A Survey of Raw Milk For Microbiological Quality and Typing of Foodborne Pathogens by MALDI-TOF MS

Tuğba CEBECİ[1]

[1] *Giresun University, Espiye Vocational School, Department of Medical Services and Techniques, Giresun,Turkey*

Abstract : This study was conducted in order to determine microbiological quality of raw cow milk samples sold in public markets and to investigate foodborne pathogens in Giresun. The samples were examined for total mesophilic aerobic bacteria, total psychrotroph aerobic bacteria, coliform, *Enterobacteriaceae*, *Micrococcus/Staphylococcus*, lactic acid bacteria, yeast and mold, *Salmonella* spp., *Escherichia coli* O157:H7, *L. monocytogenes*, *Bacillus cereus*, *Campylobacter* spp. according to Food and Drug Administration/Bacteriological Analytical Manual and International Organization for Standardization methods. Identification of the isolated pathogens was made with Matrix-assisted laser desorption, ionization time of flight mass spectrometry. The mean values of microorganism colonies detected in raw cow milk samples were as follows: total mesophilic aerobic bacteria count 5.87 log cfu/ml in 93 samples; total psychrotroph aerobic bacteria count 5.69 log cfu/ml in 95 samples; coliform count 4.85 log cfu/ml in 12 samples; *Enterobacteriaceae* count 4.84 log cfu/ml in 18 samples; *Micrococcus/Staphylococcus* count 5.17 log cfu/ml in 94 samples; lactic acid bacteria count 5.54 log cfu/ml in 97 samples, and yeast and mold 5.16 log cfu/ml in 73 samples. *Listeria monocytogenes*, *Escherichia coli* were found as 6 (6%) and 34 (34%) in 100 raw cow milk samples respectively. *Salmonella* spp., *Campylobacter* spp., *Escherichia coli* O157:H7 and *Bacillus cereus* were not detected. As a result; a statistically significant presence of bacteria threatening human health was detected in the raw cow milk samples examined. In order to resolve this problem, it is thought that further similar studies should be conducted on the basis of procuders, consumers and supervisory institutions.

Keywords: milk, microbiological quality, food pathogens, MALDI-TOF MS

Çiğ Süt Örneklerinin Mikrobiyolojik Kalitesi ve Gıda Patojenlerinin MALTI-TOF MS ile Tiplendirilmesi Üzerine Bir Araştırma

Öz: *Bu çalışma, Giresun'da pazarlarda satılan çiğ inek sütü örneklerinin mikrobiyolojik kalitesini belirlemek ve gıda patojenlerini araştırmak amacıyla gerçekleştirilmiştir. Örnekler, toplam mezofilik aerobik bakteri, toplam psikrotrof aerobik bakteri, koliform grubu bakteri, Enterobacteriaceae, Micrococcus/Staphylococcus, laktik asit bakterileri, maya ve küf, Salmonella spp., Escherichia coli O157:H7, Listeria monocytogenes, Bacillus cereus, Campylobacter spp. yönünden Food and Drug Administration/Bacteriological Analytical Manual ve International Organization for Standardization yöntemlerine göre incelenmiştir. İzole edilen patojenlerin identifikasyonları, Matriks aracılı lazer dezorpsiyon, iyonizasyon uçuş zamanı kütle spektrometresi ile yapılmıştır. Çiğ inek sütü örneklerinde belirlenen mikroorganizma kolonilerinin ortalama değerleri sırasıyla; toplam mezofilik aerobik bakteri sayısı 93 örnekte 5.87 log kob/ml; toplam psikrotrof aerobik bakteri sayısı 95 örnekte 5.69 log kob/ml; koliform sayısı 12 örnekte 4.85 log kob/ml; Enterobacteriaceae sayısı 18 örnekte 4.84 log kob/ml; Micrococcus/Staphylococcus sayısı 94 örnekte 5.17 log kob/ml; laktik asit bakteri sayısı, 97 örnekte 5.54 log kob/ml ve maya-küf sayısı 73 örnekte 5.16 log kob/ml olarak bulunmuştur. Listeria monocytogenes ve Escherichia coli, 100 çiğ inek sütü örneğinde sırasıyla 6 (%6) ve 34 (%34) olarak bulunmuştur. Salmonella spp., Campylobacter spp., Escherichia coli O157: H7 ve Bacillus cereus saptanmamıştır. Sonuç olarak; araştırmada incelenen çiğ inek sütü örneklerinde, istatistiki olarak önemli düzeyde, insan sağlığını tehdit eden bakterilerin varlığı tespit edilmiştir. Bu durumun düzeltilmesi için, üretici, tüketici ve denetleyici kurumlar bazında benzer daha çok sayıda çalışmaların yapılarak kamuoyuna duyurulması gerektiği düşünülmektedir.*

