

Detection of Antibiotic Resistance in *Staphylococcus aureus* Strains Isolated from Various Foods

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Abstract: This study was performed to determine the antibiotic resistance patterns of 27 *Staphylococcus aureus* strains, isolated from different foodstuffs, to a range of antibiotics including methicillin, penicillin, ampicillin, cefotaxime, teicoplanin, gentamicin, kanamycin, erythromycin, tetracycline, ciprofloxacin, clindamycin, chloramphenicol, quinupristin/dalfopristin and linezolid. Antibiotic susceptibility testing revealed that 88.9% (24/27) of the strains was resistant to at least one of the antibiotics. Most of *S. aureus* strains showed the resistance to penicillin (62.9%) and ampicillin (59.3%). For other antibiotics, the resistance rates were 22.2% for erythromycin, 11.1% for cefotaxime, kanamycin and tetracycline, and 7.4% for methicillin and clindamycin. Moreover, 18 of the strains had resistance to multiple antibiotics. So that, 12 strains exhibited resistance to two antibiotics, four strains to three antibiotics, one strain to four antibiotics, and one strain to six antibiotics. However, all the strains were found to be susceptible to teicoplanin, gentamicin, chloramphenicol and linezolid.

Key Words: *Staphylococcus aureus*, food, contamination, antibiotic resistance.

Çeşitli Gıdalardan İzole Edilen *Staphylococcus aureus* Suşlarında Antibiyotik Dirençliliğinin Belirlenmesi

Özet: Bu çalışma, çeşitli gıda maddelerinden izole edilen 27 adet *S. aureus* suşunun, metisilin, penisilin, ampisilin, sefotaksim, teikoplanin, gentamisin, kanamisin, eritromisin, tetrasiklin, siprofloksasin, klindamisin, kloramfenikol, kinopristin/dalfopristin ve linezolid gibi antibiyotiklere dirençlilik özelliklerini belirlemek amacıyla gerçekleştirildi. Antibiyotik duyarlılık testi, suşlardan %88.9'unun (24/27) en az bir antibiyotiğe dirençli olduğunu ortaya koydu. *S. aureus* suşlarının çoğunluğu penisilin (%62.9) ve ampisiline (%59.3) dirençlilik gösterdi. Diğer antibiyotikler bakımından dirençlilik oranları, eritromisin için %22.2, sefotaksim, kanamisin ve tetrasiklin için %11.1, metisilin ve klindamisin için %7.4 idi. Üstelik, 18 suş birden fazla antibiyotiğe karşı dirençliliğe sahipti. Öyleki, suşlardan 12'si iki antibiyotiğe, 4'ü üç antibiyotiğe, biri dört antibiyotiğe ve bir suş da altı antibiyotiğe direnç gösterdi. Bununla birlikte, tüm suşların teikoplanin, gentamisin, kloramfenikol ve linezolid'e duyarlı olduğu bulundu.

Anahtar Kelimeler: *Staphylococcus aureus*, gıda, kontaminasyon, antibiyotik dirençliliği.

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Introduction

Staphylococci are ubiquitous Gram-positive bacteria that represent part of the normal bacterial microflora of the skin and mucosal surfaces of humans and animals²¹. *Staphylococcus aureus* is widely recognised as the causative pathogen of various infections, ranging from infections of the skin and soft tissues, to septicaemia and endocarditis⁹.

Although *S. aureus* strains can harbour different virulence genes²², the most notable virulence factors associated with this microorganism are the heat-stable enterotoxins (SEs), that cause the sporadic food-poisoning syndrome or foodborne outbreaks, and the toxic shock syndrome toxin 1 (TSST-1), which diminishes the immune response of a colonized host¹⁶. It is relevant to consider that the transmission of *S. aureus* is possible either by direct contact with animals or through contaminated food⁶. Human handling of food products as well as infection/colonization of livestock or farm workers have been described as mechanisms for the contamination of food with this bacterium³.

Among the factors contributing to the virulence of this pathogen, antibiotic resistance also plays an important role²². Antimicrobial resistance is an increasingly serious public health concern in the worldwide⁷. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production^{1,13}. Increasing antimicrobial resistance and multiple resistance have resulted in increasing difficulties in the treatment of bacterial infections⁵. Among antibiotic resistant staphylococci, multidrug-resistant *S. aureus* strains are of great public concern since resistances make more difficult the treatment of infections²¹. Worldwide, methicillin-resistant *S. aureus* (MRSA) is a significant and increasing cause of nosocomial infections. Cases of illness following the consumption of MRSA-contaminated food have been reported^{10,11}.

