Animal Health, Prod. and Hyg. (2012) 1: 11 - 19

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Research Article

Biotyping of *Staphylococcus* Strains Isolated from Various Sources and Investigations on the Methicillin Resistance

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A B S T R A C T

In this study, 74 *Staphylococcus* strains were isolated from 120 milk samples obtained from dairy cows with clinical and subclinical mastitis, 42 *Staphylococcus* strains were isolated from 60 wounds and 7 *Staphylococcus* strains were isolated from 20 urine samples. Biyotyping was carried out for all *Staphylococcus* strains. Slime values (+, ++, +++, and -) of coagulase positive *Staphylococcus* strains were determined as 15%, 12%, 32.8%, and 40.2%, respectively and coagulase negative strains were determined as 10.7%, 30.4%, 16.0%, and 42.9%, respectively. 41 (33.3%) *Staphylococcus* strains were found to be resistant to methicillin. *S. aureus* strains (46%), *S. intermedius* strains (37.5%), *S. haemolyticus* strains (43%) and all *S. cohnii* subsp cohnii strains (100%) presented similar resistance to vankomisin and teikoplanin. Of 48% to all of strains were resistant to penicilin and 85% of all strains were found to be sensitive to eritromisin, 83% of all strains were found to be sensitive to sulbactam + ampicillin, and 79% of all strains were found to be sensitive to sulphamethaxazole + trimethoprim.

Keywords: Staphylococcus sp., Identification, Methisillin resistance, Antibiotic susceptibility test.

Değişik Kaynaklardan İzole Edilen *Stafilokok* Suşlarının Biyotiplendirilmesi ve Metisilin Direncinin Araştırılması

Ö Z E T

Bu araştırmada rutin teşhis laboratuarına getirilen klinik ve subklinik mastitisli ineklere ait 120 adet süt örneğinden 74 adet, 60 adet yara yeri svabı örneğinden 42 adet ve 20 adet idrar örneğinden ise 7 adet stafilokok suşu izole edildi. İzole edilen suşların identifikasyonları gerçekleştirilerek *Stafilokok* suşlarının tiplendirilmeleri yapıldı. Araştırmada koagulaz pozitif *Stafilokok* suşlarının slime değerleri (+, ++, +++, -) sırasıyla % 15, % 12, % 32.8, % 40.2; koagulaz negatif *Stafilokok* suşlarının ise slime değerleri (+, ++, +++, -) sırasıyla % 10.7, % 30.4, % 16.0, % 42.9 olarak belirlendi. Çalışmada tiplendirilmeleri gerçekleştirilen *Stafilokok* suşlarının 41 (% 33.3)'i metisiline dirençli bulundu. *S. aureus* suşları (% 46), *S. intermedius* suşları (% 37.5), *S. haemolyticus* suşları (% 43) ve *S. cohnii* subsp. cohnii suşlarının tamamı (%100) vankomisin ve teikoplanin'e aynı oranlarda dirençlilik göstermişlerdir. Tüm izolatların % 48'inin penisilin'e dirençli ve % 85 oranında eritromisine, % 83 oranında sulbactam + ampisilin'e ve % 79 oranında sulphamethaxsazole + trimethoprim'e duyarlı olduğu saptanmıştır.

Anahtar Kelimeler: Staphylococcus sp., İdentifikasyon, Methisilin Direnci, Antibiyotik Duyarlılık Testi

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Received: March 5, 2012 / Accepted: May 7, 2012

Introduction

The *Staphylococcus* genus is the microorganisms that belong to Eubacteriales order of Micrococcaceae family. *Staphylocci* are round shaped, having the diameter of 0.5-1.5 mm, mostly irregular clusters, gram positive, non motile, none sporing, facultative anaerobic bacteria. *Staphylococcus aureus* is an important pathogen for human health. They are the main cause and frequently isolated pathogens of life threatening nasocomial infections. In addition to these life-threatening infections (toxic shock syndrome, respiratory system infections, endocarditis, thrombophylebitis, septic arthritis, osteomyelitis, meningitis, sepsis, bacteriemia) these bacteria are isolated from inflammations found in various body parts (Waldvogel, 1995; Gündeş et al., 2000).

S. aureus may be resistant to miscellaneus antibiotics with different ways. The resistant formation is the result of the versatile genetical diversity. High resistancy for antibiotics reduces the success of the therapy and increases the morbidity, mortality, and the hospitality of the patients. Except *S. aureus*, in recent years coagulase negative *Staphylococci* have been reported for being serious pathogens for hospitalizing patients (Ball 1990; Kernodle et al. 1990; Lina et al. 1999). The products detected in vitro systems like slime factor, protein A, bacteriocin, gelatinase, DNAse components are the additinal factors for generation of virulance in addition to host and bacterial effect (Kotilainen, 1990).

In recent 10-15 years, it has been reported that many clinically important bacteria have developed resistancy against first choice antibiotics. The resistancy cause of *Staphylococci* against beta-lactam antibiotics is the binding of these antibiotics covalently with penicilin binding proteins (PBP) responsable for cell wall synthesis. *S. aureus* strains, which are resistant to penicillinases like methicillin, nafcillin, oxacillin should be evaluated in two groups as a new penicillin binding (PBP2a) with a new mecA coded different affinity strains and limited methicillin susceptible strains because of excess beta-lactamase production (Çetin and Ang 1962, Marples and Cooke 1988; Bradley 1992).

