YYU Veteriner Fakultesi Dergisi, 2010, 21 (3), 141 - 145
ISSN: 1017-8422; e-ISSN: 1308-3651

ORIGINAL ARTICLE

Investigation of Antibiotic Susceptibility and Presence of Plasmids in Staphylococci Isolated from Cow Milk with Subclinical Mastitis *

Afsar CELIK[1] Hasan SOLMAZ[2]

[1] *Afsar Pharmacy, Van, Turkey*
[2] *Mustafa Kemal University, Faculty of Veterinary Medicine, Microbiology Dept, Hatay, Turkey*

Received: 05.05.2010 Accepted: 04.06.2010

SUMMARY

The purpose of this study was to investigate the susceptibility of Staphylococci isolates from cow milk with subclinical mastitis to some antibiotics and to determine the presence of plasmids in isolates. For this purpose, a total of 51 Staphylococci isolates were examined. These isolates were isolated from 173 milk samples of dairy cows with subclinical mastitis. It was found that 47 (92.15%) isolates were coagulase-negative and 4 (7.84%) were coagulase-positive. These isolates were found to be susceptible to 6 different antibiotics frequently used in mastitis. Three (5.88%) of the 51 Staphylococci isolates were resistant to oxytetracycline, 3 (5.88%) to sulphamethoxazole-trimethoprim, 6 (11.76%) to novobiocin, 11 (21.56%) to erythromycin, and 24 (47.05%) to penicillin-G. Twenty seven (52.94%) isolates were susceptible to penicillin-G, 40 (78.43%) to erythromycin, 45 (88.23%) to novobiocin, 48 (94.11%) to oxytetracycline, 48 (94.11%) to sulphamethoxazole-trimethoprim, and 51 (100%) to amoxycillin-clavulanic acid. Plasmids were detected in 16 (31.37%) of the isolates. One (6.25%) isolate with plasmid was resistant to erythromycin, 2 (12.50%) to oxytetracycline and 4 (25.00%) to penicillin-G. Twelve (75.00%) of them were found to be susceptible to penicillin-G, 14 (87.50%) to oxytetracycline, 15 (93.75%) to erythromycin, and all of them (100.00%) to novobiocin, amoxicillin-clavulanic acid and sulphamethoxazole-trimethoprim. In conclusion, although plasmids were found in 16 (31.37%) of the coagulase-negative isolates, there were no plasmids in coagulase-positive isolates.

Key Words *Milk, Staphylococci, Antibiotic resistance, Plasmid, Subclinical mastitis*

Subklinik Mastitisli İnek Sütlerinden İzole Edilen Stafilokoklarda Antibiyotik Duyarlılığı ve Plazmit Varlığının Araştırılması

ÖZET

Çalışmanın amacı, subklinik mastitisli inek sütlerinden izole edilen Stafilokok izolatlarının bazı antibiyotiklere duyarlılığının araştırılması ve izolatlarda plazmit varlığının belirlenmesidir. Bu amaçla, toplam 51 Stafilokok izolatı incelendi. İzolatlar subklinik mastitisli ineklerden alınan 173 süt örneğinden izole edildi. Bu izolatlardan 47 (%92.15)'si koagulaz negatif, 4 (%7.84)'ü ise koagulaz pozitif olarak değerlendirildi. İzolatlar, mastitiste sıklıkla kullanılan 6 farklı antibiyotiğe duyarlı bulundu. İzole edilen 51 adet Stafilokok izolatından 3 (%5.88)'ünün oksitetrasikline, 3 (%5.88)'ünün sulfametoksazol-trimetoprim kombinasyonuna, 6 (%11.76)'sının novobiyosin'e, 11 (% 21.56)'inin eritromisin'e, 24 (%47.05)'ünün penisilin-G'ye dirençli; 27 (%52.94)'sinin penislin-G'ye, 40 (%78.43)'ının eritromisin'e, 45 (%88.23)'inin novobiyosin'e, 48 (%94.11)'inin oksitetrasikline, 48 (%94.11)'inin sulfametoksazol-trimetoprim kombinasyonuna, 51 (%100.00)'inin amoksisilin-klavulanik asit kombinasyonuna duyarlı olduğu tespit edildi. İzolatların 16 (%31.37) tanesinde plazmit tespit edildi. Plazmit taşıyan izolatın 1 (%6.25)'i eritromisine, 2 (%12.50)'si oksitetrasikline, 4 (%25.00)'ü penisilin-G'ye dirençli idi. İzolatların 12 (%75.00)'si penisilin-G'ye, 14 (%87.50)'ü oksitetrasikline, 15 (%93.75)'i eritromisin'e ve tamamının (%100) novobiyosin, amoksisilin-klavulanik asit ve sulfametoksazol-trimetoprim kombinasyonlarına duyarlı bulundu. Sonuç olarak koagulaz negatif suşların 16 (%31.37)'sında plazmit tespit edilmesine rağmen, koagulaz pozitif suşlarda plazmit tespit edilmedi.

