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ORIGINAL RESEARCH

Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey

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In this survey, 150 samples of raw milk, white cheese and ice cream from three different dairy-processing plants in Ankara were analysed to find out if they were contaminated with Escherichia coli*,* Staphylococcus aureus *or* Bacillus cereus*. The highest contamination percentages were found in raw milk samples as follows:* B. cereus *(90%),* E. coli *(74%) and* S. aureus *(56%) followed by cheese (70%* B. cereus*, 60%* E. coli*, and 48%* S. aureus*) and ice cream (56%* E. coli*, 36%* S. aureus *and 20%* B. cereus*). The survey showed that 2% of cheese samples were contaminated with* E. coli *O157. It was also found that the numbers of* S. aureus *and* E. coli *in raw milk, cheese and ice cream samples exceeded the numbers permitted under the Turkish Food Codex (TFC). The number of* B. cereus *in raw milk, cheese and ice cream samples was lower than the limit given in the TFC standards. The study also showed that* E. coli *and* S. aureus *exhibit resistance to ampicillin, penicillin, tetracycline, erythromycin, gentamicin and trimethoprim/sulfamethoxazole.* Escherichia coli *isolates also showed resistance to chloramphenicol and ciprofloxacin but none of them exhibited resistance to cefotaxime. All* S. aureus *isolates were found to be susceptible to cefotaxime, chloramphenicol, and ciprofloxacin.* Bacillus cereus *isolates were found to be resistant to ampicillin, penicillin and trimethoprim/sulfamethoxazole and sensitive to cefotaxime, chloramphenicol, ciprofloxacin erythromycin, gentamicin and tetracycline.*

Keywords Raw milk, White cheese, Ice cream, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*.

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INTRODUCTION

The quality of milk is evaluated by its composition and hygienic properties. Milk serves as an excellent culture medium for the multiplication of many different micro-organisms. Pathogens may be found in raw milk originating from the farm environment and could colonise dairy plant premises and consequently contaminate dairy products (Soomro *et al.* 2002).

A considerable amount of milk produced in our country is processed in dairies which do not have up to date machinery and equipment and personnel with sufficient technical knowledge. Additionally, the processes of pasteurisation are not carried out efficiently. They sell their products in the market however (Yucel and Ulusoy 2006; Sener and Cakici 2013). Cheeses and ice creams are ready-to-eat (RTE) food products that are not treated further for safety (El-Sharef *et al.* 2006). Therefore, production of cheese and ice cream in these enterprises may cause a health risk (Sener and Cakici 2013).

Escherichia coli is considered a faecal contamination indicator in foods because of its presence in the gut. The presence of *E. coli* in foods is a matter of concern because some strains may be pathogenic (Thaker *et al.* 2012). *Escherichia coli* O157:H7 serotypes, identified as enterohaemorrhagic *E. coli* (EHEC) and grouped as verotoxin-producing *E. coli* (VTEC), are recognised as the primary cause of haemorrhagic colitis (HC) and the diarrhoea-associated form of haemolytic-uremic syndrome (HUS) (Rahimi *et al.* 2011). Fermented dairy products made with unpasteurised milk are a potential vehicle for the transmission of *E. coli* O157:H7 to consumers. It has been shown that if the pathogen is present in raw milk, although it will not survive proper pasteurization, if post pasteurization occurs the organism has been shown to survive the manufacturing and ripening stages of fermented dairy products (Coia *et al.* 2001). The risk to health is high due to the low dose-response relation (10-20 cfu/g) of *E. coli O*157:H7 required to cause infection (Coia *et al*. 2001).

Staphylococcus aureus is considered to be one of the most common causes of disease worldwide (Pereira *et al.* 2009). *Staphylococcus aureus* contamination can be found in raw milk obtained from cows suffering mastitis or from food handlers who are carriers of *S. aureus* as a result of poor personal hygiene practices (Bingol *et al.* 2012). Some strains of this organism can produce food-poisoning enterotoxins if they grow excessively in foods (Pereira *et al.* 2009).

Bacillus cereus is abundant in nature and can constitute a major proportion of the microbial flora associated with food spoilage. This micro-organism has been implicated in potential food-poisoning issues (Hassan *et al.* 2010). There are many factors that make *B. cereus* a potential threat for the food industry. It can form a thermoresistant endospore, grow and survive at refrigeration temperatures and produce toxin (Khudor *et al.* 2012).

The indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance in various isolates of bacteria. Consumable animal products have been suggested as a possible source of both resistant bacteria and resistant genes that can be transferred to humans directly (Pereira *et al.* 2009). The antibiotic-resistant strains of a number of pathogenic bacteria, including *S. aureus*, *B. cereus* and *E. coli*, in foods which threaten public health have been the subject of many publications (Ozcelik and Citak 2009; Pereira *et al.* 2009; Thaker *et al.* 2012).

The present study aimed to assess the prevalence and antimicrobial resistance of *E. coli*, *S. aureus* and *B. cereus* in raw milk, Turkish white cheese and ice cream samples collected from different small dairy-processing plants in Ankara, Turkey.

MATERIALS AND METHODS

Sample collection

Between March 2012 and July 2012, a total of 150 samples consisting of raw milk (50), Turkish white cheese (50) and ice cream (50) were collected from three individual small dairy-processing plants (A, B, C) in Ankara. All samples were stored in sterile jars and transported to the laboratory in dry ice. All samples were stored at 4 °C after sampling, until the analysis was commenced. Analyses were performed within 24 h of sampling.

Microbiological analyses

Twenty-five millilitre of ice cream (molten at 40 °C for 10 min), 25 mL of raw milk and 25 g of Turkish white cheese were diluted with 225 mL of 1% sterile buffered peptone water (Oxoid, Basingstoke, UK) and homogenised in a stomacher (Lab. Lemco 400; Seward, Worthington, UK) for approximately 2 min. Decimal dilutions were prepared using the same diluents up to 10^{-6}.

For the isolation of *E. coli*, 0.1 mL of diluted samples was evenly spread on plates of eosin methylene blue agar (EMB; Oxoid). The inoculated medium was incubated aerobically at 37 °C for 24–48 h. From each plate, presumptive colonies (dark centred and flat, with or without metallic sheen) were selected, incubated on 5% sheep blood agar, confirmed by microscopic and biochemical characterisations, including Gram stain, catalase test, indole, methyl red, Voges-Proskauer test, nitrate reduction, citrate utilisation, urease production (Murray *et al.* 2003) and further identified using the API 20E kit (BioMerieux, Marcy I'Etoile, France), along with the reference strain. *E. coli* ATCC 25922 as control.

For the determination of *E. coli* O157:H7 serotype, 25 g of each sample was homogenised in tryptone soya broth (TSB; Oxoid) supplemented with novobiocin (20 mg/L) and incubated at 37 °C for 24 h. The enrichment samples were streaked onto sorbitol MacConkey agar (Merck, Darmstadt, Germany) plates supplemented with cefexime (0.5 mg/L) and potassium tellurite (2.5 mg/L) and incubated as above. After incubation, the plates were checked for the presence of sorbitol-negative, colourless colonies 1–2 mm in diameter. Subsequently, these presumptive colonies were confirmed serologically using an *E. coli* O157 latex agglutination test (Oxoid) and H7 antisera (Denka Seiken Co., Tokyo, Japan), as described by the manufacturers (AOAC 1998).

For the isolation of *S. aureus*, 0.1 mL of diluted samples was plated on Baird-Parker agar (BPA; Oxoid) supplemented with egg yolk–tellurite emulsion (Oxoid) and incubated at 37 °C for 24–48 h. Typical colonies (i.e. black, shiny, convex and with or without halo) were selected. Representatives of each colony were transferred into tubes containing 5 mL of brain heart infusion broth (BHI; Oxoid). The tubes were incubated at 37 °C for 24 h and transferred to 5% sheep blood agar and incubated at 37 °C for 24 h to obtain a pure culture. The identification was carried out using the following tests: colony morphology, Gram staining, production of coagulase, catalase and oxidation and fermentation of mannitol (Murray *et al.* 2003).

