

# Antimicrobial Susceptibility Profiles and Coagulase Gene Polymorphism of *Staphylococcus aureus* Isolated from Bovine Subclinical Mastitis <sup>[1][2]</sup>

Beytullah KENAR <sup>1</sup>  Arzu Funda BAGCIGIL <sup>2</sup> Yahya KUYUCUOGLU <sup>3</sup>  
Beren Başaran KAHRAMAN <sup>4</sup> Selahattin KONAK <sup>5</sup>

<sup>[1]</sup> This work was supported by Scientific Research Projects Committee of AKU. BAPK, Project number: 10.VF.07 (D7)

<sup>[2]</sup> This work has been presented as poster in 10<sup>th</sup> National Veterinary Microbiology Congress, 24-27 September 2012, Kusadası, Aydın, Turkey

<sup>1</sup> Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKEY

<sup>2</sup> Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY

<sup>3</sup> Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKEY

<sup>4</sup> Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, Istanbul - TURKEY

<sup>5</sup> Department Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKEY

Article Code: KVFD-2016-17247 Received: 15.12.2016 Accepted: 31.01.2017 Published Online: 10.02.2017

## Citation of This Article

**Kenar B, Bagcigil AF, Kuyucuoglu Y, Kahraman BB, Konak S:** Antimicrobial susceptibility profiles and coagulase gene polymorphism of *Staphylococcus aureus* isolated from bovine subclinical mastitis. *Kafkas Univ Vet Fak Derg*, 23 (4): 535-540, 2017. DOI: 10.9775/kvfd.2016.17247

## Abstract

The purpose of the study was to isolate *Staphylococcus aureus* from bovine subclinical mastitis, determine their antibiotic susceptibilities and investigate the coagulase gene polymorphism by using a PCR-based restriction fragment length polymorphism (RFLP) method. Milk samples from 463 CMT positive udders from 237 cows cultured. The antimicrobial susceptibility of the isolates were determined by disc diffusion method. A total of 82 out of the 83 isolates (98.8%) were found to be resistant at least one out of the 16 antibiotics studied. In this experiment 53 isolates (63.8%) were found to be resistant to penicillin; 52 (62.67%) to trimethoprim/sulphamethoxazole; 51 (61.5%) to ampicillin; 40 (48.2%) to erythromycin; 29 (34.9%) to tetracycline; 18 (21.6%) to ciprofloxacin, 16 (19.3%) to clindamycin, 13 (15.6%) to chloramphenicol; 8 (9.6%) to gentamicin; 5 (6.0%) to cefoxitin; 4 (4.9%) to vancomycin; 3 (3.6%) to cephalotin; 2 (2.4%) nafcillin; one (1.2%) to oxacillin and one to (1.2%) furazolidon. No imipenem resistance was seen in the *S. aureus* isolates. The coagulase gene polymorphism were examined by PCR amplification of coagulase gene followed by *AluI* digestion of repeating 81 bp DNA sequences. After nested PCR, double bands were produced in 8 of the isolates while there were single band in remaining 75 isolates. Following *AluI* digestion, isolates that formed single band in length of approximately 300 bp showed 3 different groups.

**Keywords:** Bovine subclinical mastitis, *Staphylococcus aureus*, Antibiotic susceptibility, Coagulase gene polymorphism

## Subklinik Mastitli İneklerden İzole Edilen *Staphylococcus aureus* İzolatlarının Antibiyotik Duyarlılık Profillerinin Çıkarılması ve Koagülaz Geni Polimorfizmine Göre Tiplendirilmesi