The numbers of methicillin-resistant *Staphylococcus aureus (*MRSA) infections have increased and their antibiotic resistancies to wide range of antibacterials have been developed. Especially VISA (Vancomicin Intermediate *Staphylococcus aureus*) strain of MRSA is the best example for this property and started to develop resistancy against vancomicin, the most effective antibiotic for MRSA. This resistancy has developed after *Enterococcus sp.* started to gain resistancy against vancomicin. It is detected that *Enterococcus sp.* has the ability to transfer the genes which gives *S. aureus* the property of resistancy against vancomicin (Töreci et al., 1985). The other concerning features of MRSA, except causing deadly infections, is its resistancy against all penicillinase resistant penicillins (methicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin), cephalosporins, clindamycin, erythromicin, tetracyclin and aminoglycosides, which makes therapy options againist MRSA infections limited (Bradley, 1992).

The resistancy ratios of MRSA strains obtained from 43 laboratories in Italy, France, Spain, Belgium, Austria, Germany, Denmark, Sweden, Holland and Switchzerland were reported as 0-57.9% to rifampin, 30.1-96.8% and 52.9-96% to clindamycin and 47.2-75.9% to trimethoprim-sulfamethoxazole (TMP-SMX) (Voss et al., 1994).

In a study carried out in Cumhuriyet University Faculty of Medicine Medical Microbiology Department (Hasbek et al. 2002), the resistancy ratio of methicillin susceptible and resistant *Staphylococci* are 10.8% and 22.4% for TMP-SMX, 4.3% and 10.8% for amoxycillin-clavulanic acid, 5.4% and 65.5% for ofloxacillin, 18.4% and 53.4% for erythromicin, 13.0% and 67.2% for rifampicin, 14.1% and 74.1% for gentamicin, 19.5% and 57.8% for clyndamicin, 42.3% and 77.5% for cephalotin and tetracyclin, 73.9% and 96.5% for penicilin G, respectively. There was not any resistant strain against vancomicin. When methicillin resistancy was ignored, the highest resistancy rate were detected for penicilin G (96.5%).

In a study conducted in Microbiology Laboratory of Fırat University Faculty of Medicine (Yakupogullari et al. 2006), the susceptibility of *S. aureus* strains for quinolones (ciprofloxacin, ofloxacin, lovoxacin, moxifloxacin) was investigated. The methicillin resistancy ratio for whole strains was detected as 49%, and ciprofloxacin susceptibility was detected in the methicillin susceptible strains as 61%, moxifloxacin susceptibility was detected as 74%; for methicillin resistant strains c iprofloxacin susceptibility was detected as 42%, moxifloxacin susceptibility was detected as 45%.

The aim of this study is serotyping *Staphylococci* strains isolated from different sources and to determine methicillin resistancy and antibiotic susceptibility of these strains.

Material and Method

In this study, a total of 200 samples from various sources (120 milk samples, 60 wound region samples, 20 urine samples) were presented to Department of Microbiology Laboratory Faculty of Veterinary Medicine Adnan Menderes University in order to isolate and to identify *Staphylococcus* strains. The samples were plated out on blood agar (Difco) containing 5% sheeep blood, and then incubated at 37°C for 24-48 hours. Gram staining was carried out on the microorganisms picked up from the colonies. Microorganisms seen as gram positive groups, showing catalase positive activity with 3% H_2O_2 were pointed out as *Micrococcaceae* family (Koneman et al. 1997).

The microorganism that would be investigated was inoculated in 1 ml Tryptic soy Broth (Oxoid) and incubated for 24 hrs at 37°C then plating out was carried out on Mueller –Hinton (Oxoid) agar. Bacitracin disks

(Oxoid) (0.04 U/ml) were placed onto inoculation zone after incubation at 37°C for 18 h, the strains resistant to disks were named as *Staphylococci*, susceptible strains were named as *Micrococci* (Koneman et al. 1997).

The strains evaluated as *Staphylococci* were seperated as coagulase positive and coagulase negative by using 1/5 diluated rabbit plasm with citrate .

Coagulase positive *Staphylococci* strains were identified as *S. aureus* and *S. intermedius* according to clumping factor, phosphatase, asetonin, urease, hemolysin, mannitol, O/F and glycose O/F reactions (Holt et al 1994; Koneman et al. 1997).

Coagulase negative *Staphylococci* strains were evaluated according to novobiocin susceptibility, urease activity, nitrate reduction, maltose, mannose, trehalose, mannitol, sucrose and lactose fermentation, arginine hydrolise activity (Koneman et al. 1997).

Investigation of Slime Factor

Slime factor was investigated with Christensen method (Christensen et al. 1983). The strains grown in sheep blood agar were inoculated into 5 ml Tryptic soy Broth and incubated at 37°C for 48 hours. The media in tubes were emptied and replaced with 0.1% safranine solution, and then shaked. After that, staining solution was emtied and tubes were shaked with water for several times. The strains were evaluated as slime (+) if the visible sheet were seen on tube wall. The ring shaped staining in the region with media was not evaluated as slime (Christensen et al. 1984).

