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DETERMINATION OF CERTAIN MICROBIOLOGICAL PARAMETERS ON CHEESE SAMPLES COLLECTED FROM SUPERMARKETS IN ERZURUM

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ABSTRACT
This study aimed to evaluate 50 different cheese samples in terms of certain microbiological parameters. The presence of *Salmonella* spp. and *Listeria* spp. in cheeses were investigated by immunomagnetic separation and conventional methods. *Staphylococcus aureus* was detected in 4 samples, while 2 samples were accepted suspicious of *Salmonella* spp. and 5/50 samples were found positive with respect to *Listeria* spp. The suspicious *Salmonella* spp. isolates were determined as *Proteus mirabilis*. Therefore, *Salmonella* spp. were not determined from the samples. The isolates of *Listeria* spp. were identified as *Listeria monocytogenes* (2/5), *Listeria grayi* (3/5). According to antibiotic test results, *Staphylococcus aureus* isolates showed susceptibility to tetracycline, trimethoprim, gentamicin, chloramphenicol, sulphamethoxazole/trimethoprim, ciprofloxacin at the level of 100%. Additionally, the *Listeria* spp. isolates displayed high susceptibility to chloramphenicol, ciprofloxacin, and ampicillin.
Keywords: Cheese; *Salmonella*; *Listeria*; Immunomagnetic separation; Antibiotic resistance

ERZURUM'DA SÜPERMARETLERDEN TOPLANAN PEYNİR ÖRNEKLERİNDE BELİRLİ MİKROBİYOLOJİK PARAMETRELERİN BELİRLENMESİ

ÖZ
Bu çalışmanın amacı 50 farklı peynir örneğini belirli mikrobiyolojik parametreler açısından değerlendirmektir. Peynirlerde *Salmonella* spp. ve *Listeria* spp. varlığı immunomanyetik separasyon ve geleneksel metotlarla araştırılmıştır. 50 örneğin 5'i *Listeria* spp. açısından pozitif bulunurken, 4'ü *Staphylococcus aureus,* 2'si şüpheli kabul edilen *Salmonella* spp. olarak belirlendi. Şüpheli *Salmonella* spp. izolatları identifikasyon aşamasında *Proteus mirabilis* olarak tanımlandığından örneklerde *Salmonella* spp. tespit edilmemiştir. *Listeria* spp. izolatları *Listeria monocytogenes* (2/5), *Listeria grayi* (3/5) olarak tanımlandı. Antibiyotik test sonuçlarına göre, *Staphylococcus aureus* izolatları tetrasiklin, trimetoprim, gentamisin, kloramfenikol, sulfametoksazol/trimetoprim, siprofloksasin antibiyotiklerine %100 seviyesinde duyarlılık gösterdi. Ayrıca *Listeria* spp. izolatları kloramfenikol, siprofloksasin ve ampisilin antibiyotiklerine karşı yüksek duyarlılık sergilemiştir.
Anahtar kelimeler: Peynir; *Salmonella*; *Listeria*; immünomanyetik separasyon; antiyotik direnç

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INTRODUCTION

Cheeses are widely consumed by the majority of people because of their high nutritional value and unique taste and flavor. More than 1000 types of cheeses are produced and consumed around the World, while approximately, 40-50 types of cheese are known in Turkey (Hayaloglu et al., 2002).

The pasteurized dairy products are considered as safe products for consumption, but pathogenic bacteria contaminating the dairy products after pasteurization can be dangerous for human health. Generally, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* have been reported as important pathogenic microorganisms that cause food-borne diseases (Mccabe-Sellers and Beattie, 2004). Generally, the sources of contamination were caused by raw milk, inadequate pasteurization of milk, post-pasteurization contamination of milk, inadequate milk handling procedures, and manufacturing process. Also, inadequate personal hygiene, ingredients, contaminated waters, post-production factors, poor sanitation, storage conditions, transportation, and temperature changes during the storage period cause spoilage and food-borne disease (Kanbakan et al., 2004; Reij and Aantraker, 2004).

Salmonella spp. are one of the most important pathogenic microorganisms that capable of causing salmonellosis in humans. *Salmonella* spreads by fecal wastes of the infected animals and humans (Ponce et al., 2008; Abulreesh, 2012).

