International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

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# Isolation and Characterisation of Staphylococcus aureus Strains from Phyllo Dough (Yufka) in Burdur Province

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**Abstract**

In this study, 11 *Staphylococcus aureus* isolates were isolated and characterized by molecular methods from 50 phylo dough samples. Antimicrobial susceptibility testing against 12 antibiotics was performed by agar disk diffusion method and resistance was detected in 10 strains. 3 strains were oxacillin resistant which are also considered as methicillin-resistant *S. aureus*. Biofilm formation test were carried out by microplate test in TSB, TSB+1% sucrose, TSB+1% glucose, TSB+4% NaCl and BHI, BHI+1% sucrose, BHI+1% glucose and BHI+4%NaCl and biofilm formation detected in 4 strains. *Coa*, *nuc* and *spa* genes were evaulated for molecular identification. Enterotoxin A, B, C, D, and E genes, toxic shock syndrome toxin gene (*tst*) and *mecA* gene were investigated. *MecA* gene not detected, enterotoxin genes detected in 10 strains and *tst* gene detected in 9 strains. Our study confirmed that possible contamination and transmission of antibiotic resistant *S. aureus* from phyllo dough.

***Keywords:*** *Staphylococcus aureus;* antibiotic resistance; biofilm; Staphylococcal enterotoxins; phyllo dough

## 1. Introduction

Staphylococci species are member of Micrococcaceae family abundant in soil, water, air, plants and skin and mucoid membranes of animals as a part of microflora. Therefore, the presence of pathogenic *Staphylococcus aureus (S. aureus)* strains in foods can be considered as an indication of both a lack of hygiene and possible infection in humans [1].

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*S. aureus* causes many infections in humans such as hospital infections, food poisoning, osteomyelitis, polyarthritis, endocarditis, toxic shock syndrome, folliculitis, conjunctivitis, urinary tract infections, pneumonia, scalded skin syndrome (CNS). The prevalence of antibiotic resistance among staphylococcal species is an important problem in the treatment of staphylococcal infections. It is well known that soon after the introduction of new antibiotics into clinical use, the microorganism acquires resistance.

Foods with known risk of staphylococcal contamination are red meat, poultry, canned foods, dairy products, sauces and bakery products [2]. Phyllo dough is also risky because it includes production stages where there is hand contact.

Within the scope of this study, the isolation, identification and molecular characterization of S. aureus strains from pastry phyllo dough sold in workshops, open markets and markets in Burdur were carried out and antibiotic susceptibility, biofilm formation and toxin gene contents, which are important virulence characteristics of the strains, were investigated.

## 2. Material and Methods

In our study, 50 different phyllo dough samples collected from workshops, open markets and markets in Burdur city center were brought to the laboratory on the same day and stored at +4 °C, subjected to bacterial examination within 24 h.

### 2.1. *Bacterial Isolation*

The phyllo dough samples (10 g) were cut under aseptic conditions with sterile scissors and forceps and transferred to the stomacher bag. Sterile peptone water (90 ml) was added and crushed until homogeneous. Samples were inoculated on Baird Parker Agar medium with supplemented egg yolk tellurite emulsion with a dragalsky spatula. After 24 h incubation at 37 °C black colonies with zone formation were selected for isolation and purification on Mannitol Salt Agar and Tryptic Soy Agar. Biochemical identification of bacteria was made by applying Gram stain, catalase, and latex agglutination tests.

### *2.2. Antibiotic Susceptibility Test*

The susceptibility of *S. aureus* strains to 12 antibiotics (penicillin G, rifampicin, cefoxitin, oxacillin, erythromycin, telithromycin, tobramycin, sulfamethoxazole-trimethoprim, ofloxacin, clindamycin, tetracycline, teicoplansin) was determined by agar disc diffusion method in Mueller Hinton Agar medium. Zone diameters around the antibiotic discs were measured after 18 hours of incubation at 35 °C. Results are evaluated according to Clinical Laboratory Standards Institute [3].

### *2.3. Biofilm Test*

The biofilm forming abilities of *S. aureus* strains were tested by microplate test in media containing TSB, TSB+1% sucrose, TSB+1% glucose, TSB+4% NaCl and BHI, BHI+1% sucrose, BHI+1% glucose and BHI+4%NaCl. 20 µl of overnight *S. aureus* strains and 180 µl of medium were added to 96-well microplates

and incubated at 37 °C for 24 hours. Unattached bacteria were removed by washing with 0.9% NaCl, adhered bacteria were fixed with methanol, stained with crystal violet, and after dissolving the dye with 33% acetic acid, absorbance values at 590 nm were measured using an Epoch (BioTek) microplate reader [4].