Anahtar Kelimeler: *süt, mikrobiyolojik kalite, gıda patojenleri, MALDI-TOF MS*

INTRODUCTION

Milk is an excellent culture medium for growth of many microorganisms due to its complex biochemical composition and high water activity. Therefore microbial content of milk is important in determination of its quality (Reta and Addis, 2015; O'Sullivan and Cotter, 2017).

The microorganisms that may be found in milk are positively or negatively affected by heat. The microorganisms found in raw milk may be beneficial or harmful for milk, and may harm human health (O'Sullivan and Cotter, 2017; Karmen and Slavica, 2008). Number and types of microorganisms found in the milk immediately after milking are influenced by several factors such as animal and equipment cleaning, season, feed and animal health. On the other hand, differences in feeding of cows and shelter strategies, milking conditions may affect presence of microorganisms that may be found in milk (Reta and Addis, 2015; Griffiths, 2010).

Sorumlu Yazar: *tgbcbcdmn@gmail.com* *Bu araştırma Giresun Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından desteklenmiştir.Proje No: FEN-BAP-A-170417-114.*

Geliş Tarihi: *11 Haziran 2019*

Kabul Tarihi: *21 Kasım 2019*

Many pathogens may be a source of infection transmission to human because of infected raw milk and products. As a result of the transmission, patients may be asymptomatic or symptomatic carriers for these pathogens. Pathogens found in raw milk and products may cause neurological damage, arthritis and renal failure (Kul et.al, 2006).

In the literature screening, there was no study about bacteria and fungi that may be found in raw milk in Giresun region. In addition, no data could be found about at what rate these pathogens may be found in human. The objective of this study was to determine microbiological quality of raw cow milk samples that were presented for sale in public open markets of districts within Giresun province, and to investigate presence of food pathogens in these samples. By this way, pathogens that may be a source of infection within the province would be determined and recommendations would be made regarding the necessary protection measures.

MATERIALS AND METHODS

Materials

In this study, 100 raw cow milk samples were collected from the public markets of Tirebolu, Keşap, Eynesil, Güce, Yağlıdere, Piraziz, Bulancak, Görele and Espiye districts of Giresun province between 2016 and 2017. Distribution of the samples collected from the districts is given in Table 1. Sample size was determined with random sampling method considering sales rates.

The samples were brought to the laboratory, paying attention to cold chain and analyzed in the same day. The samples were examined according to the Food and Drug Administration/Bacteriological Analytical Manual (FDA/BAM) and International Organization for Standardization (ISO) methods.

Table 1. Distribution of the raw milk samples studied according to districts

Sample type	District of collection	Number of studied samples (n)	Raw milk seller (n)
Raw cow milk	Bulancak	20	36
	Espiye	15	30
	Tirebolu	10	29
	Görele	12	27
	Eynesil	11	26
	Yağlıdere	11	23
	Keşap	10	18
	Piraziz	7	16
	Güce	4	11
	Total	100	216

Preparation of the samples for the analysis

In the study, buffered peptone water, modified tryptone soya broth with novobiocin (Merck 109205), listeria enrichment broth (LabM139) and campylobacter enrichment broth (LabM135) were separately added as 225 mL into sterile glass bottles. 25 mL milk was added to each bottle and homogenized for 2 minutes in the homogenizer.

Milk samples were incubated for 24 hours in modified tryptone soya broth with novobiocin at 41.5°C, listeria enrichment broth at 37°C and campylobacter enrichment broth at 41°C to provide pre-enrichment of microorganisms. Following the incubation, decimal dilutions were prepared from 10^1 to 10^5. 0.1 mL was taken from each dilution with drigalski spatula and cultured on the medium with surface plate method. Two medium was cultured from each dilution and a parallel work was done. All colonies grown in the cultured medium were counted and the living microorganisms were evaluated. The used media and conditions according to the sought microorganisms are given in Table 2.

The isolation of pathogen microorganisms after pre-enrichment on media is given in Table 3.