From among *Listeria* spp., *L. monocytogenes* cause human listeriosis and *L. ivanovii* is mainly pathogenic for animals, primarily sheep. *L. monocytogenes* is a psychrotroph microorganism and it can grow at relatively low pH and refrigeration temperature. The first major source of food contamination by *L. monocytogenes* might stem from the cross-contamination during the food processing such as cheese-making process (Arsalan and Ozdemir, 2008).

The immunomagnetic separation (IMS) technique has been used as an effective and rapid method for the detection of *Salmonella* spp. and *Listeria* spp. from foods (Lynch et al., 2004; Ayaz and Erol, 2009). Recently, immunomagnetic separation (IMS) overcomes the matrix effect and is used for the enumeration of bacteria. IMS can eliminate the potential interferences and it has been applied to conduct measurements in the food matrix, thereby bacteria can be captured easily. The IMS pretreatment can improve the concentration of target bacteria, in this way reducing analysis time and improving detection sensitivity (Bi et al., 2020; Akçınar et al., 2020).

The aim of this study was the detection of *S. aureus*, *Salmonella* spp. and *Listeria* spp. from cheese samples by IMS and conventional methods. We also determined the total aerobic mesophilic bacteria (TAMB), lactic acid bacteria (LAB), yeast/mold, psychrotrophic bacteria, and coliform bacteria counts in different cheese samples. The antibiotic susceptibility of confirmed isolates was evaluated with different antibiotics.

MATERIALS AND METHODS

For this study, A total of 50 different cheese samples were collected as 10 samples per group (Civil, Tulum, Kaşar, Lor, White Cheese) from various supermarkets, between June 2015-July 2015. These cheeses were placed in packaging material and immediately transported to the laboratory and stored at 4 °C until analysis.

For microbiological analysis, 11 g of cheese were weighed for each sample and diluted aseptically in 99 mL sterile maximum recovery diluent (MRD) (0.85% NaCl+0.1% peptone). Serial decimal dilutions of samples were prepared with MRD. Total aerobic mesophilic bacteria count was enumerated using Plate Count Agar (PCA) Merck, 1.05463) at 30±1 °C for 48 h (Harrigan, 1998; Maturin and Peeler, 1998). The counts of lactic acid bacteria (LAB) were determined on de Man, Rogosa and Sharpe Agar (MRS) (Oxoid, CM0361) at 37±1 °C for 48 h anaerobically (Dave and Shah, 1998). The yeast/molds were enumerated on Potato Dextrose Agar (PDA) (Merck, 1.10130) acidified with 10% lactic acid (Merck, Darmstadt, Germany) and incubated at 25±1 °C for 5-7 days (Herrera, 2001). The

psychrotrophic bacteria count was determined using PCA at 7-10 °C for 10 days. Total coliform counts of samples were counted by Violet Red Bile Agar (VRB) (Merck, 1.01406) at 37±1 °C for 48 h (Harrigan, 1998). The selective determination of *Escherichia coli* was performed using EC broth (Merck 1.10765) and Eosin Methylene-blue Lactose Sucrose Agar (EMB agar) (Rippey et al., 1987).

For *S. aureus* enumeration, serial dilutions of cheese homogenates were spread on Baird Parker Agar (BPA) (Merck, 1.05406) supplemented with egg yolk telluride and incubated at 37±1 °C for 24 to 48 h. Typical colonies were confirmed with coagulase, catalase activity, and DNase and TNase activity, haemolysis in blood agar tests (Bennett and Lancette, 2012).

For the isolation of *Salmonella* spp., 25 g sample was weighted to the sterile filter stomacher bags that have in 225 mL Buffered Peptone Water (BPW) and incubated at 37±1 °C for 18-24 h. Then, the other analyzes were made according to the ISO protocol (ISO, 2002; BAM, 2014). IMS method for *Salmonella* spp. were performed according to Kavaz Yüksel and Yüksel (2015) with minor modifications. 1 mL of pre-enriched sample was incubated with 20 µL Dynabeads® anti-*Salmonella* (Invitrogen, UK) in 2 mL microcentrifuge tube at room temperature for 15 min. Biochemical identification of suspicious *Salmonella* colonies was made using by API 20E test kit (BioMérieux, Inc., France) and conventional biochemical tests.

