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Characterization of *Staphylococcus aureus* strains isolated from subclinic bovine mastitis by protein patterns, antibiotic resistance and plasmid profile*

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Summary: A total of 50 *Staphylococcus aureus* strains isolated from bovine subclinical mastitis in Hatay region were characterized by protein patterns, antibiotic resistance and plasmid profiles. SDS-PAGE analysis of whole-cell protein extracts belong to *S. aureus* strains produced patterns containing 17-49 discrete bands with molecular weights of >14.4-<116 kDa. *S. aureus* strains clustered into two cluster on the basis of a computer-assisted numerical analysis. The resistance rates of 50 of *S. aureus* strains to trimethoprim-sulphamethoxazole, danofloxacin, amoxicillin-clavulanic, sulbactam-ampicillin, erythromycin, lincomycin, cefoperazone, oxytetracycline, amoxicillin, streptomycin and penicillin were 4 %, 4 %, 10 %, 14 %, 30 %, 34 %, 36 %, 38 %, 50 %, 54 % and 68 %, respectively. Plasmid profiling demonstrated that 47 of the 50 *S. aureus* strains contained plasmid ranging from 1.7 to >24 kb.

Key words: Mastitis, plasmid, protein, *S. aureus*.

Subklinik inek mastitislerinden izole edilen *Staphylococcus aureus* suşlarının protein paterni, antibiyotik direnci ve plazmid profili yönünden karakterizasyonu

Özet: Hatay bölgesindeki subklinik inek mastitislerinden izole edilen toplam 50 *Staphylococcus aureus* suşunun protein paterni, antibiyotik dirençliliği ve plazmid profili yönünden karakterizasyonu yapıldı. *S. aureus* suşlarının SDS-PAGE ile analizinde moleküler ağırlığı <14.4->116 kDa arasında değişen 17-49 bant elde edildi. Elektroforetik protein bantlarının bilgisayar destekli numerikal analizi sonucunda *S. aureus* suşları 2 gruba ayrıldı. *S. aureus* suşları trimethoprim-sülfametoksazol, danofloksasin, amoksisilin-klavulanik asit, sulbaktam-ampisilin, eritromisin, linkomisin, sefoperazon, oksitetrasiklin, amoksisilin, streptomisin ve penisilin'e sırasıyla % 4, % 4, % 10, % 14, % 30, % 34, % 36, % 38, % 50, % 54 ve % 68 oranlarında dirençli bulundu. İncelenen izolatların 47'sinde, büyüklüğü 1.7->24 kb arasında değişen plazmidler saptandı.

Anahtar sözcükler: Mastitis, plazmid, protein, *S. aureus* .

Introduction

Mastitis is one of the major causes of economic losses in dairy cattle (31). It has been known that various microorganisms cause mastitis in cows, the major microorganism is *Staphyloccocus aureus* (29). *S. aureus* infections of the udder usually results in subclinical mastitis. However infections make progress into clinical manifestation including systemic symptoms (26).

From the epidemiological point of view, it is important to determine the origin of the organisms involved in the etiology of the disease. Therefore an exact identification of bacterial pathogens is an unavoidable requirement in order to detect the reservoirs and sources of infection between animal populations (16).

There is considerable genetic heterogenecity through natural populations of *S. aureus* (12, 28). Many different techniques are available for tracing the spread of single *S. aureus* strain of human and animal origin, such as antibiotyping (1, 24), the biochemotyping (16, 17), the phage typing (30), protein electrophoresis (7, 31), plasmid profiling (4, 21), RFLP-PCR (11), RAPD-PCR (10, 20) and PFGE (3, 27). Despite the presence of all these methods, each of these techniques has advantages and disadvantages in their discriminatory power, reproducibility and typeability.

The aim of this study was to investigate the strains of *S. aureus* obtained from the cases of subclinical mastitis in Hatay according to SDS-PAGE of whole-cell protein, antibiotic resistance and plasmid profiles.

Materials and Methods

Bacterial strains

A total of 50 *S. aureus* strains isolated from subclinical bovine mastitis at 11 different localities of Hatay was studied. Isolation and identification of the strains were made as described before (14).

Total protein analysis

The total protein samples were extracted as described by Kishore et al. (13). Total protein analysis was carried out by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (15). Each run included marker proteins with known molecular weights. The gels were stained overnight with Coomassie Brillant Blue G-250 according to Bushuk et al. (6) and Demiralp et al. (9).

Cluster analysis

Different fragments on the gel were numbered sequentially, following that the presence and absence of fragments in each sample was scored (present 1, absent 0) and compared with each other according to the genetic distance method of Nei (22). Strains were then clustered by the method of unweighted pair group average linkage (UPGMA).