## Özet

Bu çalışmanın amacı subklinik mastitisli sığırlardan *Staphylococcus aureus*'u izole etmek, bunların antibiyotiklere duyarlılığını belirlemek ve bir PCR tabanlı restriksiyon fragment length polimorfizmi (RFLP) yöntemi kullanarak koagülaz gen polimorfizmi araştırmaktır. 463 sığırdan CMT pozitif olan 237 sığır memesinden süt örnekleri alınarak ekim yapılmıştır. İzolatların antimikrobiyal duyarlılığı disk difüzyon yöntemi ile belirlenmiştir. Toplam 83 izolatın 82'si (%98.8) uygulanan 16 antibiyotikten en az bir antibiyotige dirençli bulundu. Bu çalışmada 53 izolat (%63.8) penisiline, 52 izolat (%62.67 trimethoprim/sulphamethoxazole, 51 izolat (%61.5) ampiciline, 40 izolat (%48.2) eritromisine, 29 izolat (%35.0) tetrasikline, 18 izolat (%21.7) siprofloksasine, 16 izolat (%19.3) klindamisine, 13 izolat (%15.6) kloramfenikole, 8 izolat (%9.6) gentamisine, 5 izolat (%6.0) sefoksitine, 4 izolat (%4.9) vankomisine, 3 izolat (%3.6) sefalotine, 2 izolat (%2.4) nafsiline ve 1'er (%1.2) izolat ise oksasiline ve furazolidona dirençli bulundu. *S. aureus* izolatlarında imipenem dirençliliği görülmedi. Koagülaz gen polimorfizmi koagülaz genin tekrarlanan 81 bp DNA dizisinin *AluI* sindirimini müteakiben koagülaz genin amplifikasyonu ile incelenmiştir. Nested PCR'den sonra izolatların 8'inde çift bant görülmüş kalan 75 izolatta ise tek bant vardı. *AluI* sindirimini müteakiben yaklaşık 300 bp uzunluğunda tek bant oluşturan izolatlar 3 farklı grup göstermiştir.

**Anahtar sözcükler:** Sığır subklinik mastitis, *Staphylococcus aureus*, Antibiyotik duyarlılığı, Koagülaz gen polimorfizmi



## İletişim (Correspondence)



+90 505 3460685



bkenar@aku.edu.tr

## INTRODUCTION

*Staphylococcus aureus* has a wide range host spectrum and can cause serious infections by its methicillin resistant isolates. Long-term antibiotic usage is important in development of resistance against methicillin and other beta-lactam antibiotics. *S. aureus* is one of the most important pathogen for cattle mastitis and it is prevalent all around the world. Despite of strict control measures, control and eradication of *S. aureus* caused intramammary infections are quite difficult and continue as an economical problem. Antimicrobial therapy is one of the measures can be taken in order to control of staphylococcal mastitis. Detection of antibiotic susceptibilities of clinical isolates is necessary not only for treatment but also for preventing spread of resistant isolates. Regional evaluation of antibiotic susceptibilities of *S. aureus* may help veterinary surgeons [1,2]. Although most of the current *S. aureus* isolates have different genotypic and phenotypic characteristics, few are known about geographical distribution of those isolates and types of the pathogens in the herd [3]. Previously, distinct classification methods such as phage typing had been applied to both human and cattle originated *S. aureus* isolates [4,5]. Afterwards, methods such as plasmid analysis, ribotyping, pulsed-field gel electrophoresis, PCR- based fingerprinting, amplification of specific gene regions, and binary typing technics were started to be applied [1,4,6-8]. In recent years, there are some publications about genetic diversity of *S. aureus* isolates in Turkey recovered from subclinical cattle mastitis cases [2,9-13]. The aim of this study was to evaluate the biochemical capacity of the antibiotic resistances of *S. aureus* isolates recovered from subclinical cattle mastitis cases in the Middle Western Anatolia and perform molecular typing on coagulase gene polymorphism.

## MATERIAL and METHODS

### Bacteriological Studies

Milk samples were collected from 16 different dairy farms located in four different districts of Middle Western Anatolia between January-June 2010. Milk samples were collected in the mid-lactation period. California Mastitis Test (CMT) positive 463 milk samples were collected from 237 cows. The samples were inoculated onto Nutrient agar supplemented with 7% sheep blood, incubated at 37°C for 24-48 h. Eighty three *S. aureus* has been isolated and identified by the conventional tests such as Oxidase, catalase and coagulase positive (slide and tube), susceptibility to furazolidone, hemolysis, pigment formation, O/F, Baird Parker Agar (BP), Egg yolk tellurite, Mannitol Salt Agar (MSA), DNase Agar [14,15].