For the isolation of *B. cereus*, 0.1 mL of diluted samples was surface-plated on mannitol egg yolk polymyxin agar (Oxoid) and incubated at 30 °C for 24 h. From each plate, presumptive colonies (pink colonies surrounded by a zone of precipitation) were selected, incubated on 5% sheep blood agar, confirmed by Gram stain, spore stain, motility, gelatin hydrolysis, Voges-Proskauer test, anaerobic utilisation of glucose, and nitrate reduction (Murray *et al.* 2003) and further identified using the API 50 CHB (BioMerieux), along with the reference strain *B. cereus* ATCC 10876 as control.

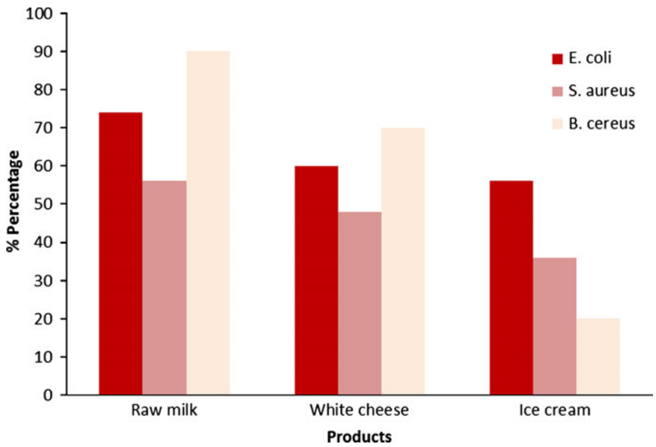

Figure 1 Prevalence of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* isolated from raw milk, white cheese and ice cream samples.

Inoculum standardisation

All the isolates of *E. coli*, *S. aureus* and *B. cereus* were cultured on Brain Hearth Infusion (BHI; Oxoid) agar plates and incubated at 37 °C for 24 h. A loopful of isolates which was taken from the suspensions in sterile normal saline were checked to match the 0.5 McFarland standards as described by Clinical and Laboratory Standards Institute (CLSI 2006).

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were performed as recommended by the CLSI (2006) on Mueller Hinton agar plates (Oxoid). All discs for disc diffusion testing were obtained from Oxoid in the following concentrations: ampicillin (10 μg), cefotaxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (30 μg), penicillin (10 μg), tetracyline (30 μg) and trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg).

Statistical analysis

The chi-square (χ^2) test was used to determine statistically significant differences amongst the samples collected from three small dairy-processing plants (A, B and C) for *E. coli*, *S. aureus* and *B. cereus* counts. *P* values of <0.05 were considered significant.

RESULTS

The prevalence, count ranges and antibiotic resistance of *E. coli*, *S. aureus* and *B. cereus* are given in Figure 1 and Tables 1 and 2. In the present study, the analyses showed that 74% of raw milk, 60% of cheese and 56% of ice cream samples were contaminated with *E. coli*. *Staphylococcus aureus* was obtained from 56% of raw milk, 48% of cheese and 36% of ice cream samples. Our results revealed that 90% of raw milk, 70% of cheese and 20% of ice cream samples were contaminated with *B. cereus*. Two percent of cheese samples were contaminated with *E. coli* O157. This pathogen was not isolated from milk or ice cream samples (data not shown). The counts of *E. coli*, *S. aureus* and *B. cereus* from each respective dairy plant was evaluated. The statistical analysis revealed that there was no significant difference among the samples collected from three small dairy-processing plants (A, B and C) with reference to *E. coli*, *S. aureus* and *B. cereus* counts ($P > 0.05$) (data not shown). *Escherichia coli*, *S. aureus* and *B. cereus* counts varied between 1.0×10^1 and 1.6×10^6 cfu/g-mL; 1.0×10^2 and 3.1×10^4 cfu/g-mL; and 1.0×10^3 and 6.6×10^3 cfu/g-mL in raw milk, cheese and ice cream samples, respectively.