For the isolation of *Listeria* spp. 25 g of cheese samples were made in 225 mL half fraser broth (Oxoid, Basingstoke, UK). The other analyses for *Listeria* spp. were made according to Hitchins et al. (2016). The obtained colonies were confirmed using mobility test, biochemical tests (Gram stain, catalase, oxidase, urea, TSI, and MR-VP, β-hemolysis, CAMP test, and production of acids from rhamnose and xylose) and API Listeria (BAM, 2014). The enrichment stage of *Listeria* isolation by IMS was made similarly to the conventional method. IMS procedure for *Listeria* spp. was performed as previously explained in *Salmonella*. Afterward, 100 µL of resuspended IMS mixture was plated on PALCAM agar, ChromoCult® Listeria Agar, Oxford Listeria agar and then same procedures were followed as described for the conventional method.

The antibiotic susceptibility tests of the isolates were made using Kirby-Bauer Disk diffusion protocol according to the standard of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014). Firstly, 5 mL sterile 0.85% NaCI solutions were prepared for each isolate and densities of them were controlled with a MacFarland 0.5 standard. Then the solutions were spread to the Mueller Hinton agar (Oxoid, CM0337) with a sterile cotton swab. Antibiotic disks including, Tetracycline (TE, 30 µg, Oxoid CT054B), Streptomycin (S, 10 µg, Oxoid CT047B), Trimethoprim (TMP, 5 µg, Oxoid CT076B), Gentamicin (CN, 10 µg, Oxoid CT024B), Kanamycin (K, 30 µg, Oxoid CT026B), Chloramphenicol (C, 30µg, Oxoid CT013B), Sulphamethox/Trimethoprim (SXT, 5 µg, Oxoid CT052B), Ciprofloxacin (CIP, 5 µg, Oxoid CT425B), Ampicillin (AMP, 10 µg, Oxoid CT003B) and Nalidixic Acid (NA, 30 µg, Oxoid CT031B) were placed onto the inoculated surface of Mueller Hinton agar. The plates were incubated at 37±1 °C for 24 hours and diameters of clear zones were measured using a ruler in terms of millimeters (CLSI, 2014).

The obtained data were analyzed using the SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the differences between results.

RESULTS AND DISCUSSION

The results of the general microbiological properties of the cheese samples are presented in Table 1.

The highest mean counts of TAMB were found in samples of 11, 15, 21, 22, 23, 26, 27, 28, 29, and 30, while the lowest mean value was determined in sample 39.

Table 1. The certain viable bacteria count of different cheese samples.

Cheese samples	TAMB count (log CFU/g)	Lactic acid bacteria count (log CFU/g)	Yeast /mold count (log CFU/g)	Psychotrophic bacteria count (log CFU/g)	*Staphylococcus aureus* count (log CFU/g)	Coliform bacteria count (log CFU/g)	EC broth results	*E. coli (EMB)*
1	7.77	8.00	7.75	7.11	<10*	5.12	+	+
2	7.48	6.63	4.21	7.94	<10	7.84	+	+
3	7.83	7.49	4.29	8.06	<10	8.11	+	+
4	7.26	6.63	3.46	7.36	<10	7.48	+	+
5	6.36	6.95	5.23	6.19	<10	<10	-	-
6	6.40	6.86	4.60	5.96	<10	<10	+	+
7	7.23	8.00	6.42	7.30	<10	4.71	+	+
8	8.52	8.16	4.45	8.08	<10	7.08	+	+
9	7.72	7.64	4.30	7.45	<10	6.45	+	+
10	7.20	8.24	5.79	8.43	<10	4.11	+	+
11	11.00	7.00	5.94	11.00	7.91	7.52	+	+
12	7.62	7.96	5.84	5.52	<10	1.70	+	+
13	7.99	8.12	6.77	7.79	<10	8.00	+	+
14	6.49	6.03	2.00	6.08	<10	5.54	+	+
15	11.00	8.48	6.11	11.00	7.36	8.00	+	+
16	8.60	7.89	2.70	8.30	<10	7.72	+	+
17	9.16	8.30	6.69	8.86	<10	8.00	+	+
18	8.14	8.16	6.38	8.45	<10	7.14	+	+
19	7.81	7.68	4.85	7.42	<10	8.00	+	+
20	9.15	9.54	6.09	9.28	<10	7.03	+	+
21	11.00	7.11	6.33	11.00	<10	2.59	+	+
22	11.00	6.44	<10	11.00	<10	2.65	+	+
23	11.00	8.02	4.02	11.00	5.36	7.88	+	+
24	7.08	7.12	3.85	5.32	<10	2.00	+	+
25	6.33	8.19	5.88	6.14	<10	<10	+	+
26	11.00	11.00	7.10	11.00	<10	3.41	+	+
27	11.00	11.00	4.37	11.00	6.48	5.90	+	+
28	11.00	11.00	3.49	11.00	<10	<10	-	-
29	11.00	11.00	4.11	11.00	<10	3.05	-	-
30	11.00	11.00	4.52	11.00	<10	1.48	+	+
31	8.45	8.63	6.83	8.48	<10	5.94	+	+
32	7.34	7.53	6.53	6.30	<10	3.98	-	-
33	8.51	8.26	3.08	5.99	<10	7.70	+	+
34	8.23	9.26	2.90	6.30	<10	6.00	+	+
35	6.13	6.14	<10	4.00	<10	4.42	+	+
36	8.78	8.38	5.82	7.95	<10	7.42	+	+
37	8.73	6.68	<10	8.77	<10	7.73	+	+
38	8.28	8.10	8.20	8.19	<10	2.30	+	+
39	5.40	6.64	7.38	4.00	<10	2.48	+	+
40	6.24	6.19	4.77	<10	<10	<10	+	+
41	6.41	5.99	5.09	7.79	<10	8.00	+	+
42	6.82	6.79	<10	5.23	<10	<10	-	-
43	8.59	8.05	5.19	7.01	<10	5.13	+	+
44	9.25	8.23	5.74	6.60	<10	5.60	+	+
45	8.61	9.11	5.56	8.35	<10	8.74	+	+
46	8.30	8.10	6.14	6.30	<10	5.78	+	+
47	8.39	7.95	4.31	8.40	<10	7.70	+	+
48	9.49	8.30	6.60	8.52	<10	7.20	+	+
49	7.90	8.49	3.48	6.92	<10	5.30	+	+
50	8.39	8.32	7.93	6.57	<10	3.30	-	-