Gram positive cocci were further identified with conventional biochemical test and API Staph (Bio Merieux, France). The isolates were kept at -70°C in Trypticase Soy

Broth (TSB) containing 15% glycerine in order to further use in molecular studies. Antimicrobial susceptibility tests of the isolates were performed in accordance with National Committee for Clinical Laboratory Standards-NCCLS [16]. The isolates were tested against to the following antibiotics: penicillin (10 IU), gentamicin (10 µg), vancomycin (30 µg), clindamycin (2 µg), trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg), cefalotin (30 µg), imipenem (10 µg), nafcillin (1 µg), furazolidone (100 µg), ampicillin (10 µg), tetracycline (30 µg), oxacillin (1 µg), chloramphenicol (30 µg), ceftiofur (30 µg), erythromycin (15 µg) and ciprofloxacin (5 µg). *S. aureus* ATCC 25923 was used as control strain [15]. Chi square test was used to evaluate the significance between antimicrobial sensitivities or resistances of *S. aureus* isolates.

### Molecular Studies

DNA extractions from *S. aureus* isolates were performed by using genomic DNA purification kit (Bio Basic Inc., Totonto, Canada). In addition to the protocol, 11 U lyso-staphin was added during lysis phase. Multiplex PCR developed by Maes et al. [17], were performed for the confirmation of *S. aureus* identification, and for the detection of methicillin resistance. Briefly 2 µL of *mecA* and *nuc* primers (10 µmol), 3 µL of 16S rRNA specific primers (10 µmol), 5 µL of dNTP mixture (2.5mM), 5 µL of 10xPCR buffer, 4 µL of MgCl<sub>2</sub> (25mM), 0.4 µL of Taq polymerase (TaKaRa, Tokyo, Japan) and 2.5 µL of template DNA were added to PCR mixture and made up to 50 µL by adding distilled water. Amplification conditions consisted of 10 min at 94°C, followed by 23 cycles of 1 min at 94°C, 1 min at 51°C, and 2 min at 72°C, with a final step of 5 min at 72°C. The amplified DNA fragments were evaluated following the gel electrophoresis on a 1.5% agarose (Bio Basic Inc., Totonto, Canada) gel stained with ethidium bromide. Nested PCR followed by *AluI* restriction enzyme dependent RFLP method was used to determine the polymorphism in coagulase gene regions of *S. aureus* isolates [18]. Primers to replicate the coagulase gene region described previously by Goh et al. [18] were used in nested-PCR assays. PCR mixture was prepared by adding 2 µL of each COA1 and COA4 primers (10 µmol), 5 µL of dNTP mixture (2.5mM), 5 µL of 10xPCR buffer, 4 µL of MgCl<sub>2</sub> (25mM), 0.4 µL of Taq polymerase (TaKaRa, Tokyo, Japan) and 26.6 µL ultra distilled water to obtain a 50 µL of final mixture, subsequently 5 µL of DNA extract was added and amplified. Fifty µL of similar mixture was prepared for the second cycle of nested PCR but this time 2 µL of COA2 and COA3 primers (10 µmol) were used as primers and 1.5 µL PCR product obtained from previous amplification as target DNA. DNA amplification was performed by pre-denaturation at 95°C for 5 min followed by 40 amplification cycles of 95°C for 30 sec, 55°C for 2 min, 72°C for 4 min and final extension at 72°C for 5 min. Ten µL of nested PCR product was digested by *AluI* restriction enzyme (TaKaRa, Tokyo, Japan) according to the manufacturer's recommended protocol. Both PCR

product and restriction digest fragments were detected by electrophoresis through a 3% agarose gel with  $\Phi$ x 174-Hae III Marker (TaKaRa, Tokyo, Japan) and 100 bp marker (Bio Basic Inc., Toronto, Canada).

Note: Ethics committee approval has been taken from AKU HADYEK on 01.04.2010 with the number 81.

## RESULTS

### Bacteriological Studies Results

Eighty-three *S. aureus* were isolated from milk samples. According to the results of susceptibility tests, 82 out of 83 isolates (98.8%) were resistant at least one of 16 antimicrobial agents involved in the study. In this experiment 53 isolates (63.8%) were found to be resistant to penicillin; 52 (62.67%) to trimethoprim/sulphamethoxazole; 51 (61.5%) to ampicillin; 40 (48.2%) to erythromycin; 29 (34.9%) to tetracycline; 18 (21.6%) to ciprofloxacin, 16 (19.3%) to clindamycin, 13 (15.6%) to chloramphenicol; 8 (9.6%) to gentamicin; 5 (6.0%) to cefoxitin; 4 (4.9%) to vancomycin; 3 (3.6%) to cephalotin; 2 (2.4%) nafcillin; one (1.2%) to oxacillin and one to (1.2%) furazolidon. No imipenem resistance was seen in the *S. aureus* isolates. There were significant differences between antimicrobial sensitivities of *S. aureus* isolates ( $\chi^2=459.03$ ;  $P<0.01$ ). Forty one isolates were multidrug resistant (resistant to four and/or more antimicrobial agents). The antimicrobial susceptibilities of those isolates were shown in Table 1.