### *2.4. Amplification of Staphyloccoccal Genes*

Cultures were grown in TSB at 37 °C overnight, and DNA extraction was performed by treatment with lysostaphin (0.1 mg/ml) and proteinase K (0.1 mg/ml). The presence of *coa* [5], *spa* and *nuc* genes specific to *S. aureus* and enterotoxin A, B, C, D, and E genes (*sea, seb, sec, sed, see*), toxic shock syndrome toxin gene (*tst*) and *mecA* gene responsible for methicillin resistance in *S. aureus* strains were investigated by PCR using gene-specific primers according to previous studies [6; 4].

## 3. Results and Discussion

During isolation studies total 96 typical staphylococci colonies were isolated and subcultured from all test materials. But it was determined that 11 isolates out of 96 bacterial isolates were *S. aureus* (11.5%) according to *coa, spa* and *nuc* gene presence. Traditional phyllo dough making is a process in which hand contact is intense, and there is a transition from the skin microbial flora of the people working in its production to the products. The examples used in this study are of the type that need to be prepared by hand completely or partially. Since our study did not aim to determine the microbial quality of food, no evaluation was made for this. In another study with phyllo dough, it was aimed to determine its microbiological quality with culture based methods. 20 samples were examined and *S. aureus* was not detected in only two of them [7].

Antibiotic susceptibility test was performed on 11 strains. All strains were susceptible rifampicin, cefoxitin, telithromycin, tobramycin, sulfamethoxazole-trimethoprim, ofloxacin, clindamycin, tetracycline and teicoplansin. Resistance to oxacillin was also detected in 3 of 9 penicillin-resistant strains. Erythromycin resistance was determined in only one strain (Table 1). Only one strain was susceptible to all tested antibiotics. Methicillin- resistant *S. aureus* (MRSA) has been causing serious health problems in recent years. It was determined that the strains with drug resistance had higher survival rates in environmental conditions [8]. High survival rate leads to further spread of these resistant strains. Antibiotic resistance and enterotoxin gene content have investigated in *S. aureus* strains from milk and dairy products, 40 (8.3%) strains have detected as MRSA according to PCR of *mecA* and culture based methods [9]. In this survey *mecA* gene was not detected in any of the strains suggests that oxacillin resistance may arise from different systems such as efflux pump systems.

Biofilm formation was observed in 4 strains which includes the all antibiotic susceptible strain. The highest biofilm formation was detected in 1% glucose supplemented media. The biofilm formation feature gives the strains a chance to long term survive in harsh conditions, which increases their virulence [1;10]

One *S. aureus* strain was found to carry the *sea* gene, one strain the *seb* gene, 4 strains the *sec* gene, and 8 strains the *sed* gene. The *tst* gene was detected in 9 strains. The results are summarized in Table 1. The presence of enterotoxin genes indicates that these strains can cause food poisoning. Although not as much as the problems caused by antibiotic resistance, food poisoning is also a disease that needs attention.

**Table 1:** Antibiotic resistance, biofilm formation, molecular characterization of bacterial isolates, enterotoxin gene content, methicillin resistance gene and toxic shock syndrome gene test results

| | Antibiotic Resistance | Biofilm Formation | *coa* | *spa* | *nuc* | *sea* | *seb* | *sec* | *sed* | *see* | *mecA* | *tst* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Y9B | Pen. | + | + | + | + | - | - | - | + | - | - | - |
| Y15A | Pen. | - | + | + | + | - | - | - | + | - | - | - |
| Y20A | Ery. | + | + | + | + | - | - | - | - | - | - | + |
| Y26B | | + | + | + | + | - | + | + | + | - | - | + |
| Y26C | Pen. | - | + | + | + | + | - | + | - | - | - | + |
| Y29A | Pen. Oxa. | - | + | + | + | - | - | + | + | - | - | + |
| Y41B | Pen. | - | + | + | + | - | - | + | - | - | - | + |
| Y43A | Pen. | + | + | + | + | - | - | - | + | - | - | + |
| Y43B | Pen. Oxa. | - | + | + | + | - | - | - | + | - | - | + |
| Y44B | Pen. Oxa. | - | + | + | + | - | - | - | + | - | - | + |
| Y44C | Pen. | - | + | + | + | - | - | - | + | - | - | + |

## 4. Conclusion

Our results confirmed that; skin contact is an important factor in the spread of *S. aureus*, different sources can be reservoirs for antibiotic resistant strains, there may be more than one mechanism that provides resistance, it should be noted that biofilm formation increases the survival rate of the strain and facilitates its spread in the environment.

The best measure that can be taken to prevent contamination in food production areas is to follow good production practices and pay attention to hygiene rules.

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