diseases related to cheese consumption are common problems in many countries. Microbial contamination in traditional cheese as the main potential risk factor is increasingly growing and has detrimental effects on public health (Belli et al., 2013). High pathogen risk in milk and dairy products originates from cow itself, inefficient animal health control, insufficient training of farmers, dairy raw milk used unpasteurized for the production, processing environment and employees about milk hygiene, utensils, post processing, retail process collection, transport and weakness in the cold chain during production processes and storage (Telli et al., 2012; Cokal et al., 2012; Belli et al., 2013). Especially traditional cheeses produced from raw milk (unpasteurized) pose a risk due to its pathogenic contents (Atasoy et al., 2003). Microbial safety of cheese is dependent on good manufacturing practices. Cheese producers have to comply with hygienic practices to ensure safety (Donnelly, 2018). Considering that traditional cheese is mostly made from raw milk, the application of food safety policy are essential in order to avoid microbial spoilage of milk, especially in local small scale dairy processing plants (Belli et al., 2013). The World Health Organization requires that small scale dairy

processing plants in the developing countries urgently comply with the Codex Alimentarius principles (WHO, 2007). Hazard Analysis of Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) are also recommended for the small production units (Belli et al., 2013).

There are limited studies based on microbial safety of traditional cheese in the province of Hatay. The present study was aimed to evaluate hygienic quality and safety of traditional cheese from local market on a small scale in Hatay province, Turkey.

## Material and Methods

### *Samples and indicator microorganisms*

In this study, totally 20 cheese samples were collected from small scaled local market in Hatay province, Turkey (*Figure 1 and Figure 2*). For all identification tests, pathogenic bacteria including *Escherichia coli* ATCC-35150, *Salmonella* spp., *Listeria monocytogenes* ATCC7644 (Remel-USA) and *Staphylococcus aureus* ATCC-25923 was used as indicator organisms for the aim of positive control. Pathogen bacteria were grown in BHI (Merck, Germany) and stocked at  $-20^{\circ}\text{C}$  in BHI supplemented with 20% (v/v) glycerol.



**Figure 1.** Traditional cheese collected from local market on a small scale in Hatay province



**Figure 2.** Hatay province of Turkey (Demirkesen, 2012)

### ***The preparation of samples for microbiological analysis***

The homogenates of cheese samples were prepared by aseptically removing 25 g of cheese into sterile filter-stomaching bags (Isolab). A 225 mL of buffered peptone water was also added into the stomaching bags containing the samples and homogeneously mixed in stomacher (Bagmixer-interscience, France) twice for 1 min. The sample homogenates were serially diluted with buffered peptone water.

### ***Isolation and enumeration of microorganisms***

The isolation of pathogenic bacteria in cheese samples was performed according to the reference methods of EN/ISO 6888 for coagulase positive staphylococci, EN/ISO 11290-1 for *L. monocytogenes* and EN/ISO 6579 for *Salmonella* spp. and EN/ISO 16649 for *E. coli* (Halkman, 2005; Turkish Food Codex, 2011).

For *E. coli* enumeration, decimal serial dilutions of the homogenate were plated in duplicate on TBX agar (Merck) plates. After incubation of plates at 44°C for 18-24 hours, the blue green colonies were counted as *E. coli*.

For coagulase positive staphylococci (*S. aureus* and other species) enumeration, decimal serial dilutions of the homogenate were plated in duplicate on Baird parker-RPF agar (Oxoid) plates. After at 37°C, for 24 hours, typical black and bright colonies were counted as coagulase positive staphylococci.

For the isolation of *Salmonella* spp., homogenate of cheese samples in erlenmeyer flask were incubated at 35-37°C for 16-20 hours. Then, 10 mL homogenate were added to RVS broth (Oxoid) as selective pre-enrichment medium and incubated at 42-43°C for 24 hours. Afterthat, inocula from the pre-enrichment medium were streaked in duplicate onto Brilliant Green agar plates (Oxoid). After incubation of plates at 37°C for 24 hours, typical *Salmonella* colonies reproduced or not on the selective agar plates.