Antibiotic susceptibility test

The susceptibility test was performed using the disc diffusion method on Mueller-Hinton Agar (Oxoid) plates (4). The following antibacterial agents (Oxoid) were used: penicillin (P) (10 U), amoxicillin (AML) (10 µg), oxytetracycline (OT) (30 µg), erythromycin (E) (15µg), streptomycin (S) (10 µg), lincomycin (L) (10 µg), trimethoprim+sulphamethoxazole (SXT) (1.25 µg-23.75 µg), danofloxacin (DFX) (5 µg), cefoperazone (CEP) (75 µg), sulbactam-ampicillin (SAM) (10 µg/10 µg) amoxicillin-clavulanic acid (AMC) (20 µg-10 µg). *Staphylococcus aureus* ATCC 25923 was used as the control strain.

Plasmid DNA analysis

Plasmid DNA was prepared according to the method described by Douglas and McKay (2). DNA was electrophoresed in 0.8 % agarose gel (19).

Results

SDS-PAGE of whole-cell protein extracts of *S. aureus* strains produced patterns containing 17 to 49 discrete bands with molecular weights of >14.4-<116 kDa (Figure). SDS-PAGE analysis of *S. aureus* isolates did not show very distinct protein profiles except strains with numbers 10, 44 and 47. Cluster dendrograms produced by genetic distances of whole cell protein showed two main clusters and average similarity among *S. aureus* strains were between 6 % and 69 %.

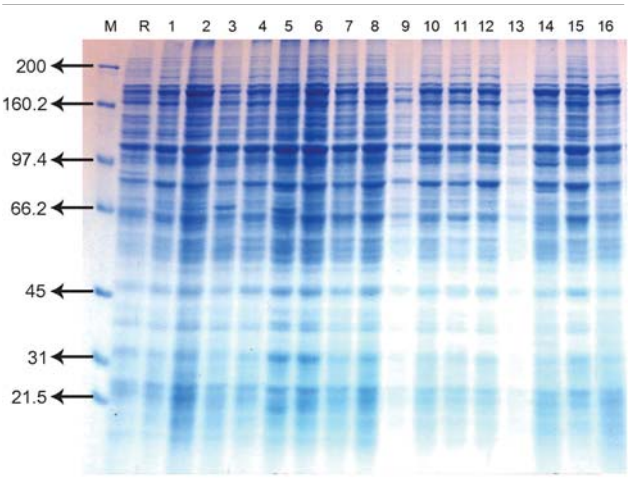

Figure. Whole cell protein profiles of *S. aureus* strains by SDS-PAGE. M: Marker (kDa), R: *S. aureus* ATTC 29213, 1-16: *S. aureus* strains

Şekil. *S. aureus* suşlarının SDS-PAGE tüm hücre protein profilleri. M: Marker (kDa), R: *S. aureus* ATTC 29213, 1-16: *S. aureus* suşları

Trimethoprim-sulphamethoxazole, danofloxacin, amoxicillin-clavulanic acid, and sulbactam-ampicillin were the most effective antibiotics for *S. aureus* strains. The susceptibility rates of *S. aureus* to these antibiotics were: 96 % for trimethoprim-sulphamethoxazole and danofloxacin, 90 % for amoxicillin-clavulanic acid, and 86 % for sulbactam-ampicillin. However high resistance rates were found to penicillin (68 %), streptomycin (54 %), amoxicillin (50 %), oxytetracycline (38 %), cefoperazone (36 %) and lincomycin (34 %) among *S. aureus* strains.

Plasmids were detected in 47 (94 %) of *S. aureus* strains. Plasmid profiles and antibiotic resistance of *S. aureus* strains are shown in Table. Molecular weight of plasmids varied from >24 kb to 1.7 kb. Seven different molecular weights were identified for each strain. Most of the strains showed only single plasmid band with size of 19.3 kb, but the rest of the strains had 2 to 4 plasmids ranging from >24 kb to 1.7 kb. The most common plasmid of 19.3 kb was detected in all strains . In contrast to other strains studied, plasmid was not detected in 3 strains of *S. aureus*. Thirtyeight of *S. aureus* strains contained single plasmid, and 9 strains contained multiple plasmids. All of the strains containing multiple plasmids with a common plasmid of 19.3 kb.

Discussion and Conclusion

The aim of this study was to investigate *S. aureus* strains from subclinic bovine mastitis collected in Hatay region by using protein patterns, antibiotic resistance, and plasmid profile analysis.