<10*: CFU/g

The cheese-making process is based on the application of lactic acid bacteria (LAB) that are expected to cause rapid acidification of milk and decrease in pH consequently (Briggiler-Marcó et al., 2007). The highest mean lactic acid bacteria counts were determined in samples 26, 27, 28, 29, and 30. The lowest mean value was found in sample 41 (5.99 log CFU/g). Yeasts and molds are important microbial contaminants for the dairy industry. The presence of theirs in cheese may cause serious problems (Borelli et al., 2006). The average numbers of yeasts/molds on the samples were between <10 CFU/g (Samples 22, 35, 37 and 42) - 8.20 log CFU/g (Sample 38). The psychrotrophic microorganisms are the most important group of microorganisms found in dairy products. Their heat-resistant enzymes such as protease and lipase cause some serious problems including proteolysis, lipolysis, rancidity and putrefaction (Özer and Yaman, 2014). The highest mean values of psychrotrophic bacteria were found in samples 11, 15, 21, 22, 23, 26, 27, 28, 29 and 30, while the lowest mean count was determined in sample 40.

The presence of coliform bacteria, *S. aureus*, *Salmonella* spp. and *L. monocytogenes* in foods are accepted as hygiene index. According to the Turkish Food Codex (Anonymous, 2011), the highest level of coliform bacteria that is allowed <10 CFU/g, also fecal coliforms should not be found in samples. On the other hand, *L. monocytogenes* and *Salmonella* spp. should not be found in 25 g cheese sample. Observing the obtained results, the coliform group microorganisms were determined as <10 CFU/g in samples 5, 6, 25, 28, 40 and 42, while the highest mean value was found in sample 45. When EC broth results were observed, only six samples (Samples 5, 28, 29, 32, 42 and 50) were found negative in terms of fecal coliform. EMB agar results showed that *E. coli* were determined samples of 44/50. The results showed that 92% (44/50) of the cheese samples were not suitable to standards in terms of coliform bacteria count and the presence of fecal coliform. On the other hand, 92% (44/50) of the cheeses were not suitable for *E. coli*. The prevalence of *E. coli* in various types of cheese samples was reported between 4% and 74.3% by several researchers (Öksüztepe et al. 2009; Brooks et al. 2012).

It can be assumed that the presence of all of these microorganisms in the observed cheeses has been attributed to the using milk, starter culture and rennet, process conditions, equipment, workers and air of the environment.

