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RESEARCH ARTICLE

Isolation of Staphylococci from Food Handlers and Investigation of Their Enterotoxigenicity and Susceptibility to Some Antibiotics

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Summary

A total of 92 isolates of staphylococcal species consisting of 7 coagulase positive staphylococci (CPS) and 85 coagulase negative staphylococci (CNS) were isolated from hands of the 25 food handlers in different restaurants. Similarly, 13 coagulase positive staphylococci and 96 coagulase negative staphylococci isolates were cultured from the nasal cavity of the workers. Only one isolate of all the hand isolates was resistant to Vancomycin. Nine of all the coagulase negative staphylococci isolate including 4 hand and 5 nasal cavity samples were resistant to Methicillin. Four of 20 coagulase positive staphylococci isolate produced staphylococcal enterotoxins (SE). Only one hand isolate of all the coagulase negative staphylococci isolates produced staphylococcal enterotoxins E. These results indicate, like before, that the food handlers would have been the main source of the staphylococcal contamination of food. It is important to note that coagulase negative staphylococci can produce staphylococcal enterotoxins and they can also cause to food poisoning.

Keywords: *Food handlers, Staphylococci, Enterotoxin, Antibiotic susceptibility*

Gıda Çalışanlarından İzole edilen Stafilokokların Enterotoksijenitelerinin ve Bazı Antibiyotiklere Duyarlılıklarının Araştırılması

Özet

Farklı restoranlarda çalışan 25 gıda personelinin ellerinden, 85 koagulaz negatif stafilokok (KNS), 7 koagulaz pozitif stafilokok (KPS) olmak üzere 92 adet stafilokok izolatı elde edilmiştir. İşçilerin burun deliklerinden 13 koagulaz pozitif stafilokok ve 96 koagulaz negatif stafilokok izolatı kültüre edilmiştir. El izolatlarından bir tanesinin Vancomycin'e, 4'ü el, 5'i de burun örnekleri olmak üzere toplam 9 koagulaz negatif stafilokok'un Methicillin'e dirençli olduğu saptanmıştır. Yirmi koagulaz pozitif stafilokok izolatından 4'ünün stafilokokkal enterotoksin (SE), ellerden izole edilen koagulaz negatif stafilokok'lardan 1'inin de stafilokokkal enterotoksin E ürettiği gözlenmiştir. Ulaşılan bulgular, gıda çalışanlarının gıdaların kontaminasyonunda önemli bir kaynak oluşturabileceğini birkere daha doğrulamıştır. Ayrıca koagulaz negatif stafilokok izolatlarının da stafilokokkal enterotoksin üretebileceği ve dolayısıyla gıda zehirlenmesi oluşturabileceği, önemli bir veri olarak belirlenmiştir.

Anahtar sözcükler: *Gıda Çalışanları, Stafilokoklar, Enterotoksin, Antibiyotik duyarlılığı*

INTRODUCTION

Staphylococcal food poisoning is caused by the ingestion of foods containing enterotoxins produced by some species of staphylococci [1,2]. It is one of the most economically important diseases in the United States, costing approximately $1.5 billion each year [3]. The disease is characterized by sudden onset of symptoms, including; nausea, vomiting, abdominal cramps, and diarrhea within 1 to 6 h after ingestion of toxin-contaminated foods. The duration is short, generally lasting from 24 to 48 h and complete recovery usually occurs within 1 to 3

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days. Staphylococcal food poisoning is generally considered a mild, self-limited illness with low mortality rate. However, the hospitalization rate has been reported to be as high as 10% [4]. The diagnosis of this food-borne illness is based primarily on recovering enterotoxigenic staphylococci and enterotoxins from leftover foods.