SDS-PAGE of proteins has been using increasingly concerning bacterial systematics both at genus and the

Table. Antibiotic-resistance patterns and plasmid profiles of *S. aureus* strains
Tablo. *S. aureus* suşlarının antibiyotik direnç ve plazmid profilleri

Isolates	No. of plasmids	Molecular weight (kb)	Antibiotic-resistance patterns
1	1	19.3	P, OT
2	1	19.3	L, S, P, AML, AMC, CEP, SAM
3	1	19.3	CEP, S, P, AML
4	1	19.3	E, OT, L, P
5	1	19.3	CEP, E, P, AML, SAM, OT
6	1	19.3	DFX, S, P, AML
7	1	19.3	-
8	1	19.3	-
9	-	-	-
10	1	19.3	P, S, AML, AMC
11	-	-	OT, L, E, S
12	1	19.3	L, CEP, AML, E, P, S
13	1	19.3	-
14	1	19.3	CEP, P, AML
15	1	19.3	S, P, AML
16	-	-	-
17	1	19.3	OT, L, P, S, SAM
18	1	19.3	CEP, P, AML, L, S
19	1	19.3	OT
20	1	19.3	OT, L, P, S
21	2	>24,19.3	L, CEP, AML, E, P, S
22	1	19.3	SAM, OT
23	1	19.3	-
24	2	19.3, 1.8	CEP, S, P, AML, OT
25	2	>24,19.3	S, P
26	1	19.3	-
27	2	19.3, 1.8	P, AML
28	1	19.3	-
29	1	19.3	E, S, P
30	2	19.3, 2	S, AMC, P, CEP
31	4	19.3, 5.2, 4, 1.8	CEP, P, S
32	3	19.3, 2, 1.8	E, S, P, AML
33	2	19.3, 1.8	L, CEP, E, P, AML, AMC, S,
34	2	19.3, 1.7	SAM, L, E, OT, CEP, S, P, AMC, AML
35	1	19.3	S, L
36	1	19.3	S, P, OT, AML, CEP, DFX
37	1	19.3	E, S, P, CEP, L, SAM, AML
38	1	19.3	E, P, OT, L
39	1	19.3	E, P, OT, L
40	1	19.3	CEP, E, P, SAM, OT, AML
41	1	19.3	S, OT
42	1	19.3	P, AML
43	1	19.3	S, P, CEP, AML
44	1	19.3	E, OT, AML
45	1	19.3	S, P, L, AML
46	1	19.3	P, OT, AML, CEP
47	1	19.3	S, P, OT, AML, L, CEP, SXT
48	1	19.3	OT, E, L
49	1	19.3	S
50	1	19.3	P, SXT, AML

species level, and more recently type determination (7, 23). The technique has been applied successfully in order to identify the strains belong to four different species of *Staphylococcus*, including *S. aureus* (25, 31). Costas et al. (8) reported that computer assisted numerical analysis by SDS-PAGE of whole-cell proteins provides additional criteria for the study of the epidemiology and evolution of *S. aureus* strains. In this study, all strains were assigned to 2 cluster by computer-derived analysis of the whole-cell protein patterns. Although minor differences were detected in strains of *S. aureus*, the whole-cell protein patterns of the *S. aureus* strains were very homogeneous, some variability existed between them with exception of 10, 44 and 47. Such a close relationship could be explained by that transmission from one quarter to another could occur. No correlation was found between the protein band(s) and antibiotic succestibility as well.

Antibiotics are commonly in use to control the causative bacteria. As a consequence of persistent antibiotic therapy, udder-pathogenic *S. aureus* strains had became increasingly resistant to antimicrobial agents. Therefore, the determination of antibiotic resistance patterns might provide important information towards specific control measures (16). In this study, most of the *S. aureus* strains were found to be resistant to penicillin, streptomycin, amoxicillin, oxytetracycline, cefoperazon and lincomycin at high rates. These high rates can be attributed to the random use of antibiotics, dry period treatments, different treatment choices used in farms of Hatay region.

The previous studies regarding antibiotic resistance in *S. aureus* revealed that resistance genes often located on plasmids (18). Aarestrup et al. (1) suggested that antibiotic susceptibility testing and plasmid profiling might be helpful in order to solve epidemiological problems concerning strains, which are assigned to the same type by other typing techniques. In this study, plasmids with varied numbers and molecular weights were found in 94 % of *S. aureus* strains in plasmid profile studies. In contrast, Lange et al. (16) and Baumgartner et al. (5) reported a high percentage of plasmid-free *S. aureus* strains from cases of bovine mastitis. Most of the plasmid harboring *S. aureus* strains resistant to different antibiotics tested at different rates, and six plasmid-harboring *S. aureus* strains were found to be sensitive to all antibiotics tested. However it could be postulated that resistance genes may exist on plasmid in *S. aureus* strains.

As a result of this study, the differentiation of the strains from each other by using antibiotic susceptibility, protein and plasmid profiles can provide a additional method to aid in the characterization of the bacteria.

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