The purpose of this work was to determine the resistance profiles to various antimicrobial agents of 27 *S. aureus* strains isolated from food samples including milk and dairy products (cheese, kefir), meat products (raw meatball, sausage) and seafood (fish, shrimp, mussel).

Materials and Methods

Bacterial strains and culture conditions

A total of 27 *S. aureus* strains that had been isolated from various foodstuffs were used in the present study. The origins of the strains used are shown in Table 1. Working stock cultures were kept frozen at -20°C in a 20% glycerol supplemented with Brain-Heart Infusion (BHI, Oxoid CM1135) broth. The cultures were activated in BHI broth at 37°C before use.

Table 1. CLSI disk diffusion breakpoints for *S. aureus* (CLSI, 2011) and diameters of the inhibition zone obtained for 27 *S. aureus* strains in disk diffusion testing

Tablo 1. *S. aureus* için CLSI disk difüzyon sınır değerleri (CLSI, 2011) ve 27 *S. aureus* suşu için disk difüzyon testinde alınan inhibisyon zon çapları

Antimicrobial agents	Disk content	The range of zone diameters (mm) in this study	CLSI breakpoints (mm) S	I	R
Methicillin	5 µg	6-38	≥14	10-13	≥9
Penicillin	10 U	9-46	≥29	-	≥28
Ampicillin	10 µg	8-48	≥29	-	≥28
Cefotaxime	30 µg	6-37	≥23	15-22	≥14
Teicoplanin	30 µg	15-24	≥14	11-13	≥10
Gentamicin	10 µg	18-26	≥15	13-14	≥12
Kanamycin	30 µg	11-28	≥18	14-17	≥13
Erythromycin	15 µg	6-30	≥23	14-22	≥13
Tetracycline	30 µg	10-31	≥19	15-18	≥14
Ciprofloxacin	5 µg	20-35	≥21	16-20	≥15
Clindamycin	2 µg	9-32	≥21	15-20	≥14
Chloramphenicol	30 µg	20-31	≥18	13-17	≥12
Quinupristin/dalfopristin	15 µg	18-29	≥19	16-18	≥15
Linezolid	30 µg	24-35	≥21	-	≥20

S, susceptible; I, intermediate; R, resistant.

Antibiotic susceptibility testing

The antibiotic susceptibility of the strains was evaluated by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute². Single colony of *S. aureus* picked from fresh cultures on Tryptone Soya Agar (TSA, Oxoid CM131), incubated aerobically 37°C for 24 h, were inoculated into Mueller-Hinton Broth (MHB, Oxoid CM405). When broth culture reached the 0.5

Table 2. Antibiotic resistance profiles of *S. aureus* strains isolated from a variety of food**Tablo 2. Gıdalardan izole edilen *S. aureus* suşlarının antibiyotik dirençlilik profilleri**

Strain no.	Source	Coagulase activity															
			ME	P	AMP	CTX	TEC	CN	K	E	TE	CIP	DA	C	QD	LIN	
1	Goat milk	Negative	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
2	Goat milk	Negative	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	Shrimp	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
4	Raw meatball	Positive	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
5	Kefir	Negative	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	Kefir	Negative	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S
7	Kefir	Negative	S	R	R	S	S	S	S	R	S	S	S	S	S	S	S
8	Shrimp	Positive	S	R	R	S	S	S	S	I	S	S	S	S	S	S	S
9	Shrimp	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
10	Fish	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
11	Fish	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
12	Shrimp	Positive	S	R	R	S	S	S	S	I	S	S	S	S	I	S	S
13	Mussel	Negative	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
14	Raw meatball	Positive	S	S	S	S	S	S	S	R	S	I	S	S	S	S	S
15	Cheese	Negative	S	R	R	S	S	S	S	R	S	S	S	S	S	S	S
16	Fish	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
17	Cheese	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
18	Shrimp	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
19	Fish	Positive	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
20	Cheese	Positive	S	S	S	R	S	S	S	I	S	S	R	S	S	S	S
22	Raw meatball	Positive	S	R	R	S	S	S	S	R	S	S	S	S	S	S	S
23	Raw meatball	Positive	S	R	R	S	S	S	S	R	S	S	S	S	S	S	S
24	Kefir	Positive	I	S	S	S	S	S	R	I	I	S	S	S	S	S	S
34	Sausage	Positive	R	R	S	R	S	S	R	S	R	S	R	S	S	S	S
35	Raw meatball	Positive	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
36	Raw meatball	Positive	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
37	Raw meatball	Positive	I	S	S	S	S	S	R	S	S	S	S	S	S	S	S