Anahtar Kelimeler *Süt, Stafilokok, Antibiyotik direnci, Plazmit, Subklinik mastitis*

INTRODUCTION

Staphylococci isolates are the causative agents of many opportunistic infections in humans and animals. Some species of these organisms are recognized as etiological agents of bovine clinical and subclinical mastitis in the world (Trinidad et al. 1990; Rajala-Schultz et al. 2004; Melchior et al. 2007), including Turkey (Alisarli and Solmaz 2003; Güler et al. 2005). Staphylococcal mastitis is encountered as infections with usually acute and rarely chronic courses in cows, sheep, and goats. The infection may cause hardening of udders, atrophying them, and in some cases death due to toxemia. The preliminary and constructive factors for appearance of the mastitis problem in cows are examined in three groups, which are the individual characteristics of the cow, the environmental conditions, and microorganisms

Corresponding author: Hasan SOLMAZ
Mustafa Kemal Universitesi, Veteriner Fakultesi, Mikrobiyoloji AD, Hatay, Turkey. e-mail: hsolmaz@mku.edu.tr
** This study was summarized from master thesis with same title.*

(Baumgartner et al.1984; Arda 1997; Anderson et al. 2006).

Resistance to antibiotics constitutes one of the most important concerns about health at the beginning of the 21st century. Also, the Staphylococci show resistance to some antibiotics widely (Kono et al.1983; Anderson et al.2006). The pharmaceutical industry has developed oxalidions, lipopeptides, injectable streptogramins, ketolides, glycylcyclines, second-generation glycopeptides, and new fluoroquinolones against these problems. However, the clinical use of these agents will constitute new selective pressures and continue triggering resistance development (Woodford 2005).

Plasmids are double-stranded autonomous DNA molecules and carry certain specific genetic information. As they convey resistance to antibiotics and heavy metal, toxin formations, pilus production and virulence factors to the bacterium, they bring some advantages to it (Arda 1997; Moroni et al. 2006; Melchior et al. 2007).

The purpose of this study was to investigate the susceptibility of Staphylococci isolates isolated from cow milk with subclinical mastitis to some antibiotics and to determine the presence and prevalence of plasmids in these isolates.

MATERIALS and METHODS

Isolation and identification of Staphylococcus spp. from milk samples

In this project, a total of 173 milk samples obtained from dairy cows in central Van and its villages between October 2006 and June 2007. Samples were collected under aseptic conditions by hand-milking in accordance with the recommendations of the International Dairy Federation (IDF) (IDF 1998). The California mastitis test (CMT) was applied to the milk samples. For this purpose, 2 ml of milk from different quarters was collected into each quadrant of a plastic CMT paddle with 4 cups. An equal amount of CMT reagent (2% aril alkaline sulfate, 0.01% bromcreosol purple, 15 ml 10% NaOH, and 1000 ml distilled water) was added to it. The mixture in the CMT paddle was rotated for 15-20 sec. The milk samples which became thicker and purple were thought to have mastitis. The samples which were found to have mastitis were put into sterile tubes and transferred to the Microbiology Laboratory, Department of Microbiology, Faculty of Veterinary Science, Yüzüncü Yıl University in shorter time by cold chain.