Identification of the isolates

Pre-definition of the obtained suspected isolates was performed, the isolates were cultured on Blood Agar Base, incubated at 37°C for 24 hours, among the suspected colonies grown in the medium, one or two colonies were collected with sterile stapula tip and spreaded to the wells on the slides of the device. Following this process, 1 µl matrix solution (cyano-4-hydroxycinnamic acid saturated in 50% acetonitrile and 2.5% trifluoroacetic acid) (VITEK MS-CHCA, bioMérieux, Inc.) was pipetted into the wells and kept for 1-2 minutes under room conditions. The slide was then inserted to the cassette, loaded to VITEK MALDI-TOF MS, and typing was performed (Rychert et al., 2013).

Statistical analysis

Analyses were conducted using the SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software. The data were tested for normality using the Ryan-Joiner Test and for homogeneity of variance using the Levene's Test prior to the analyses. One-way ANOVA or Kruskal-Wallis test followed by Tukey's or Dunn post-hoc test was used to compare the means of more than two independent groups.

RESULTS AND DISCUSSION

Milk functions as a reservoir for microorganisms. The microorganisms in raw milk may be beneficial or harmful for itself. Milk containing pathogenic microorganisms may harm human health when consumed (McSweeney et al., 2017).

Table 2. Media used in the analysis of microorganisms and incubation conditions

Microorganism group	Medium	Incubation			Source of counting method
		Temperature	Time	Conditions	
TMAB	Plate Count Agar (LabM149)	37±1°C	72 hours	Aerob	ISO 4833-2
TPAB	Plate Count Agar (LabM149)	4±1°C'	10 days	Aerob	FDA,2002; Harrigan and Mccance,1976
Coliform bacteria	Violet Red Bile Agar (LabM031)	35±1 °C	48 hours	Aerob	FDA,2002; APHA, 2001; ISO 4832-2006
Ent.	Violet Red Bile Glucose Agar (LabM088)	35±1° C	48 hours	Aerob	ISO 21528-2
Mic./Staph.	Baird–Parker agar (LabM085) egg yolk tellürit (0.5% w/v) ilaveli	37°C	24-48 hours	Aerob	Halkman,2005
LAB	Man Rogosa Sharpe Agar (LabM093)	30°C	48 hours	Anaerob	APHA, 1995; ISO/FDIS 15214
YM	Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (LabM217)	22±1°C	5 days	Aerob	ISO 21527-1

TMAB, Total mesophilic aerobic bacteria; TPAB, Total psychrotroph aerobic bacteria; Ent, *Enterobacteriaceae*; Mic./Staph., *Micrococcus* / *staphylococcus*; LAB, Lactic acid bacteria; YM, Yeast and mold

Table 3. Media used in the analysis of pathogen microorganisms and incubation conditions

Microorganism group	Medium	Incubation			References
		Temperature	Time	Conditions	
Salmonella spp.	Xylose Lysine Deoxycholate Agar (LabM032)	37±1°C	24 hours	Aerob	ISO 6579-1:2017
E. coli O157:H7	Sorbitol MacConkey agar (LabM161)	37°C'	16-24 hours	Aerob	De Boer and Heuvelink, 2000
L. monocytogenes	Oxford Agar (LabM122), Listeria Chromogenic Agar (LabMHal010)	37 °C	24-48 hours	Aerob	ISO 11290-2: 2017
Campylobacter spp.	Campylobacter Agar (LabM112)	41,5° C	48 hours	microaerophilic	ISO 10272-1:2017
B. cereus	Bacillus Cereus Medium (LabM073)	37°C	24-48 hours	Aerob	ISO 7932: 2009

E., *Escherichia*; L., *Listeria*; B., *Bacillus*

In the study, a total 100 raw cow milk samples randomly collected from 207 raw milk sellers as minimum 4 and maximum 20 samples in 9 district public markets within Giresun province were analyzed. Total mesophilic aerobic bacteria (TMAB), total psychrotroph aerobic bacteria (TPAB), coliform group bacteria, *Enterobacteriaceae*, *Micrococcus/Staphylococcus (Mic./Staph.)*, lactic acid bacteria, yeast and mold that are used in determination of microbiological quality criteria were counted in the analyzed raw cow milk samples.