S. aureus is one of the most important disease-affecting microorganisms for human health. The diseases caused by *S. aureus* occur due to the consumption of contaminated food that is containing one or more enterotoxins produced by *S. aureus* (Le Loir et al., 2003). In this study, four (samples of 11, 15, 23 and 27) cheese samples were found positive in terms of *S. aureus* presence. The highest mean *S. aureus* count was determined in sample 11 and followed by samples 15, 27 and 23, respectively. The obtained results were confirmed by the tests of coagulase, catalase activity, DNase-TNase activity and haemolysis in blood agar. After these processes, *S. aureus* was determined from four cheeses samples. According to Anonymous (2011), *S. aureus* should be found between the $1.0x10^2$-$1.0x10^3$ CFU/g. Four samples do not comply with the standards in terms of *S. aureus* count. There are several studies on the presence and growth activities of *S. aureus* in various types of cheese produced in different cities in the Turkey. Bingöl and Toğay (2017) reported that were positive for *S. aureus* and that %34 from 52 Urfa cheeses in different conditions. The results were probably caused by the using milk, production and storage conditions. Also, the manufacturing process has been demonstrated to be the source of high *S. aureus* numbers (Saadat et al., 2014).

In this research, fifty (50) cheese samples were analyzed using conventional and IMS methods in terms of the presence of *Salmonella* spp. At the end of the isolation from 2 cheese samples (2/50) were determined as suspicious *Salmonella* spp. by IMS method. Then obtained colonies were examined with some analysis. During the examined procedure, *Salmonella* Typhimurium was used as a positive culture. The suspicious colonies were analyzed in terms of their oxidase activity. Then, oxidase negative colonies were

confirmed with API 20E test kit (BioMérieux, Inc., France) and conventional biochemical tests including verification of TSI and LIA agar. Finally, the obtained API 20E results were committed to the apiweb database (https:/apiweb.biomerieux.com) and suspicion colonies were determined as *Proteus mirabilis* at the probability of 99%. In general, *Salmonella* and *Proteus* species may show similarity in terms of colony shapes and appearance in TSI and LIA agar. Some tests including hydrogen sulfide and acid production from lactose may be inadequate for the distinction of *Salmonella* spp. from other bacteria. In particular, *Proteus* species are lactose negative and sulphide positive, so they are not easily distinguishable from *Salmonella* spp. by using most commercial agar/broth (Rambach, 1990). Generally, the H_2S producing bacteria (such as *Proteus*) create colonies that have a black center with clear zone. However, XLD and XLT-4 Agars are the selective differential mediums used for the isolation and identification of gram-negative enteric pathogens. Generally, H_2S positive microorganisms, such as *Salmonella* spp. and *Proteus* spp., produces round black center colonies on these agars (Anonymous, 2014). There are wide variations among the contamination ratio of *Salmonella* spp., *Listeria* spp and other microorganisms in among cheese samples according to cheese typing. In our country and other countries, various results were also showed the presence of *Salmonella* spp. in different types of cheeses. It was detected that 6 (2.4%) out of 250 cheese samples (Colak et al., 2007) and 3 (6%) of the 50 Van otlu (herby) cheese samples (Tekinsen and Özdemir, 2006). Contrary to these findings, *Salmonella* spp. were not found in 80 white cheese and 40 cecil cheese samples by Gulmez and Guven (2001) and 50 carra cheese samples by Aygun et al. (2005). According to Pamuk and Siriken (2018), *Salmonella* spp. were isolated from 21% (21/100) of which obtained from milk products (6 of tulum cheese and 15 of fresh soft cheese) in the Afyon. In contrast, it was not isolated from cecil cheese and pasteurized milk samples.

In this study, fifty (50) cheeses were used for the isolation and identification of *L. monocytogenes* by using conventional and IMS methods. According to results, 5/50 cheese samples were determined as positive for *Listeria* spp. by these methods. The isolates confirmed with API *Listeria* (BioMérieux, Inc., France). The obtained data were transferred to the apiweb database (https:/apiweb.biomerieux.com). These colonies in two cheese samples (2/50) were determined as *L. monocytogenes* at the probability of 99%, while the other colonies in three cheese samples (3/50) were identified as *L. grayi* at the probability of 99%.