Methicillin Resistancy

The methicillin resistancy of identified *Staphylococci* were inspected by Kirby-Bauer Disc Diffusion Method with Mueller-Hinton (Difco) agar (Bauer et al. 1966, Qoronfleh and Wilkinson 1986).

Antibiotic Susceptibility Test

For antibiotic susceptibility tests, Mueller-Hinton Agar (Difco) was used with Kirby-Bauer Disc Diffusion Method (Bauer et al. 1966, Qoronfleh and Wilkinson 1986).

The used antibiotic discs and their ingredients are:

Penicillin G (Oxoid, P= 10 U), Kanamycine (Oxoid, K= 30µg), Erythromycin (Oxoid, E= 15µg), Ciprofloxacin (Oxoid, CIP= 5µg), Sulbactam+Ampicillin (Oxoid, SAM=Sulbactam 10µg, Ampicilin 10µg), Oxytetracycline (Oxoid, OT= 30µg), Gentamicin (Oxoid, CN= 10µg), Amoxicillin- Clavulanic Acid (Oxoid, AMC= 10 µg), Sulphamethaxazole+Trimethoprim (Oxoid, SXT= 25µg), Vancomycin (Oxoid, VA= 30µg), Teicoplanin (Oxoid, TEC= 30µg).

The plates were incubated at room temperature for 15 min. first, then moved to incubator at 37°C and incubated for 24 hrs. The inhibition zone diameters were calculated and shown in Table 5 (Bauer et al. 1966, Qoronfleh and Wilkinson 1986).

Results

In this study, 74 *Staphylococci* strains were isolated from 120 milk samples obtained from dairy cattle with clinical and subclinical mastitis, 42 *Staphylococci* strains were isolated from 60 wound region samples, 7 Staphylococci strains were isolated from 20 urine samples.

For milk samples, 33 (44.6%) of *Staphylococci* strains were detected as coagulase positive and 41 (55.4%) of them were detected as coagulase negative. All coagulase positive strains (33 strains-100%) were named as *S. aureus* (Table 1). 13 (31.7%) of coagulase negative strains were named as *S. hyicus*, 10 (24.4%) of them were named as *S. epidermidis*, 7 (17.1%) of them were named as *S. sciuri*, 5 (12.2%) of them were named as *S. haemolyticus*, 3 (7.3 %) of them were named as *S. lentis* and 3 (7.3%) of them were named as *S. cohnii subsp. cohnii* (Table 2).

Table 1. Biotypes of Coagulase positive strains (n=67).
Tablo 1. Koagulaz pozitif suşların biyotipleri (n=67).

Biotypes of Coagulase positive strains	Identified samples			Number of identified biotypes	Percentage of identified biotypes (%)
	Milk samples	Wound region samples	Urine samples		
S. aureus	33	22	4	59	88.0
S. intermedius	-	8	-	8	12.0

Table 2. Biotypes of Coagulase negative strains (n=56).
Tablo 2. Koagulaz negatif suşların biyotipleri (n=56).

Biotypes of Coagulase positive strains	Identified samples			Number of identified biotypes Milk samples	Percentage of identified biotypes (%) Wound region samples
	Milk samples	Wound region samples	Urine samples		
S. hyicus	13	7	1	21	37.5
S. epidermidis	10	-	1	11	19.6
S. sciuri	7	2	1	10	17.9
S. haemolyticus	5	2	-	7	12.5
S. cohnii subsp. cohnii	3	1	-	4	7.1
S. lentis	3	-	-	3	5.3

Table 3. Slime values of *Staphylococci* strains.
Tablo 3. Stafilokok suşlarının slime değerleri.

Slime values	Coagulase Positive Cocci (n=67)		Coagulase Negative Cocci (n=56)	
	Strains	%	Strains	%
Slime (+)	10	15.0	6	10.7
Slime (++)	8	12.0	17	30.4
Slime (+++)	22	32.8	9	16.0
Slime (-)	27	40.2	24	42.9

Table 4. Methicillin resistancy of *Staphylococci* strains.
Tablo 4. Stafilokok suşlarının metisilin dirençlilikleri.

Staphylococci strains	Resistant	Methicillin Intermediate	Susceptible
S. aureus (n=59)	20	10	29
S. intermedius (n=8)	3	-	5
S. hyicus (n=21)	7	5	9
S. epidermidis (n=11)	5	-	6
S. sciuri (n=10)	-	4	6
S. haemolyticus (n=7)	4	1	2
S. cohnii subsp. cohnii (n=4)	1	1	2
S. lentis (n=3)	1	1	1

Table 5. Inhibition zone diameters of used antibiotics.
Tablo 5. Kullanılan antibiyotiklere göre inhibisyon zon çapları.

Antibiotics	Resistant (R)	Intermediate (I)	Susceptible (S)
MET (5 µg)	≤ 9	10 – 13	≥ 14
P (10 U)	≤ 27	28	≥ 29
K (30 µg)	≤ 19	21 – 23	≥ 26
E (15 µg)	≤ 13	14 – 17	≥ 18
CIP (5 µg)	≤ 15	16 – 20	≥ 21
SAM (20 µg)	≤ 11	12 – 13	≥ 14
OT (30 µg)	≤ 8	17 – 20	≥ 21
AMC (30 µg)	≤ 19	-	≥ 20
CN (10 µg)	≤ 12	13 – 14	≥ 15
SXT (25 µg)	≤ 10	11 – 15	≥ 16
VA (30 µg)	≤ 9	10 – 11	≥ 12
TEC (30 µg)	≤ 10	11 – 13	≥ 14

Table 6. The distribution of antibiotic susceptibility according to Staphylococci strains.
Tablo 6. Stafilokok suşlarına göre antibiyotik duyarlılıklarının dağılımı.