For the isolation of *L. monocytogenes*, 25 g of each sample was taken aseptically and homogenized in 225 mL Fraser broth (Oxoid) using a stomacher (Bagmixer-interscience, France) for 1 min and incubated at 30°C for 48 hours. Then, inocula from the enrichment culture were streaked in duplicate onto each of two selective agars, Oxford agar (Oxoid) and PALCAM agar (Oxoid). After incubation of both selective plates at 35-37°C for 48 h, typical *Listeria* colonies reproduced or not on the selective agar plates.

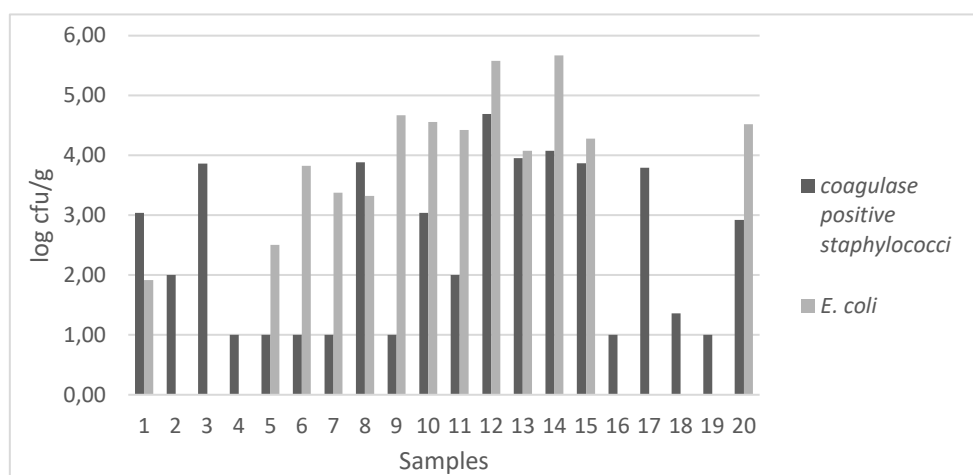
## **Results**

To assess microbiological safety of traditional cheese in Hatay province, a total of 20 cheese samples were collected from local market on a small scale in Hatay. *Table 1* represented the presence of pathogenic bacteria in traditional cheese samples from local markets on a small scale in Hatay. Additionally, viable cell counts of these pathogens which are present in these cheese were given in *Figure 3*. In this study, while *E. coli* (n=13) and coagulase positive staphylococci (n=20) were isolated in cheese samples, *Salmonella* spp. and *L. monocytogenes* were not isolated from any cheese samples. 45% of coagulase positive staphylococci exceeded the acceptable levels in Turkish Food Safety Criteria. However, since the Turkish Food Safety Criteria does not include any limitation on the number of *E. coli* that is allowed to be present in the white cheese, no comment could be made for *E. coli* levels in the present study.

**Table 1.** The presence of pathogenic bacteria in cheese from milk

Microorganism	n	m	Mean (log cfu/g)	Std. Dev	Max (log cfu/g)	Min (log cfu/g)
<i>E. coli</i>	13	UD	4.05	1.03	5.67	1.91
Coagulase positive staphylococci	20	9	2.47	1.32	4.69	1
<i>L. monocytogenes</i>	NF	NF	NF	NF	NF	NF
<i>Salmonella</i> spp.	NF	NF	NF	NF	NF	NF

n: The number of samples containing pathogens, m: The number of samples containing pathogens at unacceptable level according to Turkish Food Safety criteria, NF: not found, UD: undefined in Turkish Food Safety criteria for white cheese samples



**Figure 3.** Viable cell counts of pathogens which are present in traditional cheese

## Discussion

Farmhouse or small scale production of traditional cheeses was associated with higher microbiological risk. Food safety was seen as a critical factor in the production of such cheese types. Various small-scale production of raw milk products has performed in the world (Obrien et al., 2009). However, there is some concern that such foods may pose a risk of pathogenic bacterial food poisoning to consumers (Jorgensen et al., 2005). Similarly, in Turkey traditional cheeses were obtained with small scale production and sold in local markets. Traditional handmade cheese in Hatay, mostly reach to local market without retaining ripening duration or without providing cold chain. Thus, these cheeses are susceptible to the development of pathogenic bacteria.