In this study, The mean count of total mesophilic aerobic microorganisms was found as $7.5x10^5$ cfu/ml (5.87 log cfu/ml) in 93 samples. This value was found to be lower than the mean values (6.36-10.41 cfu/ml) obtained in different studies on raw milk. (Belbachir et al., 2015; Mesfine et al., 2015; Ibrahim et al., 2015, Göncü et al., 2017; Wanjala et al., 2017). However, it was higher than the other studies (3.17-4.57 cfu/ml) (Cempírková, 2007; Welearegay et al., 2012; Bogdanovičová et al., 2016). The results obtained were found to be similar with some studies (5-5.74 cfu/ml) (Önal and Özder, 2007; El-Diasty and El-Kaseh, 2009; Kesenkaş and Akbulut, 2010; Diler and Baran, 2014). Different results among the studies might be resulted from the study regions, methods, and environmental factors. According to the Turkish Food Codex "Raw Milk and Heat-Treated Drinking Milks Notification" no: 2009/14, it has been stated that the total bacteria count in raw cow milk should be < 100,000 cfu/mL (Anonim, 2009). In this study 93 samples were found to be higher than this value. This may be interpreted as that the samples were contaminated during or after milking. In addition, the difference between the mean values of mesophilic aerobic microorganisms was found to be statistically significant according to the variance analysis ($p<0.01$). This may be explained by higher contamination in this district compared to the other districts. It was thought that organizations should be informed about the importance of pureness of the milk.

Since some species of the general psychrotrophic bacteria that cause spoilage of milk and dairy products have ability

to produce toxins or resist against antibiotics, these species can be considered as opportunistic pathogens (Akan et al., 2014). In our study, the mean count of total psychrotroph aerobic bacteria was found as $4.9x10^5$ cfu/ml (5.69 log cfu/ml) in raw cow milk samples. This value is similar with the study by Raj et al. However, it was found to be higher than different studies (Chye et al., 2004; Cempírková 2007; Torkar and Teger, 2008). This might be resulted from that the samples collected from the district of Giresun may be source of opportunistic pathogens. According to One-way ANOVA analysis, no statistically significant difference was found between the districts in terms of the amount of total psychrotroph aerobic bacteria ($p>0.05$).

It has been reported that the presence of coliform bacteria in food may be harmful for human health (Kesenkaş and Akbulut, 2010). In this study, the mean count of coliform bacteria in raw cow milk samples was found as $7.2x10^4$ cfu/ml (4.85 log cfu/ml). This value was found to be lower compared to some studies (Ibtisam et al., 2007; El-Diasty and El- Kaseh, 2009; Uddin et al., 2011; Ibrahim et al., 2015; Tankoano et al., 2016). However, it was higher than the other studies (Kesenkaş and Akbulut, 2010; Welearegay et al., 2012; Belbachir et al., 2015; Mesfine et al., 2015). There is studies reporting similar results (Raj et al., 2010; Göncü et al., 2017; Wanjala et al., 2017). According to Kruskal-Wallis test, no statistically significant difference was found among the districts in terms of the amount of coliform bacteria (log cfu/ml) ($p>0.05$).

Enterobacteriaceae is accepted as an indicator of hygiene conditions in milk production (Bogdanovičová et al., 2016) The mean count of *Enterobacteriaceae* in raw cow milk samples was found as $6.9x10^5$ cfu/ml (4.84 log cfu/ml). This value was found to be lower compared to some studies (Ibtisam et al., 2007; Bogdanovičová et al., 2016; El-Diasty and El- Kaseh, 2009; Wanjala et al., 2017). However, this result was similar with the study by Taşçı (2011). This might be interpreted as that milking is still not performed under hygiene conditions. There was no statistically significant difference among the samples in terms of the amount of *Enterobacteriaceae* ($p>0.05$).

The mean count of mic/staph was found as $1.6x10^5$ cfu/ml (5.17 log cfu/ml). The value obtained was found to be higher than the value found in a study by Taşçı (2011) on raw cow milk (4.38 log cfu/ml). According to the One-way ANOVA analysis, there was no statistically significant difference between the samples in terms of the amount of mic/staph. ($p>0.05$).

Acid medium formed owing to the organic acids produced by lactic acid bacteria largely inhibit the development of pathogen microorganisms. The mean count of lactic acid bacteria in the raw cow milk samples was found as $3.5x10^5$ cfu/ml (5.54 log cfu/ml). This value was found to be lower than the value found by Tankoano et al. (8.00 log cfu/ml) (2016). According to One-way ANOVA analysis, there was a statistically significant difference was found among the districts ($p<0.01$). According to these data, it was thought that the pathogens that can be transmitted to people in the region of the study might be decreased. However, controlled experiments should be conducted in order to prove this hypothesis.