**Table 1.** Antimicrobial susceptibilities of *S. aureus* isolates

Antibiotic Disc	Disc Content ( $\mu$ g)	Susceptible (%)	Moderate (%)	Resistant (%)
Penicilin	10 Units	36.2	0.0	63.8
SXT*	25	33.7	3.6	62.7
Ampicillin	10	37.3	1.2	61.5
Erythromycin	15	47.0	4.8	48.2
Tetracycline	30	59.0	6.0	34.9
Ciprofloxacin	5	68.6	9.6	21.6
Clindamycin	2	72.3	8.4	19.3
Chloramphenicol	30	79.5	4.9	15.6
Gentamicin	10	84.3	6.1	9.6
Cefoxitin	30	94.0	0.0	6.0
Vancomycin	30	85.5	9.6	4.9
Cefalotin	30	95.2	1.2	3.6
Nafcillin	1	96.3	1.2	2.5
Oxacillin	1	98.8	0.0	1.2
Furazolidon	100	95.2	3.6	1.2
Imipenem	10	100	0.0	0.0
P	-	<0.01		<0.01

\*Trimethoprim/sulfamethoxazole

### Molecular Studies Results

As the result of multiplex PCR, presence of nuc gene was determined and *S. aureus* identifications were confirmed in 83 isolates which were identified by conventional methods previously. One isolate which was determined to have phenotypic resistance to meticillin was also genotypically positive in presence of mecA. All 83 *S. aureus* isolates showed differences in coagulase gene region polymorphism. After nested PCR, double bands were produced in 8 of the isolates while there were single band in remaining 75 isolates. Following *AluI* digestion, isolates that formed single band in length of approximately 300 bp were also showed 3 different groups. Fragments of 81 bp or multiples were evaluated for RFLP type classification (Table 2). As the result of RFLP typing out of 55 isolates from district A (4 farms), 44 isolates were type II and 11 isolates were type I. Out of 15 isolates from district B (5 farms) eight of them were type I, four of them were type II and three of them were type III in RFLP profile. Isolates from district C (3 farms) were distributed into type II and type IV profiles. And finally the isolates from district D (4 farms) were belonging to type I and type IV. The antimicrobial resistance profiles within the groups were shown in Table 3.

As a consequence, according to antibiogram results, staphylococci have gained resistance to some commonly used antibiotics. That's why it is recommended that it should not be used antibiotic without making an antibiogram.

## DISCUSSION

$\beta$ -Lactam antibiotics are commonly used in cattle mastitis treatment. Penicillin resistance may be related to national policies about usage of antimicrobial drugs and differences about animal raising systems [5]. The highest penicillin resistance was reported to be in Ireland (71.4%) and England (67.3%) within the European countries followed by 50% in USA. It was rather low in Denmark (18.7%) and in Norway (2%) which were the Scandinavian countries [5,19]. It was also shown by Sori et al. [20] that resistance against penicillin was quite high (87.2%) in South-West Ethiopia. There are studies about cattle mastitis showing that penicillin resistance is high in staphylococcus bacteria from isolates in different regions of Turkey. Guler et al. [2] reported the highest resistance was against penicillin and ampicillin as high as 63.3%. In Aydin region resistance against penicillin, SXT and erythromycin were 81%, 17% and 7% respectively [21]. In another study from different region, resistance of *S. aureus* isolates against penicillin G, tetracycline, erythromycin and oxacillin were 85.4%, 39.6%, 5.2% and 3.1%, respectively in mastitic cow milk [10]. The results of this study showed that penicillin resistance (63.86%) in Middle Western Anatolia of Turkey was higher than other antibacterials. This situation is relatively similar to that in other countries such as Ireland, England and USA. It is quite higher than those in Denmark

