Identification of *Staphylococcus aureus* and coagulase-negative staphylococci in some edible nuts

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**Abstract**

In this study, 217 edible nut samples have been investigated regarding *Staphylococcus aureus* and coagulase-negative staphylococci (CNS). *S. aureus* was isolated in 26 (11.98%) samples, whereas CNS were isolated in 98 (45.16%) samples. Number of samples for different isolated CNS species was as follows: *S. arlettae* 19 (8.76%), *S. saprophyticus* 14 (6.45%), *S. xylosus* 12 (5.53%), *S. epidermis* 11 (5.07%), *S. hominis* 11 (5.07%), *S. simulans* 9 (4.15%), *S. haemolyticus* 7 (3.23%), *S. lentus* 6 (2.76%), *S. equorum* 4 (1.84%), *S. sciuri* 2 (0.92%) and *S. capitis* subsp. *capitis* 1 (0.46%).

**Key words:** *Staphylococcus aureus*, coagulase-negative staphylococci, nuts.

**Introduction**

Until today, about 250 food-related diseases have been described, two thirds of which are attributed to bacteria. *Staphylococcus aureus* is a predominant species among these bacteria and causes diseases manifest with gastroenteritis via consumption of the contaminated food. Ingestion of the staphylococcal enterotoxins within the nutrient material results in staphylococcal food poisoning. Staphylococcal food poisoning occurs more commonly following acquisition of the toxins, which are formed within contaminated hand-made foods, via the alimentary tract. Five types of staphylococcal enterotoxins are known to exist, namely A, B, C, D and E. Among these toxins, which are characterized as antigenic, the type C has been classified into three subtypes, namely C1, C2 and C3. The most prevalent enterotoxigenic *Staphylococcus* species underlying food poisonings is *S. aureus*. Being naturally present in hair, skin and nasal cavity, *S. aureus* contaminates nutrient materials during food processing and preparation and therefore is one of the most important causes of food-related diseases. About 30-50% of all humans are thought to be carriers.

Researchers have reported that coagulase-negative staphylococci (CNS) may also produce staphylococcal enterotoxins and therefore are potential causes of food poisoning. Among the CNS, several species such as *S. hyicus*, *S. epidermis*, *S. xylosus*, *S. warneri*, *S. saprophyticus* and *S. lentus* are capable of producing enterotoxins. The CNS species are among the fundamental components of human dermal and mucosal floras, but are also among the most important causes of nosocomial infections with high morbidity and mortality, also causing significant economical losses. The most important 45 CNS species causing disease in humans are *S. epidermis* causing problems such as bacteremia, infections sourcing from prosthetics and catheters, wound infections, peritonitis in patients of continuous peritoneal dialysis, osteomyelitis and endophthalmitis; *S. haemolyticus* causing endocarditis, peritonitis, septicemia, urinary tract and wound infections, and *S. saprophyticus* causing urinary infections and septicemic processes, whereas *S. hominis*, *S. warneri*, *S. capitis*, *S. simulans*, *S. colnii*, *S. xylosus* and *S. saccharolyticus* are regarded as opportunistic pathogens.

The purpose of this study was to evaluate the presence of *S. aureus* and CNS in some edible nut species and to determine potential health hazards they cause.

**Materials and Methods**

**Collection of samples:** The edible nut samples that were sold without packing; hazelnut (*n* = 15), Antep’s pistachios (*n* = 15), peanuts (*n* = 18), almonds (*n* = 15), roasted chickpeas (*n* = 21), sauced roasted chickpeas (*n* = 16), melon seeds (*n* = 17), watermelon seeds (*n* = 18), pumpkin seeds (*n* = 16), Dakota’s sunflower seeds (*n* = 15), nut mixtures (*n* = 16) and sauced roasted corn (*n* = 15) were collected in different locations of Diyarbakır province of Turkish Republic and put in sterile sample pouches. The samples were transported to the laboratory in ice boxes (4°C) and immediately exposed and analyzed upon arrival.
Isolation and identification of *S. aureus* and coagulase-negative staphylococci: A piece of 11 g was taken aseptically from each sample and homogenized in 99 ml of buffered 0.1% peptone water. Samples from decimal dilutions were inoculated to Baird-Parker Agar (Oxoid CM 275+SR 054). The typical colonies formed in medium were moved to Brain-Heath-Broth medium (Oxoid CM225). In identification of isolated *Staphylococcus* spp., D Phoenix Automatic Microbiologic Identification System (BD Diagnostic Instrument Systems, Sparks, MD, USA) was also used in addition to classical biochemical tests, in accordance with the instructions of the producer company.