Staphylococci are widespread in nature. They can be found in the air, in dust, in water, and on humans and animals. The main human reservoirs of these organisms are the skin and nasal cavity [5]. About 40 to 44% of healthy humans carry staphylococci in the nose [6]. Strains present in the nose often contaminate the back of hands, fingers and face and so, nasal carriers can easily become skin carriers. Although it is difficult to determine the origin of the strains involved in staphylococcal food poisoning outbreaks, food handlers are usually regarded as one of the primary source of these organisms [7,8]. It has been reported that, one of the important pathogens often transmitted via food contaminated by infected food handlers is Staphylococcus aureus [9]. For many years, *S. aureus* was the only staphylococcal species known to produce enterotoxins [1]. An important characteristic that differentiates *S. aureus* from most staphylococcal species is its ability to produce coagulase, an enzyme that clots blood plasma. Other coagulase - positive species such as *S. hyicus* [10] and *S. intermedius* [11] have been also identified. These and several coagulase - negative species including *S. epidermidis* [12] and *S. xylosus* [13,14] have been shown to produce low levels of enterotoxins. Among them, *S. epidermidis* and *S. intermedius* were reported to be the causative agents in food-borne outbreaks [2]. Therefore, while a high correlation between coagulase production and enterotoxigenicity has been reported [12,15,16], the ability to produce coagulase should not be considered the only indication for enterotoxin production. Although several staphylococcal species have been implicated in food - poisoning incidents, *S. aureus* remains as the predominant species.

The purpose of the present study was to determine whether the food handlers from different restaurants carried coagulase positive and negative staphylococci in their nasal cavity and hands. It was also aimed to investigate enterotoxigenicity and resistance of the isolates to some antibiotics.

MATERIAL and METHODS

Sample Collection

Samples from the nasal cavity and hands of the 25 food handlers working 5 different restaurants were obtained using sterile swabs which moistured using sterile saline (0.9% NaCl) solution. Samples were taken only ones during May.

Isolation of Staphylococci from Food Hhandlers

One swab was used to swab areas in between fingers and the wrist area of the hand and another swab was used to swab the nasal cavity. Each swab collected from the nasal cavity and hands was streaked on Baird Parker Agar (BP, Merck 1.05406.0500 + egg yolk-telluride emulsion, Merck 1.03785.0100) plates and incubated at 37°C for 48 h. Total of five colonies, two typical colonies of *S. aureus* are black, shiny, convex and surrounded by clear zones of approximately 2- 5 mm and three coagulase negative staphylococci are black, shiny colonies but clear zones are absent were selected. Five colonies were transferred in to tubes containing 5 ml of Brain Heart Infusion Broth (BHI, Oxoid CM375). The tubes were incubated at 37°C for 24 h and transferred to BP agar incubated at 37°C for 48 h. Then were transferred to Nutrient Agar slants (NA, Oxoid CM3) (stock culture) for further testing.

Identification of Isolated Colonies

Each colony was transferred from Nutrient Agar to two separate test tubes containing 1 ml of BHI broth and incubated at 37°C for 24 h. Catalase and tube coagulase tests were carried out. Gram stain and staphytect plus test, latex slide agglutination test (Oxoid DR 850 B), were also performed.

Tube coagulase and staphytect plus test negative, catalase positive, gram positive coccal isolates were further analysed to differentiate between coagulase negative staphylococci (CNS) and micrococci isolates. For this purpose, glucose fermentation (GF), acid production from glycerol (GA) and response to Furazolidone and Bacitracin antibiotics were used. Glucose fermentation and GA was determined by the method described by Baker [17]. Susceptibility to a 100µg Furazolidone disk (Oxoid CT0448B) and 10 units Bacitracin (Oxoid CT0005B) were determined using the standardized CLSI 2006 disc diffusion method [18]. The method was performed using Mueller Hinton Agar (Oxoid CM0337) with 5% defibrinated sheep blood which is prepared in the lab. Zone sizes at growth inhibition were measured in millimeters after 24 h of incubation at 37°C.

Susceptibility of Identified Colonies to Some Antibiotics

Susceptibility to antibiotics was tested by the disc diffusion method as described above with Mueller Hinton Agar. The following antibiotic discs were used;

Erythromycin (15 µg, Oxoid CT0020B), Gentamycin (10 µg, Oxoid CT0024B), Methicillin (5 µg, Oxoid CT0029B), Tetracycline (30 µg, Oxoid CT0054B) and Vancomycin (30 µg, Oxoid CT0058B). The plates were incubated at 37°C for 24 h. Zone size at growth inhibition were measured and determined according to Gür [19].