ME, methicillin; P, penicillin G; AMP, ampicillin; CTX, cefotaxime; TEC, teicoplanin; CN, gentamicin; K, kanamycin; E, erythromycin; TE, tetracycline; CIP, ciprofloxacin; DA, clindamycin; C, chloramphenicol; QD, quinupristin/dalfopristin; LIN, linezolid; R, resistant, I, intermediate susceptible, S, susceptible

McFarland standard turbidity at 30°C, each suspension was spread on the surface of Mueller Hinton Agar (MHA, Oxoid CM337) plates by a sterile ecuvion. Antibiotic disks were placed aseptically on the agar surface and plates were then incubated at 37°C for 24 h. After incubation, the results were recorded by measuring the inhibition zones, and each organism was classified as resistant (R), intermediate (I) or susceptible (S) according to the guidelines of CLSI. *Staphylococcus aureus* ATCC 25923 was used as a reference strain. Table 1 shows CLSI breakpoints for disk diffusion susceptibility testing (zone diameter, mm) of *S. aureus*.

The following antimicrobial susceptibility test disks were used: methicillin 5 µg (Bioanalysis, Turkey), penicillin 10 U, ampicillin 10 µg, cefotaxime 30 µg, teicoplanin 30 µg, gentamicin 10 µg, kanamycin 30 µg, erythromycin 15 µg, tetracycline 30 µg, ciprofloxacin 5 µg, clindamycin 2 µg, chloramphenicol 30 µg, quinupristin/dalfopristin 15 µg, linezolid 30 µg (Oxoid, Basingstoke, UK).

Results

Susceptibility testing of the *S. aureus* strains isolated from different foodstuffs to 14 antimicrobials was carried out with disk diffusion method according to CLSI guidelines. Table 2 summarizes the antibiotic susceptibility profiles of the strains. All of 27 strains were found to be susceptible to teicoplanin, gentamicin, chloramphenicol and linezolid. The highest resistance percentage was obtained for penicillin (62.9%), followed by ampicillin (59.3%), erythromycin (22.2%), kanamycin (11.1%), tetracycline (11.1%), cefotaxime (11.1%), methicillin (7.4%) and clindamycin (7.4%). Table 3 shows the numbers and incidence of resistant *S. aureus* strains to antibiotics tested. Additionally, resistance to multiple antibiotics was observed among the strains.

Discussion

Due to the intensive use of antibiotics in public health and animal husbandry and the risk

of transfer of antibiotic resistance determinants, the incidence of antibiotic resistances in food associated pathogenic bacteria including *S. aureus* has been an increasing problem during the last decades¹⁷.

In the present study, 88.9% (24/27) of strains showed antimicrobial resistance properties to at least one of the antibiotics tested (Table 2). However, all 27 *S. aureus* strains were susceptible to teicoplanin, gentamicin, chloramphenicol and linezolid. The sensitivity of *S. aureus* strains to teicoplanin, gentamicin and chloramphenicol was similar to the antibiotic sensitivity of *S. aureus* isolated from rabbit meat by Rodriguez-Calleja et al.¹⁸. On the contrary, the resistance of *S. aureus* isolates was suggested to gentamicin by Pereira et al.¹⁶, by Ezzeldeen et al.⁴ and by Normanno et al.¹⁴ and to chloramphenicol by Vazquez-Sanchez et al.²⁴ and by Umoh and Odo²³.