**Phoenix ID/AST panel:** BD Phoenix™ 100 Automated Microbiology Identification System is a device which is designed for rapid identification of bacteria (ID). A hundred identification tests can be run in this system. The ID section of the system contains a series of conventional, chromogenic and florescent biochemical materials in order to identify the bacteria. There are 45 micro-wells which contain dried chemical substrate and 2 fluorescence control micro-wells in ID section of the device. The device was operated through placing pure cultures into it in accordance with the instructions of the producer company and run the fully-automated analyses procedure for evaluation.

**Results and Discussion**

Among 217 edible nut samples examined in this study *S. aureus* was found in 26 (11.98%) of all samples, while CNS were found in 98 (45.16%) samples. The numbers and proportions of *S. aureus* and CNS species in each sample are displayed in Tables 1 and 2. The highest proportion of *S. aureus* was found in watermelon seeds (50%), whereas the highest proportion of CNS was in sauced roasted corn (70%). *S. aureus* was not isolated in nuts, Antep’s pistachios, almonds, hazelnuts, pumpkin seeds and Dakota’s sunflower seeds at all. In all samples of analysis, the most commonly encountered CNS species were *S. arlettae* (8.76%) and *S. saprophyticus* (6.45%), whereas the least commonly isolated CNS species was *S. capitis* subsp. *capitis* (0.4%).

The presence of enterotoxin-producing *S. aureus* in raw and cooked food materials has been reported by some authors to range between 16 and 86% [16-19]. Danielsson and Hellberg [20] have found that 16.2% of the 111 CNS species they have isolated had the capacity to produce enterotoxins. Crass and Bergdoll [7] have isolated about 2000 *Staphylococcus* species in patients with signs of staphylococcal infection or food poisoning. Among these, 10% were found to be CNS, 33 of which had the potential of producing enterotoxins. The variation among studies is thought to source from the difference in types of food and the places from where the species are isolated [21].

Rosec et al. [4] isolated 213 *S. aureus* and 51 coagulase-negative staphylococci from 121 different foodstuff produced in France. The researchers reported that 30.5% of the *S. aureus* species were enterotoxin-producing types but there were no enterotoxin-producing bacterium among CNS.

Cunha et al. [3] have examined 88 food materials and found the presence of CNS to be 22.7%. Among them, *S. epidermis*, *S. xylosus*, *S. warneri*, *S. saccharolyticus* and *S. hominis* constituted 40, 20, 20, 15 and 5% of all CNS species, respectively. The researchers have concluded that the CNS species had the potential to produce enterotoxins and proposed that this microorganism group should be sought in food materials. In a research in Spain conducted on nasal cavity of 300 food preparation workers, 27.6% coagulase-positive staphylococci (83/300) and 39.3% coagulase-negative staphylococci (118/300) were isolated. The CNS count in 64.4% of CNS porter food processors was reported as 10¹-10⁴ cfu/swag [22].

Udo et al. [23] isolated *S. aureus* and CNS species from hands of restaurant staff who worked in 50 different restaurants in Kuwait City. The researchers reported that they isolated enterotoxigenic CNS species from restaurant staff and concluded that this bacterium should not be neglected as a cause of *Staphylococcus*-related food poisoning cases occurred due to keeping the contaminated foodstuff under conditions suitable for toxin production. Besides, the presences of *S. hominis*, *S. warneri* and *S. epidermis* were determined as 23.6, 20.6 and 3.4% respectively.

Evaluation of the results of this study suggests that dried nuts might constitute significant risks for community health. The potential source of contamination is that dried nuts are kept in inappropriate conditions and sold unpacked, exposed to heat and moisture and processed with hands. Keeping dried nuts in vacuum packets or packing them in modified atmosphere, adapted according to the quality of the product, might significantly reduce the community health risks.

**References**


### Table 1. The proportions and percents of presence of CNS in some nut species.

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<td>S. arlettae</td>
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<td>1 (4.76%)</td>
<td>1 (6.67%)</td>
<td>1 (6.67%)</td>
<td>1 (6.67%)</td>
<td>1 (5.00%)</td>
<td>7 (3.28%)</td>
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<td>5 (2.35%)</td>
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<td>1 (6.67%)</td>
<td>1 (6.67%)</td>
<td>1 (4.76%)</td>
<td>1 (6.67%)</td>
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<td>1 (6.67%)</td>
<td>1 (5.00%)</td>
<td>9 (4.15%)</td>
<td>7 (3.28%)</td>
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<td>S. simulans</td>
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<td>S. sciuri</td>
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<td>S. capitis subsp. capitis</td>
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<td>Total</td>
<td>7 (46.67%)</td>
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<td>26 (12.01%)</td>
<td>120.12%</td>
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*ND*: none detected

### Table 2. The proportions and percents of presence of *S. aureus* in some nut species.

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<tbody>
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<td>S. aureus</td>
<td>ND*</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
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*ND*: none detected