Yeast and mold may impair or decrease shelf life of milk, and may pose serious health problems for consumers (Adugna and Asresie, 2015) The mean count of yiest-mold in the raw cow milk samples was found as $1.4x10^5$ cfu/ml (5.16 log cfu/ml). This value were found to be higher than the values found in the studies conducted on raw milk. (Torkar and Teger, 2008; Kesenkaş and Akbulut, 2010; Welearegay et al., 2012; Ibrahim et al., 2015; Tankoano et al., 2016; Göncü et al., 2017). However, this value was found to be at the same level with mean $4.3×10^5$ cfu/ml, which was found by El-Diasty and El- Kaseh (2009). The amount of yiest and mold of the districts were compared with One-way ANOVA, and the difference among the districts was statistically significant ($p<0.001$). This may be explained by that infectivity of milk may differ according to the collection conditions in the districts.

Descriptive statistical values and statistical comparison for the amount of total mesophilic aerobic (log cfu/ml), total psychrotroph aerobic (log cfu / ml), coliform (log cfu/ml), *Enterobacteriaceae* (log cfu/ml), mic/staph. (log cfu/ml), lactic acid bacteria (log cfu/ml), yeast and mold (log cfu/ml) that were measured in the milk samples collected from the districts of Giresun province are given in Table 5.

The most common pathogens detected in milk-borne diseases are *Salmonella spp*., *Staphylococcus aureus* and *Escherichia (E.) coli* (Fadaei, 2014). Food poisoning, typhoid fever or paratyphoid fever may be seen by consuming *Salmonella* contaminated food (Bhan et al., 2005). *L. monocytogenes* is responsible for listeriosis, which is a serious food-borne disease. Significant number of human listeriosis cases occur due to foodborne transmission of *L. monocytogenes* (Rahman et al., 2008). *E. coli* bacteria are accepted as an important indicator of hygiene during production, storage, transport and sale processes of raw milk. *E. coli* is widely found in the intestinal flora of humans and warm-blooded animals, but it may become a pathogenic organism (Costa et al., 2009) In the present study, *L. monocytogenes* was detected in 6 (6%) and *E.coli* in 34 of 100 raw cow milk samples. *Salmonella spp*., *Campylobacter spp.* and *E. coli* O157:H7 were not detected in any sample. According to the Turkish Food Codex "Raw

Table 5. Descriptive statistics and comparison results for the study variables of milk samples

		TMAB		TPAB		C		*Ent.*		*Mic./Staph.*		LAB		YM	
D	n	M	S	M	S	Md	IQR	Md	IQR	M	S	M	S	M	S
Bl	20	4.779c	0.367	4.691	0.364	0.00	0.00	0.00	3.95	4.278	0.365	4.410b	0.360	1.410b	0.411
Es	15	5.361abc	0.013	5.361	0.029	0.00	0.00	0.00	0.00	4.637	0.356	5.191ab	0.081	3.057ab	0.512
Ey	11	5.252bc	0.045	5.290	0.088	0.00	0.00	0.00	0.00	3.776	0.625	5.149ab	0.132	4.644a	0.490
Gö	12	5.198bc	0.103	4.699	0.434	0.00	0.00	0.00	0.00	4.166	0.194	4.563b	0.430	2.342ab	0.604
Gü	4	5.410abc	0.029	5.395	0.040	0.00	4.10	0.00	4.12	4.575	0.073	4.960ab	0.099	3.610ab	1.210
Kş	10	5.774ab	0.131	4.814	0.536	0.00	0.00	0.00	0.00	5.250	0.239	5.558ab	0.169	3.180ab	0.487
Pir	7	5.266abc	0.090	5.230	0.046	0.00	5.14	0.00	5.1	3.814	0.661	4.500b	0.412	4.414a	0.349
Tir	10	6.386a	0.136	5.191	0.635	5.43	5.90	2.57	5.567	5.247	0.260	6.114a	0.083	4.675a	0.366
Ya	11	5.265bc	0.111	5.439	0.039	0.00	0.00	0.00	0.00	4.658	0.476	5.192ab	0.126	3.849a	0.749
		0.001**		0.641		0.287		0.346		0.181		0.001**		0.000***	
P-Value		(F=3.85)		(F=0.76)		(H=9.69)		(H=8.96)		(F=1.46)		(F=3.54)		(F=4.87)	