**Table 2.** Typing of isolates based on PCR and AluI digestion

Number of Isolates (%)	RFLP Type	Sizes of PCR Products (approx.bp)	AluI Profiles*		
			81 (bp)	162(bp)	243(bp)
21 (25.3%)	Type-I	300	+	+	+
51 (61.4%)	Type-II	300	+	-	+
3 (3.6%)	Type-III	300	-	-	+
8 (9.6%)	Type-IV	290, 870	+	-	+

\* Results for only fragments of 81 bp or multiples are shown

**Table 3.** Distribution of *S. aureus* isolates within dairy farms, antimicrobial sensitivities and RFLP types

		Origin of the Samples (number of farms)																Total
		A(4)				B(5)				C(3)				D(4)				
		RFLP Type				RFLP Type				RFLP Type				RFLP Type				
Antimicrobial Agent		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	
		Number of antimicrobial resistant isolates	P	6	33	-	-	5	4	1	-	-	-	-	1	1	-	-
GM	3		2	-	-	1	-	-	-	-	1	-	-	-	-	-	-	7
VA	2		1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	4
DA	1		7	-	-	4	-	-	-	-	-	-	1	2	-	-	1	16
SXT	7		30	-	-	5	-	2	-	-	2	-	4	-	-	-	2	52
CF	1		1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3
IPM	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
NAF	-		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
FX	-		-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
AMP	7		29	-	-	5	2	2	-	-	1	-	3	1	-	-	1	51
TE	3		15	-	-	6	2	1	-	-	-	-	1	-	-	-	2	30
OX	-		2	-	-	1	-	-	-	-	-	-	2	-	-	-	-	5
C	1		8	-	-	1	-	1	-	-	1	-	-	1	-	-	1	14
CTX	-		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
E	5		24	-	-	7	2	-	-	-	2	-	1	1	-	-	1	43
CIP	4	8	-	-	1	-	-	-	-	2	-	1	-	-	-	1	17	
RFLP Type	I	11	-	-	-	8	-	-	-	-	-	-	2	-	-	-	21	
	II	-	44	-	-	4	-	-	-	3	-	-	-	-	-	-	51	
	III	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3	
	IV	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	2	

P: penicillin, GM: gentamicin, VA: vancomycin, DA: clindamycin, SXT: trimethoprim/sulfamethoxazole, CF: cefalotin, IPM: imipenem, NAF: nafcillin, FX: furazolidone, AMP: ampicillin, TE: tetracycline, OX: oxacillin, C: chloramphenicol, CTX: cefoxitin, E: erythromycin, CIP: ciprofloxacin, -; no growth

and Norway. Besides the penicillin, resistance against trimethoprim/sulphamethoxazole and ampicillin were considerably high. These antimicrobial agents are usually preferred for the treatment of mastitis cases in Turkey. Coagulase production is an important phenotypic specification to identify *S. aureus*. Gene coagulase is the most important virulence factor for *S. aureus*. It is reported that both counts and localisations of *AluI* restriction regions in 3'ends of gene coagulase contain a sequence of 81 base pairs which is different between *S. aureus* isolates [18]. The classification of *S. aureus* isolates depending on gene

*coa* is a simple method for molecular typing and can be assumed as a validation test [18,22]. Aslantas et al. [9] reported that RFLP models of gene coagulase of *S. aureus* isolates from cattle mastitis showed a great diversity. They determined that coagulase gene polymorphism of *S. aureus* isolates from mastitic cow milk by RFLP method using *AluI* enzyme produced 9 different genotype strains. Rodrigues da Silva and Silva [22], reported that there were 49 different types of RFLP samples after digestion with *AluI* enzyme. Raimundo et al. [23] examined *coa* gene type of 151 samples of cattle *S. aureus* isolates from 76 farms