The behavior of *L. monocytogenes* in different kinds of cheese during ripening has been widely studied by some authors around the World and our country. Sagun et al. (2001), Colak et al. (2007) and Bouayad and Hamdi (2012). The significantly higher contamination rate was noted for *L. monocytogenes* for cheese samples. Also, other studies are reported that *L. monocytogenes* can be found more frequently in raw milk samples and soft cheeses. In soft and semi-soft cheeses, the water activity is higher than in hard cheeses, allowing the growth of *L. monocytogenes* (Farber and Peterkin, 1991). Kızanlık and Göksoy (2018) reported that 10 cheese samples were contaminated with *Listeria* spp., from 120 cheeses samples and 7 of which were contaminated with *L. monocytogenes* (1 sample) and *L. ivanovii* (6 samples) that might also cause public health risks.

Antibiotic susceptibility test was applied to *S. aureus*, *L. monocytogenes* and *L. grayi* isolates. For this purpose, 10 different kinds of antibiotics were used for the determination of antibiotic susceptibility of *S. aureus*, *L. monocytogenes* and *L. grayi* isolates (Table 2, Table 3, and Table 4).

When the effects of 10 antibiotics on the *L. monocytogenes* and *L. grayi* were examined, 5 of the isolates (5/5; 100%) were found have resistance to NA. However, all of the isolates (5/5; 100%) showed susceptibility to the C, CIP and AMP. On the other hand, 3 of the isolates (3/5; 60%) were resistance to the TE, S and K, while 2 of them (2/5; 40%) showed resistance to TMP, CN and SXT (Table 2).

Table 2. Antibiotic susceptibility of *Staphylococcus aureus*, *Listeria monocytogenes* and *Listeria grayi* isolates isolates.

Antibiotic	*Staphylococcus aureus* (n=4) %	*Listeria monocytogenes* + *Listeria grayi* (n=5) %
Tetracycline (TE)	100	40
Streptomycin (S)	75	40
Trimethoprim (TMP)	100	60
Gentamicin (CN)	100	60
Kanamycin (K)	75	40
Chloramphenicol (C)	100	100
Sulphamethox/ Trimethoprim (SXT)	100	60
Ciprofloxacin (CIP)	100	100
Ampicillin (AMP)	25	100
Nalidixic Acid (NA)	0	0

Recently, the antimicrobial resistance of bacteria has been considered as a major problem in terms of public health. The unconscious antibiotic usage of humans has caused the formation of antibiotic resistance to the pathogenic strains. Antibiotic resistance of *S. aureus* has an important problem both the veterinary and health professions (Hossein et al., 2010).

Table 3. The antimicrobial effects of different antibiotics against *Staphylococcus aureus* in cheese samples.

	Sample no			
Antibiotic	11 (*S. aureus*) (Zone diameter, mm)	15 (*S. aureus*) (Zone diameter, mm)	23 (*S. aureus*) (Zone diameter, mm)	27 (*S. aureus*) (Zone diameter, mm)
Tetracycline	20.25±0.35Db	24.10±0.14Ca	21.50±0.71Db	24.00±0.00Ca
Streptomycin	15.15±0.21Fab	14.25±0.35Ebc	14.00±0.00Gc	16.15±0.21Fa
Trimethoprim	21.75±0.35Cb	21.00±0.00Db	24.00±0.00Ca	24.00±0.00Ca
Gentamicin	18.50±0.71E	20.50±0.71D	18.25±0.35F	20.25±0.35E
Kanamycin	0.00±0.00H	20.00±0.00D	20.00±0.00E	20.00±0.00E
Chloramphenicol	27.25±0.35Aa	24.25±0.35BCb	25.25±0.35Bb	22.25±0.35Dc
Sulphamethox/ Trimethoprim	28.25±0.35Ab	25.50±0.71Bc	28.00±0.00Ab	34.50±0.71Aa
Ciprofloxacin	27.75±0.35A	28.00±0.00A	28.75±0.35A	28.75±0.35B
Ampicillin	25.00±0.00Ba	15.25±0.35Eb	12.75±0.35Hc	15.25±0.35Fb
Nalidixic Acid	11.65±0.49G	11.65±0.49F	12.00±0.00H	11.65±0.49G

Different uppercase letters indicate significant differences ($P<0.01$) among the antibiotics, while lowercase letters showed differences among the samples. Results are means (SD) of duplicate. The difference between samples is insignificant ($P> 0.01$).

Observing Table 3, the isolates of 15, 23 and 27 had the highest resistance to NA while, isolates of 11 showed the highest resistance to the K. The isolates of 11 and 27 showed susceptibility to SXT while, isolates of 15 and 23 were susceptible to CIP. According to Table 2, there were statistically significant ($p<0.01$) differences between the samples with respect to antibiotic susceptibility and resistance.