For microbiological investigation, milk samples were inoculated onto blood agar (Difco, Detroid, USA, 0045-01) with 5% sheep blood and incubated for 24 h at 37°C in aerobic condition. Among the colonies that developed at the end of the incubation, those which had the *Staphylococcus* morphology (approximately 1 mm in diameter, they are round, smooth and glistening, opaque or bright, alpha, beta, gamma and delta haemolysis) were chosen and were stained through the Gram staining method. Gram-positive coccal agents that displayed a cluster-type array at the end of the staining were inoculated onto nutrient agar (Difco, Detroid, USA, 0001-01) in order to obtain one single colony and were incubated for 24 h at 37°C in aerobic condition. The catalase (with 3% hydrogen peroxide), oxidase (with 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride absorbed to sterile filter paper), coagulase (with rabbit plasma), O/F (with glucose) tests were performed by described method. On the other hand, these isolates were harvested in mannitol salt agar (Oxoid, Hampshire England, CM85) (Koneman et al. 1988).

Bacterial strains

Fifty one *Staphylococcus* spp. were isolated in this study and they were tested by the antibiotic susceptibility test and the plasmid presence. The standard *Staphylococcus epidermidis* strain (RSE-65) from culture collection of Microbiology Department, Veterinary Faculty, Yüzüncü Yıl University, Van/Turkey used for the antibiotic susceptibility test and plasmid isolation in this project.

Antibiotic discs and antibiotic susceptibility test

Staphylococcus isolates were tested for their antimicrobial susceptibilities to amoxicillin (30 µg, Oxoid, Hampshire, England), oxytetracycline (30 µg, Oxoid), penicillin-G (10 µg, Oxoid), novobiocin (30 µg, Oxoid), erythromycin (5 µg, Oxoid), and sulphamethoxazole-trimethoprim (25 µg, Oxoid) by Bauer et al. (1966). Antibiotics used in the study were preferred for the often recommended in the treatment of mastitis in the Van region.

Plasmid isolation

The method stated by Mansi et al. (1999) was modified for plasmid isolation from Staphylococci isolates. Briefly, isolated bacteria were inoculated in 5 ml Lauria broth (tryptone 10 gr, yeast extract 5 gr, NaCl 10 gr, distilled water 1000 ml, pH: 7.2) and incubated at 37°C, overnight. After the incubation, 1 ml of the culture was centrifuged in an eppendorf tube at 5000 rpm for 2 min at room temperature (RT). The supernatant was discarded and the pellet was washed twice with TE buffer (10 mM Tris, 1 mM EDTA, pH: 8.0) (solution I). The pellet obtained was then added with 200 µl of freshly prepared solution II (1 M NaOH containing 1% SDS) and then the suspension was mixed. The eppendorf tube was kept in ice for 5 min. It was added with 150 µl of cooled solution III (11.5 ml of glacial acetic acid, 60 ml of 5 M potassium acetate, 28 ml of distilled water). The contents were mixed carefully and then centrifuged at 12.000 rpm for 5 min at 4°C. The supernatant, which contained DNA of the plasmid and some possible RNA, was transferred into a new eppendorf tube which had 20 µl of RNase in order to make sure even the slightest amount of RNA was removed. After being kept in ice for 10 min, it was added with a mixture of phenol/chloroform/isoamyl alcohol (24:25:1) (Applichem, Germany) in equal amounts to the supernatant. After the contents of the eppendorf tube were mixed completely, it was centrifuged at 12.000 rpm at 4°C for 5 min and then the clear supernatant was transferred to a new eppendorf tube. Then, the supernatant was transferred a new eppendorf tube and two volumes of ethanol (-20°C) was added and mixed well. The eppendorf tube was kept at 4°C for 60 min. The plasmid DNA was precipitated at 13.000 rpm for 10 min at RT. The supernatant was removed and it was put on paper tissue upside down so that ethanol was removed from the eppendorf tube. Plasmid DNA pellets were then washed with 1 ml of ethanol (70%) two twice at 4°C. After the supernatant's removal, big drops of ethanol were removed. The rest of the ethanol was removed and then plasmid DNA was dissolved in 50 µl of TE buffer and then the DNA was kept at 4°C for processing.