Staphylococci strains	*ANTIBIOTICS*																	
	P			*K*			*E*			*CIP*			*SAM*			*OT*		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
S. aureus (n=59)	38	5	16	44	1	14	8	6	45	15	7	37	8	4	47	10	37	12
S. intermedius (n=8)	5	-	3	6	1	1	-	-	8	-	2	6	-	-	8	1	3	4
S. hyicus (n=21)	6	3	12	13	5	3	-	2	19	-	-	21	-	3	18	2	8	11
S. epidermidis (n=11)	4	1	6	3	5	3	-	2	9	-	3	8	-	2	9	1	4	6
S. sciuri (n=10)	1	1	8	1	7	2	-	1	9	-	3	7	-	3	7	1	3	6
S. haemolyticus (n=7)	-	2	5	5	1	1	-	-	7	-	1	6	-	1	6	-	2	5
S. cohnii subsp. cohnii (n=4)	3	1	-	4	-	-	-	-	4	-	-	4	-	-	4	-	1	3
S. lentis (n=3)	3	-	-	3	-	-	-	-	3	-	-	3	-	-	3	-	1	2

Staphylococci strains	*AMC*			*CN*			*SXT*			*VA*			*TEC*		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
S. aureus (n=59)	12	7	40	24	8	27	7	4	48	27	3	29	27	2	30
S. intermedius (n=8)	-	2	6	3	-	5	-	1	7	3	1	4	3	-	5
S. hyicus (n=21)	-	3	18	6	2	13	-	5	16	-	3	18	-	-	21
S. epidermidis (n=11)	-	-	11	4	1	6	-	2	9	-	-	11	-	2	9
S. sciuri (n=10)	-	4	6	3	6	1	-	3	7	-	4	6	-	3	7
S. haemolyticus (n=7)	-	2	5	1	4	2	-	3	4	3	1	3	3	-	4
S. cohnii subsp. cohnii (n=4)	-	-	4	3	1	-	-	-	4	4	-	-	4	-	-
S. lentis (n=3)	-	-	3	-	2	1	-	1	2	-	1	2	-	-	3

For wound region samples, 30 (71.4%) of *Staphylococci* strains were detected as coagulase positive and 12 (28.6%) of *Staphylococci* strains were called as coagulase negative. For coagulase positive strains, 22 (73.3%) of them were named as *S. aureus*, 8 (26.7%) of them were identified as *S. intermedius* (Table 1). For coagulase negative strains, 7 (58.3%) of them were named as *S. hyicus*, 2 (16.7%) of the strains were named as *S. haemolyticus*, 1(8.3%) strain was named as *S. cohnii subsp. cohnii*.
For urine samples, 4 (57.1%) of *Staphylococci* strains were detected as coagulase positive and 3 (42.9%) of them were detected as coagulase negative. All of the coagulase positive strains (4 strains-100%) were typed as *S. aureus* (Table 1). For coagulase negative strains, 1 (33.3%) of them was named as *S. hyicus*, 1 (33.3%) of them was named as *S. epidermidis*, 1 (33.3%) of them was identified as *S. sciuri* (Table 2).

Slime values of *Staphylococci* strains were shown in Table 3. The methicillin resistancy of identified *Staphylococci* strains were shown in Table 4. Standard inhibition zones were evaluated when detecting the antibacterial susceptibility of *Staphylococci* (Table 5). The distribution of antibiotic susceptibility according to *Staphylococci* strains was shown in Table 6.

Discussion

Staphylococci are the genus take place in Micrococcaecae family and they have been investigated in medical investigations for many years (Archer 1990). In clinical trials, at first coagulase negative *Staphylococci* were evaluated as saprophytes and unimportant, but in recent years, they were evaluated as very important infectious agents (Töreci et al. 1985; Jawetz et al. 1987; Abigail and Dixie 1994; Ulusoy et al. 1995; Gur et al.1998).

By recent years, the cultured coagulase negative *Staphylococci*, the most often seen type of *Staphylococci* were reported as *S. epidermidis* generally found in the mucosal flora and skin (Bal et al. 1983; Akan et al. 1992). For today, the exploration of pathogenicity of coagulase negative *Staphylococci*, the manifest of *S. haemolyticus*, *S. hominis*, *S. warneri* species as infection agents in various studies (Voss et al. 1994; Akan et al. 1992), different antibiotic patterns seen between the species, the increase of resistant strains points the necessity of serotyping of coagulase negative *Staphylococci* epidemiologically and clinically (Auwera et al. 1990; Kurt et al. 1992; Devriese et al. 1994). In the study presented here, bacitracin susceptibility test was performed to gram positive and catalase positive strains in order to distinguish *Staphylococci* from *Micrococci*.