M, Mean; S, Standard error of mean; Md, Median; IQR, Interquartile range; F, One-way ANOVA; H, Kruskal-Wallis test, D, District; Bl, Bulancak; Es, Espiye; Ey, Eynesil; Gö, Görele; Gü, Güce; Kş, Keşap; Pir, Piraziz; Tir, Tirebolu; Ya, Yağlıdere; C, Coliform

, Statistically significant (p<0.01); *, Statistically significant (p<0.001)

According to Tukey test, means that do not share a letter are significantly different (p<0.05)

Milk and Heat-Treated Drinking Milks Notification", it was stated that *Salmonella spp.* should not be found in 25 mL of raw milk. Similarly in the present study, *Salmonella spp.* was not found in any raw milk sample. According to the literature; Tadesse and Solomon (2003) isolated *E.coli* by 3.3% in Jimma district of Ethiopia, Chye et al. (2004) by 64.5% in Malaysia, Zeinhom and Abdel-Latef (2014) by 16% in Egypt, and Ibrahim et al. (2015) by 80% in Cairo. In our study, *E.coli* was isolated by 34%. Different results among the studies might be resulted from the study method and sample size. *L. monocytogenes* was isolated by 6%. In similar studies, Chye et al. (2004) isolated *L. monocytogenes* by 1.9% in Malaysia, Belbachir et al. (2014) by 3% in Morocco, Seyoum et al. (2015) by 2.04%, Bogdanovičová et al. (2016) by 0.6% in Czech Republic, and Şanlıbaba and Tezel (2018) by 2% in çanakkale province. These results could be interpreted by that pathogen bacteria may be found in raw milk and products, and are important for human health.

In the study, *Salmonella spp*., *Campylobacter spp.* and *E. coli* O157:H7 were not detected. In different studies; *Salmonella spp* was not detected by Ibtisam et al. (2007) in Hartum state, and by Belbachir et al. (2015) in Morocco, *E. coli* O157: H7 and *Salmonella spp.* were not isolated by Zeinhom and Abdel-Latef (2014) in Egypt, *Salmonella spp.* and *Bacillus cereus* were not isolated by Ibrahim et al. (2015) in Cairo, and *Salmonella spp*. and *Campylobacter spp*. were not detected by Bogdanovičová et al. (2016) in Czech Republic. These results are similar to the present study. However, Chye et al. (2004) isolated *Salmonella spp*., by 1.4% in Malaysia. This difference might be resulted from the study region or the study method.

CONCLUSION

In conclusion; although *Salmonella* spp*., Campylobacter* spp*., E. coli* O157:H7 were not detected in 100 raw cow milk samples that were selected among the samples sold in public markets of 9 districts in Giresun province and were analyzed for microrganism levels, these levels were higher than the standards specified in the Turkish Food Codex Microbiological Criteria Regulation and Turkish Food Codex Raw Milk and Heat-Treated Drinking Milk Comminique in terms of mesophilic aerobic microorganisms, coliforms, *E.coli* and *L. monocytogenes*. This puts public health at risk in terms of the consumers in the study region. Determination of whether microorganisms at risk group detected in the raw milk samples are caused before, during and after milking or by marketing conditions will determine the level of impact of the raw cow milk sold under the conditions of the region on health. Organizations that control effects of the consumption of animal products as food in the regional conditions on public health (Provincial Directorates of Agriculture, Municipalities, Non-Governmental Organizations supervising Food Health, etc.) will accelerate the elimination of risk factors through similar studies.

REFERENCES

Adugna M, Asresie AA (2015) Review on microbiological quality of Ethiopian raw bovine milk. Food Sci. Qual. Man. 35:17–24.

Akan E, Yerlikaya O, Kınık Ö (2014) Psikrotrof Bakterilerin Çiğ Süt ve Süt Ürünleri Kalitesine Etkisi. Akademik Gıda 12(4): 68-78.

Anonymous (2009), Turkish Food Codex, Raw Milk and Heat Treated Drinking Milk Communiqué. Communiqué no 2009/14.

American Public Health Association (1995), Standarts Methods for the Examination of Dairy Products. American Public Health Association: New York.

American Public Health Association, APHA (2001) Compendium of Methods for the Microbiological Examination of Foods, American Public Health Assoc.: Washington, D.C.

Belbachir C, Khamri M, Saalaoui E (2015) Microbiological quality of the raw cow milk at three rural communes of the eastern region of Morocco. Inter. Food Rese. J. l22 (4): 1675-1680.

Bhan MK, Bahl R, Bhatnagar S. (2005) Typhoid and paratyphoid fever. Lancet 366: 749-762.