Escherichia coli, *S. aureus* and *B. cereus* isolates isolated from raw milk, cheese and ice cream samples were analysed in terms of their resistance to a range of antibiotics. The results of antimicrobial testing in the present study indicate that there is a high resistance of *E. coli* to ampicillin (90.5%), penicillin (82.1%) tetracycline (66.3%), erythromycin (58.4%), gentamicin (53.7%), trimethoprim/sulfamethoxazole (44.2%), chloramphenicol (29.4%) and ciprofloxacin (22.4%). None of the isolates had resistance to cefotaxime. *Staphylococcus aureus* isolates were found resistant to penicillin (97.1%), ampicillin (92.6%), tetracycline (54.3%), erythromycin (45.7%), gentamicin (41.4%) and trimethoprim/sulfamethoxazole (30%). All isolates of this species were susceptible to cefotaxime, chloramphenicol and ciprofloxacin. *Bacillus cereus* isolates were resistant to ampicillin (91.1%), penicillin (86.7%) and trimethoprim/sulfamethoxazole (27.8%). All *B. cereus* isolates which were studied were found sensitive to cefotaxime, chloramphenicol, ciprofloxacin, erythromycin gentamicin and tetracycline.

DISCUSSION

Prevalence of *Escherichia coli* in raw milk, white cheese and ice cream samples

Escherichia coli is not only regarded as faecal contaminant of milk but also an indicator of poor hygiene and sanitary practices during milking and further handling (Thaker *et al.* 2012). As shown in Figure 1, raw milk samples exhibited the highest prevalence of *E. coli* (74%). Many reports dealing with the occurrence of *E. coli* in raw milk have been evaluated. In those studies, various rates of *E. coli* were reported as 13.44%, 30%, 52.6%, 57%, 60% and 96% of examined raw milk samples by Momtaz *et al.* (2012), Thaker *et al.* (2012), Meshref (2013), Soomro *et al.* (2002), Altalhi and Hassan (2009) and Baz *et al.* (2003), respectively. There may be several reasons for these variations, such as differences in hygienic practices during milking and differences in geographic location and season. Our results showed that raw milk samples had higher *E. coli* counts (2.5×10^4–1.6×10^6 cfu/mL) than the safety limits of Turkish Food Codex (TFC) (Anonymous 2001). In a

Table 1 Counts of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* obtained from raw milk, Turkish white cheese and ice cream samples

		E. coli			*S. aureus*			*B. cereus*		
Samples	*No.of samples*	*Min (cfu/g-mL)*	*Max (cfu/g-mL)*	*TFC[a] (cfu/g-mL)*	*Min (cfu/g-mL)*	*Max (cfu/g-mL)*	*TFC (cfu/g-mL)*	*Min (cfu/g-mL)*	*Max (cfu/g-mL)*	*TFC (cfu/g-mL)*
Raw milk	50	2.5×10^4	1.6×10^6	<10	2.1×10^4	3.1×10^4	$<5 \times 10^2$	4.2×10^2	6.6×10^3	$<1 \times 10^4$
White cheese	50	1.0×10^1	1.2×10^4	<3	3.0×10^2	1.0×10^4	<2	1.0×10^3	2.6×10^3	$<1 \times 10^4$
Ice cream	50	1.0×10^1	8.6×10^2	<9	1.0×10^2	2.1×10^3	<2	1.0×10^3	2.0×10^3	$<1 \times 10^4$

[a]TFC: According to Turkish Food Codex acceptable limit for *E. coli*, *S. aureus* and *B. cereus* counts in raw milk, white cheese and ice cream samples.

Table 2 Antimicrobial resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* isolates isolated from raw milk, Turkish white cheese and ice cream samples

Antibiotic	*E. coli* (n = 95)	*S. aureus* (n = 70)	*B. cereus* (n = 90)
Ampicillin	86 (90.5)	65 (92.6)	82 (91.1)
Cefotaxime	0 (0)	0 (0)	0 (0)
Chloramphenicol	28 (29.4)	0 (0)	0 (0)
Ciprofloxacin	21 (22.1)	0 (0)	0 (0)
Erythromycin	56 (58.4)	32 (45.7)	0 (0)
Gentamicin	51 (53.7)	29 (41.4)	0 (0)
Penicillin	78 (82.1)	68 (97.1)	78 (86.7)
Tetracycline	63 (66.3)	38 (54.3)	0 (0)
Trimethoprim/ sulfamethoxazole	42 (44.2)	21 (30)	25 (27.8)

[a]Percentages are given in brackets.