In Turkey, there are many studies found about mastitis. Kaya et al. (1993) reported clinical and subclinical mastitis cases caused by *S. aureus* with a ratio of 39.40%. Erganis et al. (1995), serotyped 26 of 55 strains as *S. aureus* and 28 of 55 strains as coagulase negative *Staphylococci* in the study made in Konya region for cow and sheep mastitis. In the same study, biotyping of coagulase negative *Staphylococci* was also performed. Kırkan et al. (2005) isolated 85 (28.33%) *S. aureus* and 60 (20%) coagulase negative *Staphylococci* out of 300 mastitic milk sample in the study carried out in Aydın

province. The bacteriological cultures of coagulase negative *Staphylococci*, 20 (33.33%) strains were *S. hyicus*, 16 (26.66%) strains were *S. chromogenes*, 9 (15.00%) strains were *S. epidermidis*, 5 (8.33%) strains were *S. haemolyticus*, 4 (6.66%) strains were *S. sciuri*, 3 (5.00%) strains were *S. lentis*, and 3 (5.00%) strains were serotyped as *S. cohnii subsp. cohnii.*

In the study presented here, 33 (46%) *Staphylococci* strains were identified as coagulase positive, and 41 (55.4%) of strains isolated from milk samples were identified as coagulase negative. All of the coagulase positive strains (33 strains-100%) were identified as *S. aureus* (Table 1). For coagulase negative strains, 13 (31.7%) of them were found as *S. hyicus*, 10 (24.4%) of them were found as *S. epidermidis*, 7 (17.1%) of them were found as *S. sciuri*, 5 (12.2%) of them were found as *S. haemolyticus*, 3 (7.3%) of them were found as *S. lentis* and 3 (7.3%) of them were named as *S. cohnii subsp. cohnii* (Table 2).

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The slime activity was first detected by Christensen et al. (1985) for pathogenic *S. epidermidis* strains. Jones et al. (1992) reported that the most slime producing strain was *S. epidermidis*. Christensen et al. (1984), detected that *S. epidermidis*, *S. haemolyticus* and *S. hominis* strains produced slime activity, but *S. warneri* strains did not. Voss et al. (1994) found slime production activity ratios for of *S. epidermidis*, *S. haemolyticus* and *S. warneri* strains were 57%, for 67 %, and 0%.

In this study, slime positivity was detected at different degrees (+, ++, +++, ++++) in all coagulase positive and negative *Staphylococci* strains.

Based on the information given above, using slime production feature as a pathogenicity test instead of using sole serotyping method would be a better approach (Christensen et al. 1985; Voss et al. 1994).

In most studies, (Christensen et al. 1983; Ishak et al 1985; Akova et al.1989; Bilgehan 1993; Kiraz 1993) slime positivity was detected at a higher ratio in certainly pathogen coagulase negative *Staphylococci* than saprophyte *Staphylococci*. Christensen et al. (1984), detected slime positivity as 60% in clinical blood samples, when this ratio was 37% for contaminants. Akova et al. (1989) detected correlation between isolated coagulase negative *Staphylococci* and slime factor frequency.

In a study in Turkey (Akova et al. 1989) it was reported that slime production was a useful tool for understanding the pathogenesis of the infection. This feature found in *Staphylococci* could be demonstrated by using scanning and transmission electron microscopy, mannose spesific lectin agglutination test (Kotilainen et al. 1991), and spectrophotometric methods. In addition, tube method is also an easy, economical method to perform, and could be used easily in laboratory routines (Degener et al., 1994).

Methicillin, very resistant to penicillinase and effective for *S. aureus* and other *Staphylococci*, was started to be used in early 1960's. However within a few years, MRSA strains were started to be reported at first England then in Turkey (Cetin and Ang 1962). Although at the beginning the incidences of MRSA strains were found to be low, after 1968, hospital infections caused by MRSA were started to be reported in Turkey (Shanson et al. 1976; Gürler and Töreci, 1990).

In this study, methicillin resistancy was detected for *S. aureus* with a rate of 33.8%, for *S. intermedius* as 37.5%, for *S. epidermidis* strains as 45.5%, for *S. haemolyticus* strains as 57.1%, for *S. cohnii subsp. cohnii* strains as 25%, for *S. lentis* strains as 33.0%, no resistancy was detected in *S. sciuri* for methicillin.

The most important risk factors for colonization and infection of MRSA are age, age-dependent infections, nasal colonisation and foreign objects (catheters, tracheostomy, nasogastric tube) (Bradley 1992). In most patients infected by MRSA, the hospitalisation period is long, antibiotic usage is excess and the chance of infection with methicillin susceptible *S. aureus* seems to be high (Hershow et al. 1992). It was reported that colonisation and infection of MRSA strains appeared as sporadic, endemic and epidemic (Marples and Cooke, 1988).

MRSA is characterised with low density of protein A and strong coagulase reaction. The virulance comparison of MRSA and other *Staphylococci* is still under discussion. In addition to this, some health centers reported significant mortality rates with MRSA's (Qoronfleh and Wilkinson 1986; Marples and Cooke 1988). It was reported that MRSA colonize among the patients and infections occured by this way in England. If colonized once, it has been reported that the attempts are inssufficient to remove this colonisation so that MRSA infections occur (Marples and Cooke 1988; Franciolli et al. 1991). The *S. aureus* strains isolated in a Swiss Hospital were detected resistant agains methicillin with a resistance rate of 40-60%, beside this these strains were mostly found to be resistant to multiple antibiotics (Entenza et al. 1994).

