Brazilian Journal of Microbiology (2009) 40:254-261
ISSN 1517-8382

DETECTION OF METHICILLIN RESISTANCE AND SLIME FACTOR PRODUCTION OF *STAPHYLOCOCCUS AUREUS* IN BOVINE MASTITIS

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Submitted: June 05, 2008; Returned to authors for corrections: September 02, 2008; Approved: March 31, 2009.

ABSTRACT

This study aimed to detect methicillin resistant and slime producing *Staphylococcus aureus* in cases of bovine mastitis. A triplex PCR was optimized targetting 16S rRNA, *nuc* and *mecA* genes for detection of *Staphylococcus* species, *S. aureus* and methicillin resistance, respectively. Furthermore, for detection of slime producing strains, a PCR assay targetting *icaA* and *icaD* genes was performed. In this study, 59 strains were detected as *S. aureus* by both conventional tests and PCR, and 13 of them were found to be methicillin resistant and 4 (30.7%) were positive for *mecA* gene. Although 22 of 59 (37.2%) *S. aureus* isolates were slime-producing in Congo Red Agar, in PCR analysis only 15 were positive for both *icaA* and *icaD* genes. Sixteen and 38 out of 59 strains were positive for *icaA* and *icaD* gene, respectively. Only 2 of 59 strains were positive for both methicillin resistance and slime producing, phenotypically, suggesting lack of correlation between methicillin resistance and slime production in these isolates. In conclusion, the optimized triplex PCR in this study was useful for rapid and reliable detection of methicillin resistant *S. aureus*. Furthermore, only PCR targetting *icaA* and *icaD* may not sufficient to detect slime production and further studies targetting other *ica* genes should be conducted for accurate evaluation of slime production characters of *S. aureus* strains.

Key words: *Staphylococcus aureus*, *icaA*, *icaD*, slime, mastitis

INTRODUCTION

S. aureus is an important etiologic agent of mastitis in ruminants and also has an economical importance in cattle industry (43,47). Several tecniques are used to characterize the bovine *S. aureus* strains in veterinary microbiology. In addition to the phenotypic methods, Polymerase Chain Reaction (PCR) is valuable in identification and genotypic characterization of the *S. aureus* strains (36,42). Several efforts to removig this pathogen from farms are hampered by some factors. One of these factors is antibiotic resistance (22). One of the major mechanisms of resistance to β-lactam antibiotics is β-lactamase producing by staphylococci. This enzyme hydrolyzes the β-lactam ring and causes inactivation of β-lactams. In the early 1950s, it has been aware of the effectiveness of penicilin in treatment of *S. aureus* infections because of β-lactamase-producing plasmids. In 1959, methicillin, synthetic, penicillinase-resistant penicillin, was introduced and solved problem in clinical practice, for a time. However, by 1960, *Staphylococcus aureus* strains were found to be resistant to the new semi-synthetic β-lactams (methicillin, oxacillin, flucloxacillin), and became known as methicillin-resistant *S. aureus* (MRSA). This type of resistance was termed "intrinsic resistance" because it was not due to destruction of the antibiotic by β-lactamase (12). Methicillin resistance in *S. aureus* is mediated by the production of an altered penicilin-binding protein (PBP2a), a transpeptidase. *mecA* encodes this enzyme involved in cell-wall peptidoglycan synthesis. Unlike conventional PBPs of *S. aureus*, PBP2a does not bind to β-lactam antibiotics with high affinity (13,28,32,38,41). A distinctive feature of methicillin

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resistance is its heterogenous nature, with the level of resistance varying according to the culture conditions and β-lactam antibiotic being used. Heterogeneous strains can be considered to be composed of two populations of cells: relatively susceptible cells and highly resistant cells. Another type of methicillin resistance is borderline (or low level) resistance. Borderline strains characterized by methicillin MICs at or just above the susceptibility breakpoint (e.g., oxacillin MICs of 4-8 µg/ml) and may divided into two categories on the basis of presence of *mecA* gene (12). Strains not contain *mecA* differ from the others that contain *mecA* in the absence of highly resistant clones. The reasons for *mecA*-negative borderline resistance may be the modifications of normal PBP genes, the overproduction of β-lactamase and meticillinase (40).