by using Coag2 and Coag3 primers and found 6 types of PCR. Su et al.<sup>[3]</sup> investigated coa gene diversity in *S. aureus* isolates from 4 countries. They reported 5 genotypes were dominant for each country. However, dominant types changed according to the geographical regions. Karahan and Cetinkaya<sup>[24]</sup> reported that 83.9% were produced single band and 16.1% produced 2 bands after coa gene amplification in PCR results of 161 coa positive *S. aureus* isolates. They found that 23 different types of restriction profiles in RFLP results by using *AluI* enzyme. Guler et al.<sup>[2]</sup> investigated 125 *S. aureus* isolates which had antibiotic resistance and found that there were 4 types of coagulase gene. In the present study, 90.4% of the isolates produced single band and 9.6% of them produced double bands after coa gene amplification. In this study, 51 out of 83 *S. aureus* isolates were obtained from 7 dairy farms and each of them produced bands approximately 300 bp in length. After RFLP those isolates produced 2 bands (81, 243 bp). Remaining 32 isolates were obtained from 9 different dairy farms and produced 2 types of PCR products of 300 and 290, 870 bp. It was also found that 24 samples to have 2 different types of 300 bp product after RFLP (81, 162, 243 bp and 243 bp) while PCR products of 290, 870 bp in length were produced 2 bands of 81, 243 bp in length after RFLP. This study showed that there were 4 different types of *S. aureus* as Type I, II, III and IV in Middle Anatolia region upon classification by PCR-RFLP. Besides Type II was the most common one to be present in 61.4% of the isolates. Eventually, 33 out of 51 Type II isolates were found to be resistant to penicillin. 6 out of 8 Type IV isolates were resistant to trimethoprim/sulfamethoxazole and, 13 out of total 21 Type I isolates were resistant to ampicillin amongst antibiotics involved in the trial. All of 83 isolates were sensitive to imipenem. However, only 1 isolate were resistant to nafcillin and furazolidon (*Table 3*).

## ACKNOWLEDGEMENT

This experiment was supported by AKU-BAP under the project number 10. VF.07. The authors would like to thank to expert Zahide Kose for her great support in laboratory studies, Assist. Prof. Dr. Ibrahim Kilic for his valuable contribution on statistical analysis and Prof. Dr. Erol Sengör for his valuable support in preparation of this work for publication. We also would like to thank intern students S Yilmaz, OH Karatas, S Kestane, I Orhan, M Colakoglu, A Fiskin, O Ture, EM Ozdemir and S Bilici for their assistance during the laboratory works.