MRSA caused hospital infection are generally seen worldwide. In spite of epidemiological differences of MRSA strains, the resistancy features show correlation in all countries (Marples and Cooke 1988). Resistancy for methicillin in *Staphylococci* infections is a criterion for the usage of beta-lactam antibiotics, and it is pointed that beta-lactam antibiotics are not advised for therapy of methicillin resistant strains infections (Boyce et al. 1998).

Ciprofloxacin is the most frequently used antibiotic in recent years in the therapy of MRSA. But as a result of this antibiotic usage, highly dramatic resistance increasement (49-76%) is reported from many countries (Auwera et al. 1990; Raviglione et al. 1990; Harnett et al. 1991). Cross resistancy is seen between the resistancy of quinolones (Harnett et al. 1991).

In this study, 25.4% of the isolated *S. aureus* strains were found to be resistant to ciprofloxacin with but no resistancy was detected for the other isolates which were a remarkable finding. This might be due to the rare usage of ciprofloxacin in the treatment of MRSA infections in this region of Turkey.

In this study, 46.0% of *S. aureus* strains, 37.5% of *S. intermedius* strains, 43.0% of *S. haemolyticus* strains and all *S. cohnii subsp. cohnii* strains showed resistancy against vancomicin and teicoplanin.

The development of antibiotic resistancy in coagulase negative *Staphylococci* is a problem for therapy and eradication of hospital infections (Gray et al. 1984; Younger et al. 1987;Maple et al. 1989; Auwera et al. 1990; Kotilainen et al. 1990; Franciolli et al. 1991). In the early years of penicilin usage, *S. epidermidis* strains were susceptible to Penicillin with a susceptiblity rate of 80.0%, but since 1940's, a resistancy have developed and nowadays it has reached up to 85.0-90.0% (Ludlam et al. 1989; Kloos and Smith 1994). Ulusoy et al (1995) reported penicilin resistancy with a resistancy rate of 79.9%, whereas Kurt et al. (1992) reported this rate as 64.0%. Ciftcioglu (1991) detected penicilin resistancy with a ratio of 38.2% for S*. aureus* strains isolated from healthy individuals.

In a study conducted in by Bal et al. (1983), 564 agent was isolated from 568 mastitic milk samples and coagulase positive strains were susceptible to tetracyclin, ampicillin, erythromicin, penicilin and neomicin with a resistancy rates of 43.2%, 60.6%, 55.5%, 64.7%, and 71.6%, respectively. 87.5% of the *S. aureus* strains isolated were resistant to penicilin.

In this study, 48% of all isolates were resistant to penicilin and 85% of them were susceptible to erythromicin, 83% of them were susceptible to sulbactam+ampicillin and 79% of them were susceptible to sulphamethaxsazole + trimethoprime.

In the veterinary and medical field, when concerning the spread of multiple antibiotic resistancy and infections, and especially when concerning that these situation should effect patients found in risc group, it is important that the correct typing of coagulase positive and negative Staphylococci should be carried out and pathogenic character of the strains should be distinguish correctly. Methicillin, vancomicin and teicoplanin resistancy seen in *Staphylococci* isolated from different sources, may play an important role in forming of disease pathogenesis.

In this study, it was seen that *Staphylococci* isolated from different sources played an important role for infections. According to these data, the isolation and identification methods of *Staphylococci* should be placed in routine diagnosis, the methicillin resistancy should be investigated and antibiotic susceptibility tests should be generalised.

Acknowledgements

This work was the review of the master of science thesis of the first author.

References

Abigail AS and Dixie DW (1994). Bacterial pathogenesis. 1 st. ed. Washington D.C., ASM Press, 30-41.

Akan ÖA, Özalp M, Şener B, Hayran M and Baykal M (1992) Stafilokokların biyokimyasal tiplendirilmesi ve bilgisayar kullanımı. Mikrobiyoloji Bülteni, 26, 103-107.

Akova M, Gür D, Akalın HE and Baykal M (1989). Klinik önemi olan *Staphylococcus epidermidis* suşlarının saptanmasında slime testinin yeri. İnfeksiyon Dergisi, 3, 321-326.

Archer GL (1990). *Staphylococcus epidermidis* and other coagulase negative staphylococci. In : Mandel, G.L., Dougles, R.G., Benett, J.E. (eds.). Principles and Practice of Infectious Diseases. 3rd ed., Melbourne, Churchill Livingstone, pp. 1511-1517.

Auwera PV, Godard C and Denis C (1990). In vitro activities of new antimicrobial againts multiresistant *Staphylococcus aureus* isolated from septisemic patietns during a Belgian national survey from 1983 to 1985. Antimicrob Agents Chemother, 34, 226.

Bal Ç, Altun B and Anğ Ö (1983). Üriner sistem enfeksiyonu ve kolonizasyonunda *Staphylococcus haemolyticus*. Türk Mikrobiyoloji Cemiyeti Dergisi, 23, 159-164.

Ball P (1990). Emergent resistance to ciprofloxacin amongst Pseudomonas aeruginosa and *Staphylocooccus aureus*: clinical significance and therapeutic approaches. J Antimicrob Chemother, 26, 165-179.