Bogdanovičova K, Vyletělova-Klimešova M, Babak V, Kalhotka L, Kolačkova I, Karpiškova R (2016) Microbiological quality of raw milk in the Czech Republic. Czech J. Food Sci. 34:1–8.

Cempíriková R (2007) Contamination of cow's milk by psychrotrophic and mesophilic microflora in relation to selected factor. Czech Journal of Animal Science 52:378-393.

Chye FYF, Abdullah A, Ayob MK (2004) Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol 21:535-541.

Costa D, Vinue L, Poeta P, Coelho AC, Matos M, Saenz Y, Somalo S, Zarazaga M, Rodrigues J, Torres C (2009) Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers. Veterinary Microbiology, 138: 339–344.

De Boer E, Heuvelink AE (2000) Methods for detection and isolation of shiga toxin producing *Escherichia coli*. J Appl Microbiol 88: 133-43.

Diler A, Baran A (2014) Determination Of Some Quality Characteristics of Raw Cow Milk on Smallholder Dairy Farms in Hinis District of. Alınteri 26(B) :18-24.

El-Diasty Eman M, El-Kaseh RM (20099 Microbiological monitoring of raw milk and yoghurt samples collected from El-Beida city. Arab J. Biotech. 12(1): 57-64.

FDA (2002) Food and Drug Administration, *Bacteriological Analytical Manual*. 9th Ed., AOAC International: Arlington, VA, USA.

Fadaei A (2014) Bacteriological quality of raw cow milk in Shahrekord, Iran. Veterinary World 7 (4): 240-243.

Griffiths MW (2010) The microbiological safety of raw milk. In Griffiths MW (eds.), Improving the Safety and Quality of Milk.Woodhead Publishing: Cambridge, UK, 27-63.

Göncü B, Çelikel A, Akın MB, Akın MS (2017) A Research on the Determination of Chemical and Microbiological Properties of Street Milk Sold in Şanlıurfa. HU J. of Eng. 02:15-23.

Halkman K (2005) Gıda mikrobiyolojisi uygulamaları. Başak Matbaacılık Ltd. Şti: Ankara, Turkey.

Harrigan WF, Mccance ME (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press Inc. Ltd., London.

Ibrahim GA, Sharaf OM, Abd El-Khalek AB (2015) Microbiological quality of commercial raw milk, domiati cheese and kareish cheese. Middle East Journal of Applied Sciences 5(1): 171–176.

Ibtisam E, El Zubeir M, Ahmed Mahbora IA (2007) The hygienic quality of raw milk produced by some dairy farms in Khartoum State. Sudan Research Journal of Microbiology 2:988–991.

ISO/FDIS 15214 (1998) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria-Colony count technique at 30 _C. International Organization for Standardization: Genova, Switzerland.

ISO 4832 (2006) Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms -- Colony-count technique, International Organization for Standardization: Genova, Switzerland, 2006; pp. 1-6.

ISO 21527-1 (2008) Microbiology of the food and animal feeding stuffs - Horizontal method for the detection and enumeration of yeasts and moulds - Part 1: Colony-count technique in products with water activity greater than 0,95, International Organization for Standardization: Genova, Switzerland.

ISO 7932 (2009) Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of presumptive Bacillus cereus -- Colony-count technique at 30 degrees C. International Organization for Standardization: Genova, Switzerland.

ISO 4833-2 (2013) Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 degrees C by the surface plating technique. International Organization for Standardization: Genova, Switzerland.

ISO 21528-2 (2017) Microbiology of the food chain -- Horizontal method for the detection and enumeration of Enterobacteriaceae -- Part 2: Colony-count technique. International Organization for Standardization: Genova, Switzerland.

ISO 6579-1 (2017) Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp. International Organization for Standardization: Genova, Switzerland.

ISO 11290-2 (2017) Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 2: Enumeration method. International Organization for Standardization: Genova, Switzerland.

ISO 10272-1 (2017) Microbiology of the food chain -- Horizontal method for the detection, enumeration of Campylobacter spp. -- Part 1: Detection method. International Organization for Standardization: Genova, Switzerland.

Karmen GT, Slavica GT (2008) The Microbiological Quality of Raw Milk after introducing the two Day's milk collecting system. Acta agriculturae Slovenica 92(1): 61-74.

Kesenkaş H, Akbulut N (2010) Determination of milk quality produced from middle and large scale dairy farms and informal/street milk quality sold in izmir. Ege Üniv Ziraat Fak Derg 47 (2):161-169.