In brief, as known, cheese produced with unpasteurized milk may result in foodborne outbreaks. On this sense, food industry which purposes microbiological safety prefers the use of pasteurized milk for the production of cheese. However, sensorial properties of cheeses from unpasteurized milk and pasteurized milk are different from each other and according to consumer demands both type cheese were also sold on the market. Therefore the achievement of microbiological safety in traditional cheese is essential during processing including manual handling, molding and ripening. Additionally, concentration of brine solution, storage conditions, ingredients, cheese composition and packaging etc. play an important role on microbial charge of cheese. The safety of traditional cheese

could particularly ensure with microbial risk assessments based on ripening duration, water activity and storage conditions (Choi et al., 2016).

*L. monocytogenes*, *S. aureus*, *E. coli* and *Salmonella* spp. are well-recognized foodborne bacterial pathogens. Traditional cheese from raw milk could support the growth of such foodborne pathogens. Therefore, there is concern for microbiological safety of domestically produced traditional cheeses (Esho et al., 2013). If pathogens are present in raw milk, they can be present throughout a cheese. Raw milk or contaminated milk was seen as the source of *L. monocytogenes*, however the primary route of *Listeria* contamination of dairy products results from environmental contamination during processing (Donnelly, 2018). Various reports detected its presence in milk and cheese (Akkaya and Alişarlı, 2006). In addition to this, traditional cheeses are at risk of containing pathogenic bacteria due to post-processing contamination that can occur during manufacture, as well as during ripening and washing, or at retail (Donnelly, 2018). The incidence of high *E. coli* and *S. aureus* count in food is considered as indicator for poor hygienic condition in production process (Obrien et al., 2009; Samouris et al., 2011; Esho et al., 2013).

The result of this study showed that 65% and 100% of cheese samples contains *E. coli* and coagulase positive staphylococci, respectively. The presence of *E. coli* and coagulase positive staphylococci showed that microbiological quality of traditional white cheese samples was generally poor because of inadequate hygienic conditions during production (Hayaloğlu et al., 2002; Ozer et al., 2004; Telli et al., 2012; Belli et al., 2013). In general while the presence of *S. aureus*, *L. monocytogenes* and *Salmonella* spp. in cheese stemmed from raw milk, the presence of *E. coli* associated with inappropriate or hygienic conditions (Torkar et al., 2006; Obrien et al., 2009; Pacheco and Galindo, 2010; Yücel and Anıl, 2011). According to microbiological criteria linked to food safety policy in Turkey, while the presence of *L. monocytogenes* and *Salmonella* spp. are not allowed in cheese, the presence of coagulase positive staphylococci are limited between 2 and 3 log cfu/g (Turkish Food Codex, 2011). Similarly, microbiological criteria prepared by European commission associated with food safety and hygiene referred the same limits with regard to *L. monocytogenes*, *Salmonella* spp. and coagulase positive staphylococci in cheese. Our results about the presence of *L. monocytogenes* and *Salmonella* spp. in cheese samples comply with microbiological criteria. However, the amount of coagulase positive staphylococci isolated from some cheese samples used in this study has exceed the limit values. As seen in *Figure 3*, higher amounts of coagulase positive staphylococci than microbiological criteria were found as 4.69 and 4.68 log cfu/g in samples 12 and 14. Although microbiological criteria for *L. monocytogenes*, *Salmonella* spp. and coagulase positive staphylococci in cheese are described by Turkish Food Safety criteria and European Commission microbiological criteria, *E. coli* are not specified. In case of using unpasteurized raw milk, producers must process products under equivalent temperature condition (63°C for 30 min) to eliminate the risk of pathogenic bacteria. Thus, *E. coli* are not expected to exist due to the heating process in the production of traditional cheese. Additionally, postproduction contamination, poor hygienic practices and equipment are the challenging factors in cheese-related foodborne illness (Esho et al., 2013). As shown in *Table 1*, traditional white cheese in Hatay province contained detectable numbers of coagulase positive staphylococci between 1 and 4.69 log cfu/g and *E. coli* between 1.91 and 5.67 log cfu/g. Similar to our results, according to the previous reports based on Turkish traditional white cheese, *E. coli* and *S. aureus* amounts in white cheese were between 0.60-5.15 log cfu/g and 1.30-1.70 log cfu/g, respectively, whereas *Salmonella*