Enterotoxin Testing

All the CPS isolates and selected CNS isolates from hands and nasal cavity swabs were analysed for detecting staphylococcal enterotoxins. Selected colonies were inoculated 5 ml of BHI broth and incubated at 37°C for 24 h. and then centrifuged for 15 min at 5.000 g. Supernatant of culture extracts were filtered using sterile filter (0.2 µm, Sartorius CE-0297). A 100 µl of filtrate from each culture extracts were used staphylococcal enterotoxin analysis. Staphylococcal enterotoxins were detected by the sandwich enzyme immunoassay test kit RIDASCREEN SET A, B, C, D, E (R-Biopharm AG, D-64293, Germany). The test was performed by following the manufacturer's instructions.

RESULTS

A total of 250 isolates, 125 isolates from hands and 125 isolates from nasal cavity swabs, were examined. The 20 isolates were identified as CPS including 13 (10.4%) isolates from the nasal cavity and 7 (5.6%) isolates from the hands of workers. The CPS isolates were found to be egg yolk reaction positive except 3 isolates (2 isolates from nasal, one isolate from hand samples). 96 (76.8%) of 125 isolates from nasal swabs and 85 (72.8%) of 125 isolates from hand swabs were identified as CNS.

Resistance to different antimicrobial agents was detected in all CPS and CNS isolates. Five isolates (5.21%) were resistant to Methicillin, 27 (28.42%) to Erythromycin, 34 (36.17%) to Tetracycline and one isolate expressed medium level resistance to Vancomycin and Gentamicin. The results of antibacterial susceptibility of nasal CNS were shown in *Table 1*.

The results of antibacterial susceptibility of hand CNS isolates were shown in *Table 2*. While 4.70% isolates were resistant to Methicillin, 1.19% to Vancomycin, 33.33% to Erythromycin and 29.11% to Tetracycline, 3.53% isolates showed medium resistance to Methicillin, 2.38% to Vancomycin.

All the nasal and hand CPS isolates were found to be susceptible to Methicillin, Vancomycin and Gentamicin. One nasal CPS isolate and two hand CPS isolates were found to be resistant to Erythromycin and Tetracycline.

All the CPS (n:20) isolates and 52 (28.73%) of 181 CNS isolates from hands and nasal cavity swabs were analysed for detecting staphylococcal enterotoxins. Only one CNS isolate from hand swabs produced SEE. However, 4 CPS isolates 2 from nasal cavity and 2 from hand swabs, produced SE. One isolate from nasal cavity swabs produced SEA, SEC, SED and SEE. One isolate from hand swabs produced SEE and others produced SEC. None of them produced SEB.

Table 1. *Antibacterial susceptibility of nasal CNS isolates*
Tablo 1. *Nasal KNS izolatlarının antibakteriyel duyarlılığı*

Antibiotics	Numbers of Isolates	Susceptible (%)	Resistant (%)	Medium Resistant (%)
Methicillin	96	87 (90.63)	5 (5.21)	4 (4.16)
Vancomycin	96	95 (98.96)	0	1 (1.04)
Gentamicin	95	94 (98.95)	0	1 (1.05)
Erythromycin	95	68 (71.58)	27 (28.42)	0
Tetracycline	94	59 (62.77)	34 (36.17)	1.(1.06)

Table 2. *Antibacterial susceptibility of hand CNS isolates*
Tablo 2. *El KNS izolatlarının antibakteriyel duyarlılığı*

Antibiotics	Numbers of Isolates	Susceptible (%)	Resistant (%)	Medium Resistant (%)
Methicillin	85	75 (91.77)	4 (4.70)	3 (3.53)
Vancomycin	84	81 (96.43)	1 (1.19)	2 (2.38)
Gentamicin	83	82 (98.8)	0	1 (1.2)
Erythromycin	84	55 (65.48)	28 (33.33)	1 (1.19)
Tetracycline	79	55 (69.63)	23 (29.11)	1 (1.26)

DISCUSSION

In recent years, much attention has been given to food production, processing, packaging, transportation and storage. Therefore when foods are not produced and stored in proper conditions and or if any kind of damage occurs, they may be contaminated with infectious or toxigenic microorganisms, thus becoming a source of illness for humans. One aspect in the investigations of food poisoning outbreaks is to determine how the implicated food becomes contaminated. It is recognized that food handlers are the major source of contamination with staphylococci. High frequency of carrier status among food handlers has been identified by several investigators and many investigation studies conducted on staphylococci carrier status in humans in many countries [20-25], showed that 30 to 50% of them were carriers at any given time. Pereira et al.[25] examined 55 healthy food handlers in a large industrial kitchen in Belo Horizonte (Brazil) and found that 32 (58.2%) were carriers of *S. aureus* and 17 (30.9%) carried enterotoxigenic strains in their nasal cavity, throat and under fingernails. In the present study, 20 of 25 food handlers were found to be colonized by staphylococci.