Agarose gel electrophoresis

The plasmid samples were analyzed by electrophoresis on 0.7% (w/v) agarose gel at 80 mV for 2 h and stained with ethidium bromide (0.5 µg/ml). Ten µl of extracts with suspected plasmids and 3 µl loading buffer were mixed and separated (Mansi et al.1999).

Statistical analysis

The results of antibiotic susceptibility test and plasmid isolation were evaluated in percentage terms (Sümbüloğlu and Sümbüloğlu, 2002).

RESULTS

Staphylococci isolates

Fifty one *Staphylococcus* spp. were isolated from 173 milk samples. It was found that all the isolates examined in Gram positive coccus that displayed a cluster-type array, catalase, coagulase, oxidase, O/F (fermentative) tests and mannitol salt agar were positive. Forty seven isolates were found to be coagulase-negative and 4 were coagulase-positive.

Antibiotic susceptibility test

It was found that 3 (5.88%) of the 51 Staphylococci isolates were resistant to oxytetracycline, 3 (5.88%) to sulphamethoxazole-trimethoprim, 6 (11.76%) to novobiocin, 11 (21.56%) to erythromycin, and 24 (47.05%) to penicillin-G. Twenty seven (52.94%) isolates were susceptible to penicillin-G, 40 (78.43%) to erythromycin, 45 (88.23%) to novobiocin, 48 (94.11%) to oxytetracycline, 48 (94.11%) to sulphamethoxazole-trimethoprim, and 51 (100%) to amoxicillin-clavulanic acid (Table 1).

Table 1. Results of antibiotic susceptibility test

Tablo 1. Antibiyotik duyarlılık test sonuçları

Antibiotics	Resistant		Susceptible	
	No	%	No	%
Oxytetracycline	3	5.88	48	94.11
Novobiocin	6	11.76	45	88.23
Erythromycin	11	21.57	40	78.43
Penicillin-G	24	47.05	27	52.94
Amoxicillin- clavulanic acid	0	0	51	100
Sulphamethoxazole-trimethoprim	3	5.88	48	94.11

Plasmid detection

It was found that 16 (31.37%) of the 51 Staphylococci isolates were contained plasmids in different masses. All the plasmids were detected in coagulase-negative isolates. Fourteen of the plasmids were of various masses and were resistant to the same antibiotic and was also different, while 2 plasmids were both the same masses (Figures 1 and 2).

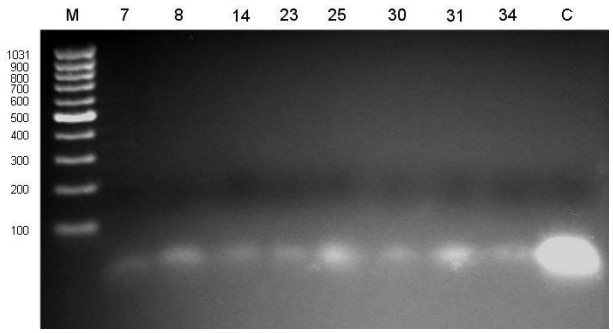

Figure 1. The view of the plasmids in agarose gel, M: Marker (Gene Ruler 100bp DNA Ladder, Fermentas), C: Positive control (standard Staphylococcus epidermidis strain -RSE-65), 7, 8, 14, 23, 25, 30, 31 and 34: The number of coagulase negative isolates.

Şekil 1. Plazmitlerin agaroz jeldeki görüntüsü.