## REFERENCES

- Aarestrup FM, Wegener HC, Rosdahl VT:** Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. *Vet Microbiol*, 45, 139-150, 1995. DOI: 10.1016/0378-1135(95)00043-A
- Güler L, Ok U, Gündüz K, Gülcü Y, Hadimli HH:** Antimicrobial susceptibility and coagulase gene typing of *Staphylococcus aureus* isolated from bovine clinical mastitis cases in Turkey. *J Dairy Sci*, 88, 3149-3154, 2005. DOI: 10.3168/jds.S0022-0302(05)72998-2
- Su C, Herbelin C, Frieze N, Skardova O, Sordillo LM:** Coagulase gene polymorphism of *Staphylococcus aureus* isolates from dairy cattle in different geographical areas. *Epidemiol Infect*, 122, 329-336, 1999.
- Zadoks RN, van Leeuwen WB, Kreft D, Fox LK, Barkema HW, Schukken YH, van Belkum A:** Comparison of *Staphylococcus aureus* isolates from bovine and human skin, milking equipment, and bovine milk by phage typing, pulsed-field gel electrophoresis, and binary typing. *J Clin Microbiol*, 40, 3894-3902, 2002. DOI: 10.1128/JCM.40.11.3894-3902.2002
- Vintov J, Aarestrup FM, Zinn CE, Olsen JE:** Association between phage types and antimicrobial resistance among bovine *Staphylococcus aureus* from 10 countries. *Vet Microbiol*, 95, 133-147, 2003. DOI: 10.1016/S0378-1135(03)00156-1
- Myllys V, Asplund K, Brofeldt E, Hirvela-Koski V, Honkanen-Buzalski T, Junttila J, Kulkas L, Myllykangas O, Niskanen M, Saloniemi H, Sandholm M, Saranpaa T:** Bovine mastitis in Finland in 1988 and 1995-Changes in prevalence and antimicrobial resistance. *Acta Vet Scand*, 39, 119-126, 1998.
- Fitzgerald JR, Hartigan PJ, Meaney WJ, Smyth CJ:** Molecular population and virulence factor analysis of *Staphylococcus aureus* from bovine intramammary infection. *J Appl Microbiol*, 88, 1028-1037, 2000. DOI: 10.1046/j.1365-2672.2000.01071.x
- Saidi R, Cantekin Z, Khelef D, Ergün Y, Solmaz H, Kaidi R:** Antibiotic susceptibility and molecular identification of antibiotic resistance genes of *Staphylococci* isolated from bovine mastitis in Algeria. *Kafkas Univ Vet Fak Derg*, 21, 513-520, 2015. DOI: 10.9775/kvfd.2014.12836
- Aslantas Ö, Demir C, Türütoğlu H, Cantekin Z, Ergün Y, Doğruer G:** Coagulase gene polymorphism of *Staphylococcus aureus* isolated from subclinical bovine mastitis. *Turk J Vet Anim Sci*, 31, 253-257, 2007.
- Ünal N, İstanbulluoğlu E:** İnsan ve sığır kökenli *Staphylococcus aureus* izolatlarının fenotipik ve genotipik özelliklerinin araştırılması. *Ankara Üniv Vet Fak Derg*, 56, 119-126, 2009.
- Karahan M, Acik MN, Cetinkaya B:** Investigation of virulence genes by PCR in *Staphylococcus aureus* isolates originated from subclinical bovine mastitis in Turkey. *Pak Vet J*, 31, 249-253, 2011.
- Türkyılmaz S, Yıldız Ö, Oryaşın E, Kaynarca S, Bozdoğan B:** Molecular identification of bacteria isolated from dairy herds with mastitis. *Kafkas Univ Vet Fak Derg*, 16, 1025-1032, 2010. DOI: 10.9775/kvfd.2010.2300
- Ünal N, Yıldırım M:** Antibiotic resistance profiles of staphylococci species isolated from milks, teat skins and noses mucous of cows. *Kafkas Univ Vet Fak Derg*, 16, 389-396, 2010. DOI: 10.9775/kvfd.2009.833
- Bisping, W, Gunter A:** Colour Atlas for the Diagnosis of Bacterial Pathogens in Animals. Paul Parey Scientific Publishers Berlin and Hamburg. Germany, 1998.
- Quinn PJ, Carter ME, Markey BK, Carter GR:** Clinical Veterinary Microbiology. 2<sup>nd</sup> edn., Mosby, London, UK. 1998.
- National Committee for Clinical Laboratory Standards-NCCLS:** Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard, 8<sup>th</sup> edn., NCCLS document M2-A8, Wayne, PA 2003.
- Maes N, Magdalena J, Rottiers S, de Gheldre Y, Struelens MJ:** Evaluation of a triplex PCR assay to discriminate *Staphylococcus aureus* from coagulase negative staphylococci and determine methicillin resistance from blood cultures. *J Clin Microbiol*, 40, 1514-1517, 2002. DOI: 10.1128/JCM.40.4.1514-1517.2002
- Goh SH, Byrne SK, Zhang JL, Chow AW:** Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. *J Clin Microbiol*, 30, 1642-1645, 1992.
- Moon JS, Lee AR, Kang HM, Lee ES, Joo YS, Park YH, Kim MN, Koo HC:** Antibiogram and coagulase diversity in staphylococcal enterotoxin-producing *Staphylococcus aureus* from bovine mastitis. *J Dairy Sci*, 90, 1716-1724, 2007. DOI: 10.3168/jds.2006-512
- Sori T, Hussien J, Bitew M:** Prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in dairy farms of Jimma Town, South West Ethiopia. *J Animal Vet Adv*, 10, 745-749, 2011. DOI: 10.3923/javaa.2011.745.749

---

**21. Kirkan S, Goksoy EO, Kaya O:** Identification and antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative staphylococci from bovine mastitis in the Aydin region of Turkey. *Turk J Vet Anim Sci*, 29, 791-796, 2005.

**22. da Silva ER, da Silva N:** Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in southern Brazil. *Can J Vet Res*, 69, 260-264, 2005.

**23. Raimundo O, Deighton M, Capstick J, Gerraty N:** Molecular typing of *Staphylococcus aureus* of bovine origin by polymorphisms of the coagulase gene. *Vet Microbiol*, 66, 275-284, 1999. DOI: 10.1016/S0378-1135(99)00020-6

**24. Karahan M, Çetinkaya B:** Coagulase gen polymorphism detected by PCR in *Staphylococcus aureus* isolated form subclinical bovine mastitis in Turkey. *Vet J*, 174, 428-431, 2007. DOI: 10.1016/j.tvjl.2006.05.016