Bauer AU, Kirby WM, Sherris JC and Tarck M (1966). Antibiotic susceptibility testing by a standardized single disc method. Journal of Clinical Pathology, 45, 493-494.

Bilgehan H (1993). Temel mikrobiyoloji ve bağışıklık bilimi. 5. Baskı, Ankara, Barış Yayınları, Fakülteler Kitabevi, 212-240.

Boyce JM, Medeiros AA and Rimland D (1998). Detection and traetment of infections caused by Staphylococcus aureus resistant of penicillinase resistant penicillins. Journal of Infectious Disease, 157, 602-605.

Bradley SDF (1992). Methicilin resistant *Staphylococcus aureus* infection. Clinics in Geriatric Medicine, 8, 853-868.

Christensen GD, Parisi JT, Bisno AL, Simpson WA and Beachey EH (1983). Characterization of clinically significant strains of coagulase negative Staphylococci. Journal of Clinical Microbiology, 18, 258-269.

Christensen GD, Simpson WA, Bisno AL and Beachey EH (1984). Adherence of slime producing strains of *Staphylococcus epidermidis* on the human celluler immune response. Lancet, 1, 365-367.

Christensen GD, Simpson WA, Younger JJ, Baddour LM and Barret FF, Melton DM and Beaches EH (1985). Adherence of coagulase-negative staphylococci to plastic tissve culture plates: a quantitave model for the adherence of staphylococci to medical devices. Journal of Clinical Microbiology, 22, 996-1006.

Çetin ET and ANĞ Ö (1962). Staphylococci resistant to methicillin. British Medical Journal, 2, 51-52.

Çiftçioğlu N (1991). Bronşial astım ve rinitli hastaların burun sekresyonlarından izole edilen koagulaz negatif stafilokokların tiplendirilmeleri, bunlara karşı uygulanan bazı patojenite testleri ve antibiyotiklere duyarlılıkları. Ankara Üniversitesi Tıp Fakültesi Sağlık Bilimleri Enstitüsü. Doktora Tezi.

Devriese LA, Laevens H, Haesebrovek F and Hommez J (1994). A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Research in Veterinary Science, 57, 240-244.

Entenza JM, Fluckiger U and Glauser MF (1994). Antibiotic treatment of experimental endocarditis due to methicilin resistant *Staphylococcus epidermidis*. Journal of Infectious Disease, 174, 100.

Erganiş O (1994). Mikrobiyoloji ve İmmünoloji. 2. baskı. Konya Sağlık Bilimleri Enstitüsü Yayınlar No: 11, Konya, 268.

Erganiş O, Kuyucuoğlu Y and Ok Ü (1995). İnek ve koyun mastitislerine sebep olan koaguloz negatif ve koagulaz pozitif stafikokların biyotiplendirilmesi. Veterinarium , 6, 23-27.

Franciolli M, Bille J and Glauser MP (1991). Betalactam resistance mechanismsof methicilin resistance *Staphylococcus aureus*. Journal of Infectious Disease, 163, 514-523.

Gray ED, Peters G, Verstegen M and Regelmann WE (1984). Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. The Lancet, 18, 365-367.

Gündeş G, Karadenizli A and Willke A (2000). Hastane infeksiyonu etkeni olarak izole edilen *Staphylococcus aureus* suşlarında çoğul antibiyotik direncinin değerlendirilmesi. İnfeksiyon Dergisi, 15, 303-306.

Gür D, Özalp M and Sümerkan B (1998). Prevalance of antimicrorobial resistance in respiratory tract pathogens from five centers in Turkey. 8th International Congress of Infectious Diseases, Boston, Abst. no. 82011.

Gürler N and Töreci K 1990. Stafilokoklarda antibiyotiklere direnç gelişimi ve yarattığı sorunlar. İnfeksiyon Dergisi, 4, 699-716.

Harnett N, Brown C and Krishnan C (1991). Emergence of quinolone resistance among clinical isolates of methicilin resistance *Staphylococcus aureus* in Ontario, Canada. Antimicrob Agents Chemother, 35, 632.

Hasbek M, Hakgüdener Y, Kaya S and Bakici MZ (2002). Stafilokoklarda metisilin direncinin farklı yöntemlerle belirlenmesi ve çoğul antibiyotik direnci. Cumhuriyet Üniversitesi Tıp Fakültesi Dergisi, 24,179-184.

Hershow RC, Kayr WF and Smith NL (1992). A comparison of clinical virulance of nozocomially acquired methicilin resistant and methicilin sensitive *Staphylococcus aureus* infection in a univercity hospital. Infection Control and Hospital Epidemiology, 13,587-593.

Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST (1994). Bergey's Manual of Determinative Bacteriology. Ninth Edition. Williams & Wilkins, Baltimore, Maryland, USA, 532, 544-551.

Jawetz E, Melnick JL and Adelberg EA (1987). Rewiew of medical microbiology. 7th ed., California, Appleton & Lange, 217-223.

Jones JW, Scott RJD, Morgan J and Pether JVS (1992). A study of coagulase negative staphylococci with reference to slime production, adherence, resistance patterns and clinical significance. Journal of Hospital Infection, 22, 217-227.