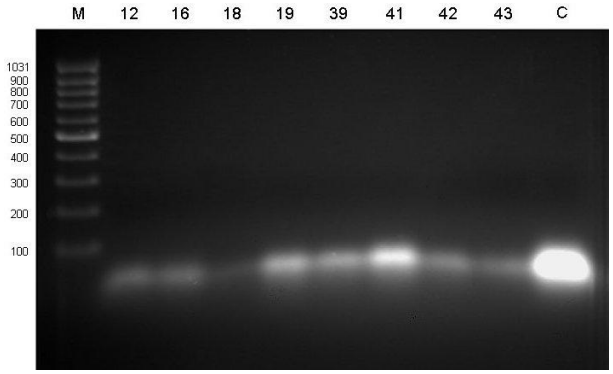

Figure 2. The view of the plasmids in agarose gel, M: Marker (Gene Ruler 100bp DNA Ladder, Fermentas), C: Positive control (standard Staphylococcus epidermidis strain -RSE-65), 12, 16, 18, 19, 39, 41, 42 and 43: The number of coagulase negative isolates

Şekil 2. Plazmitlerin agaroz jeldeki görüntüsü

Comparison of antibiotic test results and plasmid presence

One isolate (6.25%) that having plasmid was resistant to erythromycin and 2 (12.50%) were resistant to oxytetracycline and 4 (25%) to penicillin-G. Twelve of them (75%) were susceptible to penicillin-G, 14 (87.50%) to oxytetracycline, 15 (93.75%) to erythromycin, and all of them (100%) to novobiocin, amoxicillin-clavulanic acid and sulphamethoxazole-trimethoprim (Table 2).

Table 2. Comparison of antibiotic susceptibility test with the presence and absence of plasmid in Staphylococci isolates

Tablo 2. Plazmit taşıyan ve taşımayan stafilokok suşlarının antibiyotik duyarlılık test sonuçlarının karşılaştırılması

	Plasmid (-) (n=35)				Plasmid (+) (n=16)			
	Resistant		Susceptible		Resistant		Susceptible	
Antibiotics	No	%	No	%	No	%	No	%
Oxytetracycline	1	2.85	34	97.14	2	12.50	14	87.50
Novobiocin	6	17.14	29	82.85	0	0	16	100
Erythromycin	10	28.57	25	71.42	1	6.25	15	93.75
Penicillin-G	20	57.14	15	42.85	4	25	12	75.00
Amoxicillin- clavulanic acid	0	0	35	100	0	0	16	100
Sulphamethoxazole-trimethoprim	3	8.57	32	91.42	0	0	16	100

It was found that of 19 (37.25%) Staphylococci isolates that were susceptible to all of the antibiotics, 9 (17.64%) of them contained plasmids. Eighteen (35.29%) isolates were found to be resistance to any antibiotic and 5 (9.80%) of them contained plasmids. It was also determined that 18 isolates were resistance to 2 different antibiotics and they were contained plasmids (Table 3).

Table 3. Comparative results of antibiotic susceptibility test and the plasmid presence in Staphylococci isolates

Tablo 3. Plazmit taşıyan stafilokok suşları ile karşılaştırmalı antibiyotik duyarlılık test sonuçları

Susceptibility to antibiotics*	Number of isolates (n)	Strains containing plasmids	
		Number	%
I	19	9	47.36
II+	18	5	27.77
III+	12	2	16.66
IV+	2	0	0.00
Total	51	16	31.37

*I: Strains resistant to all of the antibiotics, II: Strains resistant to only one antibiotic, III: Strains resistant to two antibiotics, IV: Strains resistant to more than two antibiotics