previous study conducted in Turkey, *E. coli* counts ranged between 3.6×10^2–1.1×10^5 cfu/mL in raw milk samples (Yucel and Ulusoy 2006). White cheese is widely consumed by the Turkish population and the manufacturing process is generally traditional. *Escherichia coli* and *E. coli* O157 were isolated from 60% and 2% of examined cheese samples, respectively. Aráujo *et al.* (2002) detected *E. coli* in 97.7% of cheese samples in Brazil. This value is higher than that obtained from this study. Vural *et al.* (2010) found that 7.62% of Turkish traditional cheese samples were contaminated with *E. coli* O157 while Bingol *et al.* (2012) reported an incidence of 2%. In Iran, 4.2% of traditional cheese samples were positive for *E. coli* O157 (Rahimi *et al.* 2011). The presence of various types of *E. coli* in cheeses can be attributed to different factors such as the use of raw milk, inadequate pasteurisation or postprocessing contamination. The intestinal tract of dairy cattle was reported as the principal reservoir of *E. coli* O157 (Coia *et al.* 2001). Therefore, preventing faecal material from contaminating milk is an important step in reducing the prevalence of *E. coli* O157 in raw milk and its products. In the present study, the count of *E. coli* was determined to range from 1.0×10^1 to 1.2×10^4 cfu/g in cheese samples. In Turkey, several investigators reported higher *E. coli* counts than that found in this study; 3.6×10^2–1.1×10^5 cfu/g (Yucel and Ulusoy 2006), 1.0×10^1–1.8×10^6 cfu/g (Bingol *et al.* 2012), 1.2×10^2–3.6×10^8 cfu/g (Sener and Cakici 2013). *Escherichia coli* was also detected in ice cream samples (56%) with counts of 1.0×10^1 to 8.6×10^2 cfu/mL. In some studies conducted in different cities of Turkey, *E. coli* was detected in 29.2% and 20.5% of ice cream samples and the counts varied between 1.1×10^2 and 8.4×10^2 cfu/g (Yaman *et al.* 2006; Caglayanlar *et al.* 2009). It was observed in our study and above-mentioned studies that cheese and ice cream samples did not conform to TFC (Anonymous 2001). Similar results were obtained by Baraheem *et al.* (2007) who showed that *E. coli* counts in 75% of Kariesh cheese and 42.5% of ice cream samples did comply with Egyptian standards (absent in 1 g) for cheese and ice cream. El-Sharef *et al.* (2006) isolated *E. coli* from 6% of ice cream samples. They reported that *E. coli* counts in ice cream samples were higher than the limits permitted by the Libyan standards for ice cream (<10 cfu/g). Possible reasons for the high counts could be the following: infected food handlers who practice poor personal hygiene; water infected by human contaminants; improper cooling after milking and improper heat treatment. High counts of *E. coli* contribute to the poor hygienic quality of milk, cheese and ice cream. It is also thought that high *E. coli* counts imply a risk of presence of other enteric pathogens in the sample. Therefore, farmers must be educated in safe handling techniques and proper personal hygiene practices, and an effective food inspection system should be provided by the relevant authorities (Meshref 2013).

Prevalence of *Staphylococcus aureus* in raw milk, white cheese and ice cream samples

