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# *Short communication:* Prevalence, antimicrobial resistance, and resistant traits of coagulase-negative staphylococci isolated from cheese samples in Turkey

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## ABSTRACT

A total of 17 coagulase-negative staphylococci (CNS) isolates obtained from 72 cheese samples were included in this study. Coagulase-negative staphylococci isolates obtained in this study comprised 6 (35.3%) *Staphylococcus saprophyticus*, 3 (17.6%) *Staphylococcus epidermidis*, 2 (11.8%) *Staphylococcus hominis*, 2 (11.8%) *Staphylococcus haemolyticus*, 1 (5.9%) *Staphylococcus xylosus*, 1 (5.9%) *Staphylococcus vitulinus*, 1 (5.9%) *Staphylococcus lentus*, and 1 (5.9%) *Staphylococcus warneri*. The disc diffusion assay revealed that the highest occurrence of resistance was found for penicillin (76.5%), erythromycin (35.3%), tetracycline (29.4%), and trimethoprim-sulfamethoxazole (17.6%) among CNS isolates. However, all CNS isolates were found to be susceptible to vancomycin, streptomycin, linezolid, and gentamycin. Of the isolates, 64.7% carried at least one of the following antimicrobial resistance genes: *mecA*, *tet*(M), *erm*(B), *blaZ*, *ant*(4′)-*la*, *aph*(3′)-*IIIa*, and *lnu*(A). The results suggest that improved hygienic conditions, such as safer handling of raw milk, proper cleaning, and sanitation during the manufacturing in the dairies, are urgently needed in Turkey.
**Key words:** coagulase-negative staphylococci, antimicrobial resistance, cheese

## Short Communication

*Staphylococcus* spp. are gram-positive and nonmotile bacteria that have been divided into 2 groups: coagulase-positive and -negative staphylococci (Podkowik et al., 2013). Staphylococci are widely distributed in nature, and the primary habitat of staphylococci is the skin and mucous membranes of humans and warm-blooded animals. Staphylococci are highly tolerant to environmental extremes; for instance, they are able to grow well in a wide pH range (4.8–9.4) and have a high degree of tolerance to NaCl (7.5–10%; Schleifer and Kloos, 1975).

Although CNS have been considered as nonpathogenic normal commensal flora, recent studies have shown CNS to be one of the most common causes of nosocomial infections, particularly in immunocompromised and hospitalized patients (Koksal et al., 2009). In addition, CNS are often found in foods including meat, milk, and dairy products, and some strains are used in the preparation of fermented foods as a starter culture (Zell et al., 2008). Epidemiological studies showed that dairy products can be contaminated with CNS by using unpasteurized raw milk and also during manufacturing practices by workers. A case study of food poisoning outbreaks linked CNS strains with contaminated unpasteurized milk (Do Carmo et al., 2004).The safeness of CNS strains in food has been questioned due to the fact that some CNS strains isolated from foods have the ability to produce several virulence factors, such as staphylococcal enterotoxins (Do Carmo et al., 2004; Bertelloni et al., 2015; Nunes et al. 2015). In addition, antimicrobial resistance and resistance determinants in CNS strains from foods has gotten attention because of its ability to transfer resistance genes to other bacterial species, including *Staphylococcus aureus* (Hiramatsu et al., 2001). The objective of the current study was to assess the distribution of CNS in different types of cheese samples in Turkey. Antimicrobial resistance profiles of isolated CNS together with antimicrobial resistance genes were also determined.

A total of 72 cheese samples consisting of 11 different types (6–7 samples each of White, Kaşar, Tulum, Ezine, Antep, Sürk, Lor, Van Otlu, Civil, Örgü, and Dil) were analyzed in this study. These cheese samples were produced from raw or pasteurized cow, goat, and sheep milk. All cheese samples were collected from supermarkets, open-air markets, and retail shops between January and June 2014 in Hatay province, Turkey. All samples were transferred to the laboratory in an ice box and stored at 4°C before analysis.

Isolation of staphylococci was carried out by suspending 10 ± 1 g of each cheese sample in buffered peptone

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water (90 mL). The suspension was then enriched at 37°C for 24 h. After an overnight incubation, mannitol salt agar plates were inoculated with 0.1 mL of the enriched suspension and incubated at 37°C for 24 h (Chajęcka-Wierzchowska et al., 2015). The selected staphylococci colonies (2–3 small pink or red colonies per plate for presumptive staphylococci) were streaked on blood agar plates and incubated at 37°C for 24 h. The isolates were initially identified as *Staphylococcus* spp. by PCR amplification of the 16 ribosomal DNA gene using primers as described by Strommenger et al. (2003). All PCR-positive isolates were then examined for coagulase production using rabbit plasma with EDTA lyophilized according to the manufacturer's instructions (Bactident Coagulase, Merck, Darmstadt, Germany). The identification of the coagulase negative isolates to the species level was further done by using VITEK2 automated system (bioMérieux, Marcy-l'Étoile, France).