+Multi-drug resistance isolates

DISCUSSION

Staphylococci are important agents of subclinical and clinical mastitis in dairy cows (Trinidad et al. 1990; Rajala-Schultz et al. 2004; Melchior et al. 2007). In the study of Rajala-Schultz et al. (2004), which was conducted to determine the antimicrobial susceptibility of mastitis pathogens from cow milk with subclinical mastitis, it was stated that 78% of the 202 bacteria that were isolated consisted of coagulase-negative Staphylococci. Costa et al. (2000) reported that 45 *Staphylococcus* spp. isolates from parenchyma of the udders of dairy cows killed in a slaughterhouse, 33 and 12 were coagulase-negative and coagulase-positive, respectively. In this study, 47 of the 51 *Staphylococcus* spp. isolated from dairy cows with subclinical mastitis were found to be coagulase-negative, while 4 were coagulase-positive. The percentages of coagulase-negative and positive *Staphylococcus* spp. were agreement with others (Costa et al. 2000; Rajala-Schultz et al. 2004).

Resistance to antibiotics is the biggest problem encountered in the treatment of bacterial infections (Moroni et al. 2006; Melchior et al. 2007). There are a number of studies related to antibiotic susceptibility of Staphylococci isolated from cases of subclinical and clinical mastitis. (Boynukara et al. 1991; Costa et al. 2000; Alişarlı and Solmaz 2003; Güler et al. 2005; Moroni et al. 2006).

In the study of Boynukara et al. (1991), in which the susceptibility of 16 coagulase-positive *Staphylococcus* spp. isolated from cow milk with subclinical mastitis were tested to 13 different antibiotics and informed that 81.2% of the isolates were resistant to penicillin-G, 73.0% to trimethoprim-sulphametoxazole, 62.5% to colistin sulphate and chlortetracycline, 56.2% to neomycin and carbenicillin, 50.0% to ampicillin, 43.7% to streptomycin and tetracycline, 31.2% to rifamycin, 25.0% to erythromycin, and 6.0% to oxytetracycline. In another study by Watts and Salmon (1997) reported that 70 of the 100 coagulase-positive *Staphylococcus* spp. originated from intramammal infections in cows were susceptible to erythromycin. Alişarlı and Solmaz (2003) examined the 80 CMT-positive and 20 CMT-negative milk samples from the Van region and samples of washing water taken from the teat skin of cows from which milk samples were obtained before milking in terms of coagulase-positive Staphylococci isolates. The researchers isolated coagulase-positive Staphylococci isolates in 38 (38.0%) of the teat skin samples, in 19 (23.7%) of CMT-negative and in 12 (60.0%) of CMT-positive milk samples. On the other hand, the same researchers informed that coagulase-positive *Staphylococcus* spp. were found to be susceptible to sulbactam-ampicillin (32.0%), to amoxicillin (42.0%), to cloxacilline (44.3%), to cefaperazone (68.0%), to oxytetracycline (75.0%), to erythromycin (83.0%) and to gentamicin (100.0%). Güler et al. (2005) reported that 79 (29.8%) of 265 coagulase-positive Staphylococci isolates were susceptible to penicillin, ampicillin, amoxicillin-clavulanic acid, kanamycin–cephalexin, oxacillin, oxytetracycline, trimethoprim–sulphametoxazole and enrofloxacin. The researchers stated that 63.3% of the strains were resistant to penicillin and ampicillin, while they observed no resistance to amoxicillin-clavulanic acid and kanamycin-cephalexin and to oxacillin and enrofloxacin. Anderson et al. (2006) reported that 37 of coagulase-positive *Staphylococcus* spp. isolated from dairy cow with subclinical mastitis and all of them were resistant to penicillin-G, 1 isolate to erythromycin, and 9 isolates to both penicillin and erythromycin. In this study, it was found that 3 (5.88%) of the 51 *Staphylococcus* spp. were resistant to oxytetracycline, 3 (5.88%) to sulphamethoxazole-trimethoprim, 6 (11.76%) to novobiocin, 11 (21.56%) to erythromycin, and 24 (47.05%) to penicillin-G, and 27 of them (52.94%) were susceptible to penicillin-G, 40 (78.43%) to erythromycin, 45 (88.23%) to novobiocin, 48 (94.11%) to oxytetracycline, 48 (94.11%) to sulphamethoxazole-trimethoprim, and 51 (100.0%) to amoxicillin-clavulanic acid. According to these results, the two antibiotics to which the greatest resistance were found as penicillin-G (47.05%) and erythromycin (21.56%).