spp. and *L. monocytogenes* were generally not present from the cheese samples (Hayaloğlu et al., 2002).

The presence of *Staphylococcus aureus*, *Salmonella* spp. and *L. monocytogenes* were mostly found during the cheese making process in small-scale raw milk cheese production. The main reason for such contamination is considered as the use of raw milk (Jorgensen et al., 2005; Kaynar et al., 2005; Jakobsen et al., 2011; Esho et al., 2013; Rola et al., 2016). *E. coli* are one of indicators of fecal contamination during cheese processing. Especially, cheese produced from unpasteurised milk (raw) can contain *E. coli*. Because *E. coli* is seen as a hygiene indicator for raw milk cheese, a manufacturer need to attempt to implement an appropriate HACCP plan. (Samouris et al., 2011; IDF, 2016). *E. coli* and *S. aureus* are the dominant pathogenic microflora in traditional cheese (Ozer et al., 2004). Previous studies were usually referred *E. coli* and *S. aureus* as the main contamination sources of cheeses whereas contamination of cheese by *Salmonella* spp. and *L. monocytogenes* stated as very few or none (Torkar et al., 2006; Obiren et al., 2009; Pacheco and Galindo, 2010). The presence of *L. monocytogenes* and *Salmonella* spp. in traditional cheese samples was highly rare and generally at rate of between 1 and 3% or none (Kara, 1999; Kaynar et al., 2005; Torkar et al., 2006; Keskin et al., 2006; Jakobsen et al., 2011; Telli et al., 2012; Esho et al., 2013). The present results confirmed this literature information.

*S. aureus* is common in milk and milk products and could cause intoxications with its toxins during processing (Ünlütürk, 2003). In international literature information, coagulase positive staphylococci were isolated on farms with small scale production of saw milk cheeses in Poland and their concentrations were ranged from approximately 0.5 to 7.5 log cfu/g (Rola et al., 2016). For example, Jorgensen et al. (2005) detected the presence of *S. aureus* in raw milk cheese with small scale production at levels of 3 log cfu/g. On the other hand, *E. coli* amounts of cheese made with raw milk were ranged from 0.46 to 5.38 log cfu/g (Samouris et al., 2011).