Staphylococcus aureus may access raw milk by direct excretion from the udder of infected animals with clinical or subclinical staphylococcal mastitis. In the present study, contamination with *S. aureus* was recorded in 56% of raw milk samples. *Staphylococcus aureus* counts (2.1×10^4–3.1×10^4 cfu/mL) were higher than the maximum limits recommended by TFC (Anonymous 2001) for raw milk. Compared to our results, higher contamination rates of raw milk with *S. aureus* were reported as 100% (Gundogan *et al.* 2006b), 83% (Bartolomeoli *et al.* 2009) and 66.7% (André *et al.* 2008) from Turkey, Brazil and Italy, respectively. In contrast, in Slovakia, Belickova *et al.* (2001) did not detect any *S. aureus* in milk and milk products. Robinson (2002) stated that *S. aureus* counts in raw milk should not exceed the limit of 100 cfu/mL. The results generated in this study indicate that all of the samples tested would fail this criterion. Similar results obtained by Meshref (2013) reported that all raw milk samples collected in Egypt had *S. aureus* levels above the recommended numbers established by Robinson (2002). Storage under high environmental temperatures, permitting growth of *S. aureus*, can stimulate the production of *S. aureus*, enterotoxin in raw milk and cheese (Meshref 2013). Therefore, time and temperature controls to prevent the growth of the organism is the primary control measure for *S. aureus*. Personal hygiene is also important to prevent contamination of product from food handlers (Can and Celik 2012). High prevalence of *S. aureus* in cheese samples (48%) detected in this study is in agreement with the rates reported by Ertas *et al.* (2010) and André *et al.* (2008) who indicated that 60% and 70.8% of cheese samples, respectively, were contaminated with *S. aureus*. As this pathogen is inactivated by pasteurisation, it should not be present in pasteurised products. The presence and numbers of these agents make it possible to conclude the hygienic status of the product. Compared to our results, lower contamination rates of different types of cheeses with *S. aureus* were reported as 36–26.7% in Turkey (Tasci *et al.* 2011; Bingol *et al.* 2012) and 3.8% in Egypt (El-Sharoud and Spano 2008). The results in the work reported here showed that *S. aureus* counts (3.0×10^2–1.0×10^4 cfu/g) in cheese samples were above the limits established by TFC (Anonymous 2001). The high *S. aureus* count of cheese samples in this study is in agreement with other recently carried out studies in other regions of Turkey (Ertas *et al.* 2010; Bingol *et al.* 2012) and emphasise the need for urgent action by the regulatory agencies to safeguard consumer health. Likewise, Aráujo *et al.* (2002) reported that 8 (17.7%) of 45 cheese samples were above the limits established by Brazilian legislation for *S. aureus* ($\leq 10^3$ cfu/g). In Germany, Akineden *et al.* (2008) detected one of 50 cream cheese and one of 56 semihard cheese samples having *S. aureus* concentration higher than 10^5 cfu/g. It is generally considered that the numbers of *S. aureus* need to be $>10^5$ cfu/g of food for the production of sufficient toxin to cause illness (Bingol *et al.* 2012). However, neither the absence of *S. aureus* nor the presence of a small number of organism can provide complete assurance that milk and dairy products are safe. Because, even in the case of complete alleviation or reduction of *S. aureus*, enough toxin could have been produced and still cause symptoms of staphylococcal food poisoning (Meshref 2013). Many studies on the microbiological quality of ice cream samples have shown remarkable abundance of samples not compliant with standards in Turkey (Kanbakan *et al.* 2004; Gundogan *et al.* 2006b; Yaman *et al.* 2006). The authors highlighted the inadequate handling of pasteurisation processes, postprocessing contamination from water, unsatisfactory conditions of utensils and inadequate sanitary habits by the handlers and vendors of the products. The detection of *S. aureus* in 36% of ice cream samples in the present study with counts of 1.0×10^2 and 2.1×10^3 cfu/mL reflects this situation, indicating the possible risk to public health caused by the consumption of ice cream. High incidences of *S. aureus* in ice cream samples and the counts above the safe limit level have been reported by other researchers around the world, such as Warke *et al.* (2000) (100%), Aráujo *et al.* (2002) (77.7%) and Zakary *et al.* (2011) (50%).