In a study conducted by Udo et al.[26], the researchers found that 81.61% CNS from hand and only 7% CNS from the nasal cavity of the same workers. Francisco Polledo et al.[16] also found that CNS constituted 39.3% and CPS 27.6% of the nasal flora of food handlers. Our results from hands were similar to Udo et al.[26] with 72.8% CNS while results of nasal cavity (76.8% CNS) of the same restaurant workers was higher than Udo et al.[26] and Francisco Polledo et al.[16]. Differences from results may reflect differences in different populations living in different geographical regions as mentioned by Udo et al.[26]. At the same time, workers work in different foods so it is possible that, they introduce a lot of flora to their fauna of microorganisms from these foods.

Udo et al.[26] found that all CNS isolates (n: 155) were susceptible to Vancomycin, Gentamicin, Streptomycin. In our study one (1.19%) hand isolate were resistant to Vancomycin while two (2.38%) isolates were shown medium resistance to Vancomycin. However, all the CPS isolates were found to be susceptible to Vancomycine. Ligozzi et al.[27] was conducted a study to evaluate the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram positive cocci and they used 100 clinical isolates of CNS. Their results also showed that all CNS isolates were susceptible to Vancomycin. Similarly to results of Udo et al.[26], our results also showed that the incidence of antibiotic resistance was lower than that obtained from the skin flora of hospitalized patient and clinical speciment [28-30].

Investigation of SE production from food served in restaurants was shown that five different SE can be detected from those foods and these SE are SEA, SEB, SEC, SED and SEE [31]. It is important to know source and distribution of these enterotoxigenic staphylococci to protect from food poisoning. Francisco-Polledo et al.[16] investigated 201 staphylococci isolates from food workers' nasal cavity for production of enterotoxins. They found that 36 CPS strains produced enterotoxins and distribution of enterotoxins were SEA (12 isolates), SEB (8 isolates), SEC (7 isolates), SED (2 isolates), SEE (2 isolates), SEA + SED (4 isolates) and SEB+SEC (one isolate). In their study, none of the coagulase negative isolates produced enterotoxin. In another study, 207 isolates of *S. aureus* from nasal cavity of restaurant workers were investigated for staphylococcal enterotoxin production and found that 55 isolate produced SE. They found that 18 strains produced SEA, 14 strains produced SEC, 13 strains produced SED and 9 strains produced SEB and SEE [4]. Udo et al.[26] also found that 8% of the CNS (including one nasal isolate) and 12.5% of *S. aureus* from the hands of the food handlers produced one or combination of staphylococcal enterotoxins. In the present study only one hand isolate (1.9%) of 52 CNS isolates and four (2 hand, 2 nasal cavity) isolates (20%) of 20 CPS was produced staphylococcal enterotoxin.

Despite the fact that only one of the CNS produced SE, its detection was significant because it confirms that CNS from different sources can produce SE [32].

The search for food borne pathogenic microorganisms is a common practice at the Public Health Laboratory, but examination for food handlers is sometimes neglected during the investigation of an outbreak. Food handlers must be considered a potential source of enterotoxigenic staphylococci, and the identification of the enterotoxin produced by strains isolated from both food handler and incriminated food will help trace the agent's profile. Although in some countries individuals colonized with staphylococci are not allowed to handle food, this is not a practical solution to the problem, because it is difficult to control. The best solution is the proper training of food handlers in order to prevent the contamination of vulnerable foods, and to instruct them on the need of proper storage of such foods. It is recommended that three approaches to reduce the incidence of food borne disease attributed to food handlers can be used: conducting training and education programs, implementing a Hazard Analysis and Critical Control Points system, and supporting certification of food service manager [9]. In conclusion it is important to know that CNS can also be cause of staphylococcal food poisoning along with *S. aureus* and food handlers could

be contaminate the food easily, if the food not handle carefully.

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