Table 4. The effects of different antibiotics on the *Listeria grayi* and *Listeria monocytogenes* in cheese samples.

Antibiotic	Sample no				
	1 (*L. grayi*) (Zone diameter, mm)	6 (*L. grayi*) (Zone diameter, mm)	30 (*L. grayi*) (Zone diameter, mm)	38 (*L. monocytogenes*) (Zone diameter, mm)	47 (*L. monocytogenes*) (Zone diameter,mm)
Tetracycline (TE)	8.00±0.00Gb	33.50±2.12Aa	8.25±0.35Db	11.25±0.35Db	11.00±0.00Db
Streptomycin (S)	14.00±0.00Fb	18.50±2.12Ba	13.90±0.14 CDb	8.25±0.35Ec	0.00±0.00Ed
Trimethoprim (TMP)	20.50±0.71Ca	25.50±4.95ABa	21.00±0.70ABCa	0.00±0.00Fb	0.00±0.00Eb
Gentamicin (CN)	16.25±0.35Eab	23.50±6.36ABa	14.15±0.21BCDab	10.25±0.35Db	10.25±0.35Db
Kanamycin (K)	17.25±0.35Eab	25.00±7.07ABa	16.00±0.00BCab	10.25±0.35Db	10.25±0.35Db
Chloramphenicol (C)	20.50±0.71Cbc	33.00±1.41Aa	19.25±0.35ABCc	23.25±0.35Bb	23.25±0.35Bb
Sulphamethox/ Trimethoprim (SXT)	18.75±0.35Da	19.00±1.41Ba	17.50±0.71ABCa	0.00±0.00Fb	0.00±0.00Eb
Ciprofloxacin (CIP)	22.75±0.35Bab	21.50±0.71ABbc	24.75±0.35Aa	20.50±0.71Cbc	19.50±0.71Cc
Ampicillin (AMP)	25.75±0.35Ab	28.50±0.71ABa	22.00±0.00ABc	26.00±0.00Ab	25.75±0.35Ab
Nalidixic Acid (NA)	0.00±0.00H	0.00±0.00C	0.00±0.00E	0.00±0.00F	0.00±0.00E

Different uppercase letters indicate significant differences (P<0.01) among the antibiotics, while lowercase letters showed differences among the samples. Results are means (SD) of duplicat

As seen in Table 4, all of the *L. grayi* isolates (1, 6 and 30) showed the highest resistance to NA, while each of them had the highest susceptibility to the different antibiotics including AMP (isolate 1), TE (isolate 6) and CIP (isolate 30).

Currently, there is inadequate information about the antibiotic susceptibilities of *Listeria* spp. in cheeses. Some studies accorded that *L. monocytogenes* has become resistant to antimicrobial drugs currently in use for both human and veterinary medicine. Observing the *L. monocytogenes* isolates, the isolates of 38 and 47 had the highest resistance to TMP, SXT and NA. Also, all of the isolates showed highest susceptibility to the AMP. According to the Table 4. *L. grayi* and *L. monocytogenes* isolates showed statistically significant differences ($p<0.01$) in each other in terms of antibiotic susceptibility. Observing the statistical evaluations, the TMP, SXT and NA could be grouped together with respect to the effect on sample of 38 while, S, TMP, SXT and NA showed similar effect on sample 47. Also, TE, CN, C and K had similar effect on the samples of 38 and 47. Similar results were also reported by Pesavento et al. (2010) for *Listeria* spp. isolates were isolated from foods.

CONCLUSIONS

In conclusion, certain microbiological characteristics and antibiotic susceptibilities of *S. aureus*, *Salmonella* spp. and *Listeria* spp. were revealed in 50 cheese samples. The obtained results indicated that some of the cheese samples were not suitable to standards with respect to the presence of pathogenic and other bacteria contents. Therefore, it can be said the use of stronger hygienic practices has high importance during cheese manufacturing, marketing and storage. Immunomagnetic separation (IMS) is accepted as an alternative method to the conventional method for the detection of *Salmonella* and *Listeria* due to rapid, sensitive and effective. The determined *S. aureus, L. monocytogenes* and *L. grayi* isolates were analyzed in terms of their antibiotic susceptibilities and resistances. The results of the tests showed that the obtained isolates had quite significant antimicrobial resistance to antibiotics.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

AKY and MY performed research. AKY analyzed data, and wrote the paper. All authors contributed to the article and approved the submitted version.

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