Kaya O, Erganiş O and Kuyucuoğlu Y (1993). Cow mastitis, caused by microorganisms and their susceptibities to antibiotics. Türk Veteriner Hekimleri Birliği Dergisi, 5, 49-50.

Kernodle DS, Classen DC, Burke JP and Kiser AB (1990). Failure of cephalosporins to prevent *Staphylococcus aureus* surgical wound infections. The Journal of the American Medical Association, 263, 961-966.

Kirkan Ş, Göksoy EO and Kaya O (2005). Identification of and antimicrobial susceptibility to *Staphylococcus aureus* and coagulase negative staphylococci from bovine mastitis in the Aydin region of Turkey. Turkish Journal of Veterinary and Animal Science, 29, 791-796.

Kiraz N (1993). Koagulaz negatif stafilokokların slime oluşturmaları ve bazı antibiyotiklerin slime oluşumuna etkileri. 4. Ulusal Enfeksiyon Hastalıkları Kongresi, 27-30 Nisan, İzmir.

Kloos WE and Smith PB (1994). Staphylococci. In: Lenette, E.H., Balows, A., Hausler, W.J., Shadomy, H.J. (eds): Manuel of Clinical Microbiology. 4th ed., Washington D.C., ASM Press., pp. 143-153.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC and Winn WC JR (1997). Color atlas and textbook of diagnostic microbiology. Fifth edition. Lippincott, Philadelphia, New York, 539-565.

Kotilainen P (1990). Association of coagulase negative staphylococcal slime production and adherence with the development and outcome of adult septicemias. Journal of Clinical Microbiology, 28, 2779-2785.

Kotilainen P, Maki J, Oksman P, Viljanen MK, Nikoskelaainen J and Huovinen P (1990). Immunochemical analysis of the extracellular slime substance of *Staphylococcus epidermidis*. Europian Journal of Clinical Microbiology, Infectious Diseases, 9, 262-270.

Kotilainen P, Nikoskelainen J and Huovinen P (1991). Antibiotic susceptibility of coagulase negative staphylococcal blood isolates with special reference to adherent, slime-producing *Staphylococcus epidermidis* strains. Scandinavian Journal of Infectious Diseases, 23: 325-332.

Kurt H, Tural D, Tekeli E and Onul M (1992). Stafilokokların antibiyotiklere invitro duyarlılığı. Ankara Üniversitesi Tıp Fakültesi Mecmuası, 45, 541-548.

Lina G, Quaglia A, Reverdy ME, Leclercq R and Etienne J (1999). Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among staphylococci. Antimicrobial Agents Chemotherapy, 43, 1062-1066

Ludlam HA, Noble WC, Marples RR and Phillips I (1989). The evaluation of atyping scheme for coagulase negative staphylococci suitable for epidemiological studies. Journal of Clinical Microbiology, 30, 161-165.

Maple PA, Hamilton-Miller JM and Brumfitt W (1989). World wide antibiotic resistance in methicilin resistant *Staphylococcus aureus*. Lancet, 1, 537-540.

Marples RR and Cooke EM (1988). Current problems with methicilin resistance *Staphylococcus aureus*. Journal of Hospital Infection, 11, 381-392.

Qoronfleh MW and Wilkinson BJ (1986). Effects of growth of methicilin resistant and susceptible *Staphylococcus aureus* in the presence of beta lactams on peptidoglycan sturcture and susceptibilty to lytic enzymes. Antimicrobial Agents Chemotherapy, 29, 250-257.

Raviglione MC, Boyle JF and Mariuz P (1990). Ciprofloxacin resistant *Staphylococcus aureus* in an acute care hospital. Antimicrobial Agents Chemotherapy, 34: 20-50.

Shanson DC, Kensit JG and Duke R (1976). Outbreak of Hospital Infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methiciline. Lancet, 2, 1347-1348.

Töreci K, Gürler N and Çalangu S (1985). Methicillin-resistance in *Staphlococcus aureus* strains isolated in İstanbul. Ankem Dergisi, 2, 265.

Ulusoy S, Çetin B and Arda B (1995). Metisiline dirençli *Staphylococcus aureus* kökenlerinin antibiyotik direnci. İnfeksiyon Dergisi, 9, 7-10.

Voss A, Milatovic D and Wallrauch-Schwarz C (1994). Methicillin-resistant *Staphylococcus aureus* in Europe. European Journal of Clinical Microbiology & Infectious Diseases, 13, 50-55.

Waldvogel FA (1995). *Staphylococcus aureus*, In: Mandell Gl, Bennett Je, Dolin R (Editors). Principles and practice of infectious diseases. 4. Baskı, New York: Churchill Livingstone, 1754-1776.

Yakupoğullari Y, Gündüz A, Özcan M, Doğukan M, Seyrak A and Yilmaz M (2006). Staphylococcus aureus suşlarının siprofloxasin, ofloksasin, levofloksasin, ve moksifloksasin duyarlılıkları, Fırat Tıp Dergisi 11, 45-47

Younger JJ, Christensen GD, Bartley DL, Simmons JCH and Barrett FF (1987). Coagulase negative staphylococci isolated from cerebrospinal shunts: importance of slime production, species identification and shunt removal to clinical outcome. The Journal of Infectious Diseases, 156, 548-554.