In previous studies based on microbiological quality of white cheese in Turkey, the presence of *S. aureus* and *E. coli* were found about between 40% and 100% and their amounts were varied from 1 and 6 log cfu/g. For instance, Torkar et al. (2006) investigated the occurrence of pathogenic bacteria in cheese produced at small dairy processing plants. Accordingly, all tested samples contained coagulase staphylococci (approximately 4 log cfu/g) and *E. coli* (approximately 6 log cfu/g) whereas *Salmonella* spp. and *L. monocytogenes* were not detected. Ozer et al (2004) reported that at the end of the 90-day storage period (at different brine concentration) in white Urfa cheese samples, *S. aureus* and *E. coli* were respectively found at level of between 3 and 4 log cfu/g and 0 and 3.5 log cfu/g however, *Salmonella* spp. could not detected. As seen, up to 90 days *S. aureus* and *E. coli* protected their viability. Our result also supported the hypothesis that the presence of *E. coli* and coagulase positive staphylococci in traditional cheese produced from raw milk in local market is possible. Additionally, Cokal et al (2012) reported that while contamination levels of *L. monocytogenes*, *E. coli* and *S. aureus* in Turkish traditional cheese samples were found 5%, 43% and 100%, respectively, none of food samples contained *Salmonella* spp. In accordance with this, the highest pathogen contamination arisen from coagulase positive staphylococci and followed *E. coli*. In the present study none of 20 samples had the presence of *L. monocytogenes* and *Salmonella* spp., however it does not mean that *L. monocytogenes* is not present in the whole cheese samples sold in Hatay. If more cheese samples were collected in Hatay, the prediction of pathogen contamination levels could be increased.

Keskin et al. (2006) investigated microbiological quality of the white cheese sold on street markets. The presence of 86% *E. coli* and 66% *S. aureus* were detected and were not present *Salmonella* spp. and *L. monocytogenes*. Başkaya et al. (2006) determined approximately 2 log cfu/g *E. coli* and *S. aureus* in civil cheese (another Turkish traditional cheese). Bilge and Karaboz (2005) detected the presence of *Staphylococcus aureus* (about 2-3 log cfu/) in some white cheese marketed in Izmir. Our results are in accordance with these previous reports.

To improve the food safety in small-scale cheese production, producers should be increasingly aware of the importance of some pre-requisites and process control points. As process control in cheese production, producers could focus on the quality of milk, regular cleaning of equipment and facilities, optimal concentrations of brine and processing factors such as time, pH and temperature. To reveal strengths and weaknesses of small scale cheese production, survey including questions such as personnel, education, dairy premises, utensils, production volume, hygiene barriers, control measures, water supply, pasteurization conditions and procedures for handling starter cultures should be implemented to producers (Rosengren et al., 2012; Damico et al., 2017). For example, by food safety authority of Ireland, a workbook has been produced to assist farmhouse cheesemakers meet their legal obligation to develop and implement a food safety management system based on the principles of HACCP (Hazard Analysis and Critical Control Point), also referred to as HACCP-based procedures. This workbook provides a controlled approach by describing and recording each step during individual operations in cheesemaking, from raw material production/purchasing through to the storage and dispatch of cheese (Food Safety Authority of Ireland, 2019). On this sense, small scale cheese producers need to develop and implement HACCP plan based on their specific productions (Zhao et al., 2003; Hof et al., 2008). HACCP system as one of the most important systems in quality assurance programs is a plant-specific and product-specific quality system. The application of HACCP plan in small-scale cheese plant could ensure the food safety via the identification of microbiological hazards (Nasr et al., 2017).

## Conclusion

The present study concluded that the microbiological quality and hygienic status of traditional cheese analyzed in this study were mainly favorable. Most of traditional cheese samples analyzed in this paper complied with the microbiological criteria notifications on food safety by Turkish government. In Turkey, cheese is automatically produced in industrial plants with large food factories or traditionally on a small scale in local markets. Handmade cheeses with raw milk could have deficiencies from utensils, staff and other unstable processing conditions in terms of hygienic quality. In particular, raw milk constitutes the critical control point in the production process of traditional cheeses. Pathogen risk is inevitable in cheeses manufactured from low microbial quality milk. As a matter of fact such contaminated cheese could result in the outbreaks of foodborne pathogens including *L. monocytogenes*, *Salmonella* spp., *E. coli* and *S. aureus*. Policies on food safety and protection have focused on ensuring food safety in conventional products. On this sense, for the sustainability of traditional cheeses, microbial safety must be ensured with a HACCP or other food safety plan in manufacturing field and producers should focus on the hygienic conditions during milking and cheese processing.



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