Prevalence of *Bacillus cereus* in raw milk, white cheese and ice cream samples

Because *B. cereus* is widely distributed in the environment, the organism can be introduced into the milk from soil, air, water, feeds, pasture, udder and excreta from the cows and milking equipment. *Bacillus cereus* was isolated from mastitic cows, particularly those kept in barns (Hassan *et al.* 2010). Frequent occurrence of *B. cereus* in raw milk samples (90%), which could be explained by the unsatisfactory hygienic conditions during milking and further handling on dairy plants, was in agreement with the results of other authors. In Egypt, *B. cereus* has been found in 26.7% and in 30% of raw milk samples (Ayoub *et al.* 2003; Hassan *et al.* 2010). Khudor *et al.* (2012) reported that 32.7% of raw milk samples examined in Iraq were contaminated with *B. cereus*. The prevalence of *B. cereus* in raw milk samples varied from 6.34 to 75.3% in Turkey (Gundogan and Arik 2005; Dikbas 2010). Spores of *B. cereus* are very adhesive to surfaces of dairy equipment used in dairy plants. The strong adhesive capacity of spores is mainly due to their relatively high hydrophobicity, low surface charge and morphology (Citak *et al.* 2010). Its spore-forming property enables *B. cereus* to grow in pasteurised milk and dairy products (Iurlina *et al.* 2006). We found 70% of cheese samples were contaminated with *B. cereus*. There are many studies stemming from Turkey and other countries concerning the incidence of *B. cereus* in cheese samples. Molva *et al.* (2009) studied different cheese samples collected from different regions of Turkey and 6% of them were found to contain *B. cereus*. Citak *et al.* (2010) reported that 20% of Turkish white cheese samples were contaminated with *B. cereus*. Schlegelova *et al.* (2003) suggested that the most important factor causing diseases related to *B. cereus* can be improper storage or incorrect temperature. They also showed that milk products (cream cheese and butter) that were heat-treated during the manufacturing process were significantly more contaminated with *B. cereus* strains (54–65%) than unheated products (3.2%). Likewise, Iurlina *et al.* (2006) reported that the incidence of the *B. cereus* in the samples of Port Salut Argentino cheeses is 50%. They think that the absence of *B. cereus* in Quartirolo cheese is the result of the process differences of these two types of cheeses; that is, in case of Quartirolo cheese the cooking step is excluded. After cooking, spores can germinate and vegetative cells of *Bacillus* spp. can grow well in the absence of a competitive microflora (Iurlina *et al.* 2006). In Poland, *B. cereus* strains were isolated from 14.1% of the mould cheese samples (Berthold 2007), while in Iraq, the percentage of these bacteria was 16.6% and 18% in soft cheese and curled cheese, respectively (Khudor *et al.* 2012). In the present study, the incidence of *B. cereus* in ice cream samples was 20%. Likewise, Yaman *et al.* (2006) reported an incidence of 19% in open ice cream samples and they indicated that the *B. cereus* count of the ice cream samples was 10^4. *Bacillus cereus* was present in 100% (Ozcelik and Citak 2009) and 48% (Citak *et al.* 2010) of ice cream samples with counts of 6.0×10^3 cfu/g. Outside of Turkey, the ratio of ice cream containing *B. cereus* was reported as 40% (Warke *et al.* 2000), 48% (Hassan *et al.* 2010) and 62% (Messelhausser *et al.* 2010) from India, Egypt and Germany, respectively. Differences between the results may be based on the differences in the cheese and ice cream production techniques, and whether the milk used was raw or pasteurised. The cheese and ice cream samples were obtained from several sources and storage conditions which bring about different results. In the present study, *B. cereus* counts ranged from 1.0×10^3 to 6.6×10^3 cfu/g-mL in raw milk, cheese and ice cream samples. In accordance with our findings, the colony counts of *B. cereus* are lower than the limit given in the TFC (Anonymous 2001). Consequently, it does not create any potential hazard. It has been shown that counts exceeding 10^7 cfu/g-mL are required before appreciable levels of toxin are produced in milk and dairy products (Citak *et al.* 2010). However, it should be stressed that, under appropriate conditions, these microorganisms can multiply rapidly and produce toxins to induce symptoms of food poisoning. Poisoning from *B. cereus* can be prevented by storing the food either refrigerated or room temperature according to the type of product. It is important to note that reheating food that has been ‘temperature-abused’ will not make it safe (Ozcelik and Citak 2009).

Antibiotic resistance of *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* isolates

β-Lactams, tetracycline, erythromycin, aminoglycosides and fluoroquinolone group antibiotics are often used in food-producing animals for prophylactic and growth promoter agents. Such uses of antimicrobial agents may contribute to the emergence of resistant *E. coli*, *S. aureus* and *B. cerus* strains from milk and meat products.