It was observed that the resistance rates of the strains isolated in this study against erythromycin (21.56%) and oxytetracycline (5.88%) were close to the rates of the isolates in Boynukara et al. (1991), Watts and Salmon (1997), and Alişarlı and Solmaz (2003) against the same antibiotics, and to the rates of the isolates in Moroni et al. (2006) and Güler et al. (2005) against oxytetracycline. Besides, the resistance of the isolates in Boynukara et al. (1991), Güler et al. (2005), and Anderson et al. (2006) against penicillin-G was higher than that of the isolates in this study against penicillin-G (47.05%).

Kono et al. (1983) reported that the genes of resistance to penicillin and erythromycin could be found on the same plasmid. In our study, however, even though sequence-based genetic examination was not carried out, it was observed that 6 of the strains from which plasmids were detected were resistant to both penicillin and erythromycin, but plasmid masses were all different (Figure 1 and 2).

Although it was stated in Baumgartner et al. (1984) that the strains did not have antibiotic resistance among 85 coagulase-positive Staphylococci isolates isolated from cows with chronic mastitis in 18 farms did not contain plasmids, resistance to antibiotics was also found in strains in which no plasmids were detected in our study. These results indicate that antibiotic resistance may not be related to the presence of plasmids.

Arslan et al. (2004) examined the antibiotic susceptibility of 40 coagulase-positive and 10 coagulase-negative *Staphylococcus* spp. isolated from cows with mastitis using standard bacteriological methods and their plasmid profiles. It was stated that 38 (95.0%) of 40 coagulase-positive and 9 (90.0%) of 10 coagulase-negative Staphylococci isolates were contained plasmids. However, in our study, while plasmids were detected in 16 of 47 coagulase-negative Staphylococci isolates and none of the 4 coagulase-positive Staphylococci isolates were contained plasmids.

Piccinini and Zecconi (2001) reported that one or more than one plasmids in 9 different masses in 3 of 4 coagulase-positive Staphylococci isolates were detected. However, no plasmids were detected in coagulase-positive Staphylococci isolates isolated in our research. On the other hand, 14 different masses of plasmids were detected in 16 (31.37%) of the coagulase-negative isolates (Figure 1 and 2).

Plasmids were detected in 16 (31.37%) of the Staphylococci isolates in this study. Fourteen of the plasmids were of various masses and the masses of the plasmids detected in different strains that were resistant to the same antibiotics were also different, while 2 plasmids were both the same mass (Figure 1 and 2). All of the plasmids were detected in coagulase-negative strains. Seven of the strains containing plasmids (43.75%) were resistant to antibiotics.

In conclusion, high resistance seen in animals with subclinical mastitis against certain antibiotics clearly indicates that the effectiveness of antibiotic treatment administered without carrying out antibiogram tests will be low. The fact that 16 (31.37%) strains with plasmids were found in 51 Staphylococci isolates show that plasmids have considerable importance in resistance transfer; however, the fact that resistance to various antibiotics was observed in strains with no plasmids detected show that antibiotic resistance is not only dependent on the presence of plasmids.

ACKNOWLEDGEMENT

This study summarized from the master thesis of Afsar CELIK. It was supported by the Office for Scientific Research Projects of University of Yuzuncu Yil, Van, Turkey (Project No. 2006-SBE-110).

REFERENCES

Alişarlı M, Solmaz H (2003). The pathogenic properties and the varied antibiotic sensitivities of *S. aureus* isolated from teat skin and raw milk of dairy cows (Article in Turkish). *Atatürk Üniv Ziraat Fak Derg,* 34, 333-339.

Anderson KL, Lyman RL, Bodeis-Jones SM, White DG (2006). Genetic diversity and antimicrobial susceptibility profiles among mastitis-causing Staphylococcus aureus isolated from bovine milk samples. *Am J Vet Res,* 67,1185-1191.