Kul E, Erdem H, Atasever S (2006) Effect of Different Udder Traits on Mastitis and Somatic Cell Count Dairy Cows. J. of Fac. of Agric.OMU 21(3): 350-356.

McSweeney, P.L.H.; Fox, P.F.; Cotter, P.D.; Everett, D.W. Cheese, Fourth edition; Elsevier Academic Press: London, United Kingdom, 2017; 1-1302.

Mesfine S, Feyera T, Mohammed O (2015) Microbiological Quality of Raw Cow's Milk from Four Dairy Farms in Dire Dawa City, Eastern Ethiopia. World J. Dairy Food Sci. 10(1): 09-14.

O'Sullivan O, Cotter PD (2017) Microbiota of Raw Milk and Raw Milk Cheeses. In McSweeney PLH, Fox PF, Cotter PD, Everett DW (eds.), *Cheese*, Elsevier Academic Press, London, United Kingdom, 301-316.

Önal AR, Özder M (2007) Trakya'da Özel Bir Süt İşleme Tesisi Tarafından Değerlendirilen Çiğ Sütlerin Somatik Hücre Sayısı ve Bazı Bileşenlerinin Tespiti. Tekirdağ Ziraat Fak. Derg. 4 (2): 195-199.

Rahman MM, Mashiar Rahman M, Arafat SM, Rahman A, Khan MZH, Rahman MS (2008) Microbiological Quality Assessment of a Local Milk Product, Kwacha Golla, of Bangladesh. J.Korean Soc.Appl.Biol.Chem. 51 (4):251-257.

Raj JM, Kumar A, Bhattacharya A, Rekha VBA (2010) Study on Microbial Quality of Raw and Pasteurized Milk Sold in Puducherry, India. J. Vet. Pub. Hlth. 8(2):121-125.

Reta AM, Addis AH (2015) Microbiological quality assessment of raw and pasteurized milk. Int. J. Food Sci. Microbiol. 2(6):87-91.

Rychert J, Burnham CA, Bythrow M, Garner OB, Ginocchio CC, Jennemann R (2013) Multicenter evaluation of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Gram positive aerobic bacteria. J Clin Microbiol. 51: 2225–31.

Seyoum ET, Woldetsadik DA, Mekonen TK, Gezahegn HA, Gebreyes WA (2015) Prevalence of Listeria monocytogenes in raw bovine milk and milk products from central highlands of Ethiopia. J. Infect Dev. Ctries. 9: 1204-1209.

Şanlıbaba P, Tezel BU (2018) Prevalence and Characterization of Listeria Species from Raw Milk and Dairy Products from Çanakkale Province. Turkish Journal of Agriculture - Food Science and Technology 6(1): 61-64.

Tadesse G, Solomon GS (2003) Assessment of the bacteriological quality of milk at dairy farms and individual breeders in Jimma town, South western Ethiopia. Ethiopian Journal of Health Science 13(1): 21-29.

Tankoano A, Kabore D, Savadogo A, Soma A, Fogny NF, Sereme DC, Hounhouigan JD, Lingani HS, (2016) Evaluation of microbiological quality of raw milk, sour milk and artisanal yoghurt from Ouagadougou, Burkina Faso. Afr J Microbiol Res. 10(16):535–541.

Tasci F (2011) Microbiological and chemical properties of raw milk consumed in Burdur. J. Vet. Adva. 10:635-641.

Torkar KG, Teger SG (2008) The microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agri Slovenica. 92(1):61–74.

Uddin MA, Motazzim-ul-Haque HM, Noor R (2011) Isolation and Identification of Pathogenic *Escherichia coli, Klebsiella* spp. and *Staphylococcus* spp. in Raw Milk Samples Collected from Different Areas of Dhaka City. Bangladesh Md. Stamford Journal of Microbiology 1(1): 19- 23.

Wanjala GW, Mathooko FM, Kutima PM, Mathara JM Microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi region. African Journal of Food, Agriculture, Nutrition and Developement 17 (1): 11518-11532.

Welearegay H, Yilma Z, Tekle-Giorgis Y (2012) Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, southern Ethiopia. Agricultural Research and Reviews 1(4):132-142.

Zeinhom MMA, Abdel-Latef GK (2014) Public health risk of some milk borne pathogens. BeniSuef University Journal of Basic and Applied Sciences 3(3): 209–215.