As it is shown in Table 2, resistance to ampicillin (90.5%) and penicillin (82.1%) was most common amongst *E. coli* strains. This is not surprising because β-lactams are commonly used antibiotics for the treatment of *E. coli* infections in humans and animals. According to literature data, *E. coli* strains produce an extended spectrum of Amp-C-like β-lactamases (Meyer *et al.* 2008). In the present study, 66.3%, 58.4%, 53.7%, 44.2%, 29.4% and 22.1% of the *E. coli* isolates were resistant to tetracycline, erythromycin, gentamicin, trimethoprim/sulfamethoxazole, chloramphenicol and ciprofloxacin, respectively, but none of them had resistance to cefotaxime. Momtaz *et al.* (2012) in Iran, investigated milk and milk-based foods for the occurrence and antimicrobial resistance patterns of *E. coli*. The foods included were bovine, ovine, caprine, buffalo, camel and donkey milk and cheese, butter and ice cream. These researchers reported that resistance to tetracycline, penicillin, enrofloxacin, nitrofurantoin and ciprofloxacin was seen in 58.8%, 46.07%, 45.09%, 3.92% and 7.84% of the *E. coli* isolates respectively. Ampicillin-, tetracycline-, chloramphenicol-, erythromycin- and trimethoprim-/sulfamethoxazole-resistant *E. coli* strains obtained from various foods have also been described previously (Gundogan *et al.* 2006a;.; Meyer *et al.* 2008; Akond *et al.* 2009).

According to the results reported here, all *S. aureus* isolates were susceptible to cefotaxime, chloramphenicol and ciprofloxacin while resistant to penicillin (97.1%) and ampicillin (92.6%) in accordance with natural resistance for β-lactams of *Staphylococcus* spp. induced by exposure to penicillins. Most of the isolates were resistant to tetracycline (54.3%), erythromycin (45.7%), gentamicin (41.4%) and trimethoprim/sulfamethoxazole (30%). Different rates of ampicillin, penicillin, tetracycline and gentamicin resistance have been reported for *S. aureus* obtained from different sources. Gundogan *et al.* (2006b) reported that 110 *S. aureus* strains in raw milk, pasteurised milk and ice cream samples were resistant to penicillin (96.3%). André *et al.* (2008) reported that *S. aureus* isolates isolated from raw milk and cheese samples were resistant to penicillin (69.9%), tetracycline (24.7%) and erythromycin (5.5%) but not against ciprofloxacin and gentamicin. Can and Celik (2012) observed that most of the *S. aureus* strains isolated from Turkish cheeses were resistant to ampicillin and erythromycin (50%), followed by tetracycline (25%), similar to this study. Citak and Duman (2011) reported that 40.2% and 36.9%, respectively, of *S. aureus* isolates were resistant to tetracycline and erythromycin. In a study conducted in Portugal by Pereira *et al.* (2009) on the resistance of isolates from various foods, 70% and 73%, respectively, of *S. aureus* isolates were resistant to ampicillin and penicillin.

As it shown in Table 2, *B. cereus* isolates were resistant to ampicillin (91.1%), penicillin (86.7%) and trimethoprim/sulfamethoxazole (27.8%). Ozcelik and Citak (2009) reported that *B. cereus* isolates recovered from ice cream samples were resistant to ampicillin (29.5%) penicillin (29.5%) and trimethoprim/sulfamethoxazole (12%). Likewise, high frequencies of ampicillin (100%), penicillin (45.8%) and trimethoprim/sulfamethoxazole (38.2%) resistance were reported in the isolates obtained from white cheese and ice cream (Citak *et al.* 2010). In a study of Dikbas (2010), 100% of *B. cereus* recovered from raw milk, chickens, cereals and meats were resistant to penicillin. Although all *B. cereus* isolates were susceptible to cefotaxime, ciprofloxacin erythromycin, chloramphenicol, gentamicin and tetracycline, *B. cereus* may acquire these drug-resistant phenotypes, as antibiotics are frequently used in animals feed and in chemotherapy.

CONCLUSIONS

The results obtained in this study in Turkey showed a high incidence of *E. coli*, *S. aureus* and *B. cereus* in raw milk samples examined. Our results suggest that raw milk, cheese and ice cream produced by these processing plants represent a potential health risk. This is because some strains of these organisms are capable of producing toxins. Our results also indicate that antibiotic-resistant strains are common in raw milk and dairy products in Turkey. The presence of these bacteria in cheese and ice cream samples seemed to be related to the use of raw milk and unhygienic production processes and storage conditions. Therefore, Turkish regulatory agencies should require dairy-processing plants to adopt quality guarantee systems such as Hazard Analysis and Critical Control Points (HACCP) system and a better control system to prevent the presence of these products on the market.

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