As shown in Table 3, the overall antimicrobial susceptibility profiles revealed that the highest percentage of resistance among the strains was detected for penicillin (62.9%), followed by ampicillin (59.3%) and erythromycin (22.2%). The lower resistance rates was observed for kanamycin, tetracycline and cefotaxime (11.1%), methicillin and clindamycin (7.4%). Several authors have reported resistance of *S. aureus* strains obtained from different types of food to a variety of antibiotics. The rates of resistance to penicillin in our work compare with those found by Pereira et al.¹⁶ and Umoh and Odo²³, who, among *S. aureus* from different food origins, observed 73% and 56.7% of resistance to this antibiotic, respectively. Much lower prevalence rates of penicillin resistance were reported for *S. aureus* strains in studies conducted by Shahraz et al.²⁰ (18.7%), by Peles et al. (15) (30.5%), by Rodriguez-Calleja et al.¹⁸ (26.9%) and by Spanu et al.²² (18%). However, Vazquez-Sanchez et al.²⁴ found a high incidence of penicillin resistant *S. aureus* (100%) in fishery products from retail outlets in Galicia (Spain). We found that 59.3% of strains were resistant to ampicillin. Different rates of ampicillin resistance were suggested for *S. aureus* strains obtained from various food samples in Portugal (70%)¹⁶, in Nigeria (50%)²³, in Italy (18%)²² and in Italy (46.4%)¹⁴.

In this investigation, it was found that six (22.2%) of the strains were resistant to erythromycin. Other some workers have also indicated erythromycin resistance with incidence rates of 20.3%²⁰ and 32.0%¹⁴. On the other hand, our

result disagreed with Peles et al.¹⁵ and Vazquez-Sanchez et al.²⁴ who reported that all of *S. aureus* strains from bovine milk in Hungary and fishery products in Spain, respectively, were susceptible to erythromycin.

In our study, 11.1% (3/27) of *S. aureus* strains exhibited resistance pattern to tetracycline, which is below (from 16.7% to 82.4%)^{4,18,23,24} or above (from 0.7% to 6.0%)^{16,22} the incidence rates of tetracycline resistance reported by some other groups.

Table 3. Numbers and incidence of resistant *S. aureus* strains isolated from food-stuffs

Tablo 3. Gıda maddelerinden izole edilen dirençli *S. aureus* suşlarının sayıları ve insidensi

Antibiotics	Resistant strains	
	Number	Incidence (%)
Methicillin	2	7.4
Penicillin	17	62.9
Ampicillin	16	59.3
Cefotaxime	3	11.1
Teicoplanin	0	0
Gentamicin	0	0
Kanamycin	3	11.1
Erythromycin	6	22.2
Tetracycline	3	11.1
Ciprofloxacin	0	0
Clindamycin	2	7.4
Chloramphenicol	0	0
Quinupristin/dalfopristin	0	0
Linezolid	0	0

Methicillin-resistant *S. aureus* (MRSA) was first reported in the United Kingdom (UK) in 1961¹², and MRSA infections had become a global health issue due to the severity of the illness they may cause¹³. MRSA, posing a potential threat to human health, in food samples analysed in several surveys from different parts of the world has been found, with following rates reported: Iran 89%²⁰, Malaysia 20%¹⁹, Egypt 12.6%⁴, Turkey 97.2%⁸, Italy 3.7%¹³. The present survey also showed that two (7.4%) out of 27 *S. aureus* strains had resistance to methicillin. However, some early reports suggested the absence of methicillin-resistant *S. aureus* in bovine milk¹⁵, fishery products²⁴, raw ewe's milk cheese²², food samples implicated in suspected food poisoning complaints and foodborne outbreaks³.

According to Table 2, only two strains had intermediate resistance to quinupris-

tin/dalfopristin and one strain to ciprofloxacin. All the remaining strains were shown to be susceptible to these antibiotic agents. Concerning ciprofloxacin contrary data were reported by Shahraz et al.²⁰ and Vazquez-Sanchez et al.²⁴ who recorded 14% and 100% of *S. aureus* isolates from packaged hamburgers and fishery products, respectively, were resistant for this antibiotic.

18 strains showed multidrug resistance: one was resistant to six antibiotics (methicillin, penicillin, cefotaxime, kanamycin, tetracycline, clindamycin), one strain to four antibiotics, four strains to three antibiotics and twelve strains to two antibiotics.

In conclusion, most of *S. aureus* strains were resistant to one or more antimicrobial agents. Our results showed that animal originated foods harboured the antimicrobial resistant strains of *S. aureus* and can be a likely vehicle for transmission of multidrug resistant strains. Therefore, the monitoring for the emergence of antibiotic resistance in foods and the prudent use of antibiotics in veterinary medicine and animal production is essential for public health.

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