The antimicrobial resistance profile of selected isolates was examined by using the disc diffusion method as described by the Clinical Laboratory Standards Institute (CLSI, 2015). Eleven antibiotics from different classes were included in the assay, including gentamicin (10 μg/disc), streptomycin (25 μg/disc), levofloxacin (5 μg/disc), vancomycin (30 μg/disc), linezolid (30 μg/disc), erythromycin (15 μg/disc), penicillin (10 μg/disc), tetracycline (30 μg/disc), chloramphenicol (30 μg/disc), clindamycin (10 μg/disc), and trimethoprim-sulfamethoxazole (1.25/23.75 μg/disc). The bacterial inoculum was prepared from an overnight culture and density of inoculum was standardized to 0.5 McFarland (BioSAN, Riga, Latvia). Bacterial suspension (200 μL) was spread onto Muller Hinton agar plate. Antibiotic discs were placed on the surface of the agar and inoculated plates were incubated at 37°C for 24 h under aerobic conditions. *Staphylococcus aureus* (ATCC 29213) was used as a quality control strain. At the end of the incubation, the inhibition zones were measured in the plates with a ruler and recorded in millimeters. The results were interpreted by the CLSI (2015) guidelines and intermediary resistance phenotypes were accepted as susceptible for this study.

Because the disc diffusion assay has been not recommended for the reporting of methicillin resistance, the *mecA* gene was screened in all CNS isolates by PCR, which was accepted to be the gold standard for identification of methicillin resistance (Zhang et al., 2009; CLSI, 2015). All isolates were examined for the presence of *mecA* gene using PCR as described previously (Choi et al., 2003). Multiplex PCR assay for detection *tet*(K), *tet*(L), and *tet*(O) genes coding efflux proteins and *tet*(M) gene coding ribosomal protection proteins were performed as described by Strommenger et al. (2003). To determine macrolide resistance genes [*erm*(A), *erm*(B), and *erm*(C)], PCR was performed according to Jensen et al. (1999). In addition, all isolates were screened for the presence of aminoglycosides resistance-associated genes [*aac*(6′)-*Ie*-*aph*(2″)-*Ia*, *ant*(4′)-*la*, and *aph*(3′)-*IIIa* (Choi et al., 2003), *blaZ* (Vesterholm-Nielsen et al., 1999), and *lnuA* genes (Lina et al., 1999)], also determined by PCR.

A total of 17 staphylococci isolated from cheese samples were positive for 16S rDNA genes and were further identified to species level by using automated VITEK 2 system. These CNS isolates were belong to 8 species which were distributed as follows: *Staphylococcus saprophyticus* (n = 6/17; 35.3%), *Staphylococcus epidermidis* (n = 3/17; 17.6%), *Staphylococcus hominis* (n = 2/17; 11.8%), *Staphylococcus haemolyticus* (n = 2/17; 11.8%), *Staphylococcus xylosus* (n = 1/17; 5.9%), *Staphylococcus vitulinis* (n = 1/17; 5.9%), *Staphylococcus lentus* (n = 1/17; 5.9%), and *Staphylococcus warneri* (n = 1/17; 5.9%).

The results of the antimicrobial susceptibility tests revealed that 88.2% (n = 15/17) of CNS strains displayed antimicrobial resistance to at least one antibiotic (Table 1). In addition, 5 CNS isolates (29.4%) showed multidrug resistance (3 or more class of antimicrobials). As presented in Table 1, CNS isolates were 76.5% resistant to penicillin, 35.3% resistant to erythromycin, and 29.4% resistant to tetracycline. Resistance was also detected to trimethoprim-sulfamethoxazole (17.6%), levofloxacin (11.8%), clindamycin (5.9%), and chloramphenicol (5.9%). No isolate was resistant to gentamycin, vancomycin, streptomycin, and linezolid. *Staphylococcus vitulinis* was found to be susceptible against all antibiotics tested.

In the current study, all CNS isolates were screened for selected resistance genes. As presented in Table 1, 3 isolates of *S. epidermidis* and 1 *S. haemolyticus* isolate were positive for the *mecA* gene. The 4 tetracycline-resistant isolates were found to carry the *tet*(M) gene, whereas only 2 erythromycin-resistant isolates contained *erm*(B) gene. Eight CNS isolates were confirmed to harbor the *blaZ* gene. Even though none of the CNS isolates were found to be resistant to gentamycin or streptomycin, the PCR results showed that *aph*(3′)-*IIIa* (n = 4) and *ant*(4′)-*la* (n = 2) genes were present in 6 isolates. In addition, the *lnu*(A) gene was detected in 2 isolates.

Coagulase-negative staphylococci used to be considerably less important due to their nonpathogenic characteristics when compared with *S. aureus*. As a result, a limited number of studies on the CNS isolated from foods were performed even though CNS are very common on dairy products, with the main source of CNS coming from raw milk and the hands of employees.