Arda M (1997). *Temel Mikrobiyooji*. (1. Baskı), Ankara, Medisan Yayınları. PP: 183–201.

Arslan E, Açık L, Uçan US, Çelebi A (2004). Antimicrobial resistance and plasmid typing of coagulase-positive *Staphylococcus* species isolated from bovine mastitis (Article in Turkish). *S Ü Fen Ed Fak Fen Derg,* 24, 45- 50.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol,* 45, 493–496.

Baumgartner A, Nicolet J, Eggimann M (1984). Plasmid profiles of *Staphylococcus aureus* causing bovine mastitis. J Appl Bacteriol, 56, 159-163.

Boynukara B, Timurkan H, Kuyucuoğlu Y (1991). A research on the antibiotic susceptibility of *Staphylococcus aureus* strains isolated from dairy cows (Article in Turkish). *Veterinarium,* 2, 13–16.

Costa EO, Benites NR, Guerra JL, Melville PA (2000). Antimicrobial susceptibility of *Staphylococcus* spp. isolated from mammary parenchymas of slaughtered dairy cows. *J Vet Med B Infect Dis Vet Public Health,* 47, 99–103.

Güler L, Ok U, Gündüz K, Gülcü Y, Hadimli HH (2005). Antimicrobial susceptibility and coagulase gene typing of *Staphylococcus aureus* isolated from bovine clinical mastitis cases in Turkey. *J Dairy Sci,* 88, 3149–3154.

IDF (1998). *Milk and milk products.* Enumeration of coliforms. IDF standard 73B, International Dairy Federation, Brussels.

Koneman EW, Allen SD, Dowell VR, Janda WM, Sommers HM, Winn, WC (1988). *Color atlas and textbook of diagnostic microbiology.* (Third edition), Pennsylvania, Lippincott Company, PP: 337-343.

Kono M, Sasatsu M, Koji OHK, Shiomi Y, Hayasaka T (1983). Mechanism of resistance to some cephalosporins in *Staphylococcus aureus*. *Antimicrob Agents Chemother,* 23, 938-940.

Mansi M, Anderson KJ, Inche CA, Knowles LK, Platt DJ (1999). Isolation and curing of the *Klebsiella pneumoniae* large indigenous plasmid using sodium dodecyl sulphate. *Res Microbiol,* 151, 201–208.

Melchior MB, Fink-Gremmels J, Gaastra W (2007). Extended antimicrobial susceptibility assay for *Staphylococcus aureus* isolates from bovine mastitis growing in biofilms. *Vet Microbiol,* 125, 141-149.

Moroni P, Pisoni G, Antonini M, Villa R, Boettcher P, Carli S (2006). Antimicrobial drug susceptibility of *Staphylococcus aureus* from subclinical bovine mastitis in Italy. *J Dairy Sci,* 89, 2973–2976.

Piccinini R, Zecconi A (2001). Relationship among plasmids recovered from *Staphylococcus aureus*, milk leukocytes, and antimicrobial resistance. *J Dairy Sci,* 84, 2641–2648.

Rajala-Schultz PJ, Smith KL, Hogan JS, Love BC (2004). Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. *Vet Microbiol,* 102, 33–42.

Sümbüloğlu K, Sümbüloğlu V (2002). Biyoistatistik. (10. Baskı), Ankara, Hatiboğlu Yayınevi.

Trinidad P, Nickerson SC, Luther DG (1990). Antimicrobial susceptibilities of *Staphylococcal* species isolated from mammary glands of Unbred and Primigravid dairy heifers. *J Dairy Sci,* 73, 357-362.

Watts JL, Salmon SA (1997). Activity of selected antimicrobial agents against strains of *Staphylococcus aureus* isolated from bovine intramammary infections that produce ß-lactamase. *J Dairy Sci,* 80, 788–791.

Woodford N (2005). Biological counterstrike. Antibiotic resistance mechanisms of Gram-positive cocci. *Clin Microbiol Infect,* 11, 2–21.