It is noted that several studies concerning the evaluation of rapid methods for diagnosis of intrinsic resistance of oxacillin have been reported. Among them, PCR has succesfully used for the detection of *mecA* gene (3). Most of the clinical isolates show hetereogenous resistance in routine culture conditions and therefore detection of the presence of *mecA* gene by PCR is accepted as "gold standard". Detection of methicillin resistance is influenced by several factors as *mec* regulatory genes, β-lactamase regulatory genes and *fem* genes (12,40). Detection of *mecA* gene is the most reliable and fundamental method of identifying methicillin-resistant *Staphylococcus aureus* (3).

Several factors such as exotoxins, surface proteins (44) and extracellular polysaccharides (1) having important roles in virulence of *S. aureus* isolated from mastitis cases have been reported. Furthermore, it has been determined that production of slime factor in *S. aureus* strains causing mastitis was an important virulence factor affecting pathogenesis (44). It is considered that the first step in mastitis progress is adhesion of *S. aureus* to mammary epithelial cells (14) and slime factor plays an important role for adhesion and colonization (10,47). Production of slime factor also plays an important role in antibiotic resistance and it has been reported that slime producing strains are more resistant to antibiotics than non-slime producing strains (4). Intracellular adhesin is encoded in the *ica* locus containing *icaA*, *icaB*, *icaC*, *icaD* genes in *S. aureus* strains (17,34). *icaA* gene encodes N-acetylglucosaminyltransferase. Further, *icaD* plays an important role in expression of this enzyme. *icaA* and *icaD* were found to be in high prevalance among *S. aureus* mastitis isolates and this finding confirms that *ica* locus has a potential role as a virulence factor in the pathogenesis of mastitis in ruminants (47). Although production of slime factor has been well characterized in other staphylococci isolated from different infections, there is a little information in literature about the formation of slime factor and detection of *ica* locus in *S. aureus* mastitis isolates.

In this study, we aimed to determine the methicillin resistance and slime factor production of *S. aureus* in bovine mastitis phenotypically and genotypically.

MATERIALS AND METHODS

Bacterial isolates and phenotypic identification

The bacteria used in this study consisted of 161 *Staphylococcus* spp. isolated from subclinic mastitic milk samples. The staphylococcal isolates were identified morphologically and biochemically by standart laboratory procedures (35). For discrimination of *S. aureus* from coagulase-negative stahylococci (CoNS), the coagulase test was performed.

DNA extraction

Staphylococcus strains were inoculated on Triptycase Soy Agar. After incubation period, fresh colonies were suspended in 500 µl of DEPC-treated water (DNase-RNase free). The suspension was held in a 100ºC of water bath for 10 min. After centrifugation at 10 000 rpm for 5 min, the supernatant containing bacterial DNA was used as a template for subsequent PCR mixture (50).

Triplex PCR

A triplex PCR assay was performed to discriminate the *S. aureus* from other staphylococci and determinate the methicillin resistance, genotypically. For the detection of 16S rRNA (*Staphylococcus* spp. specific) 5'-AAC TCT GTT ATT AGG GAA GAA CA-3' was used as the forward primer and 5'-CCA CCT TCC TCC GGT TTG TCA CC-3' was used as the reverse primer. For the detection of *nuc* (*S. aureus* specific) gene 5'-GCG ATT GAT GGT GAT ACG GTT-3' was used as forward primer and 5'-AGC CAA GCC TTG ACG AAC TAA AGC-3') was used as reverse primer. Two primer, Forward: 5'-GTA GAA ATG AC GAA CGT CCG ATA-3'and Reverse: 5'-CCA ATT CCA CAT TGT TTC GGT CTA A -3' were used to detect *mecA* (methicillin resistance specific) gene.

Five microliter of the rapid extracted DNA was used as a template in a 25 µl PCR mixture containing 1XPCR buffer (50 mm KCl, 20 mM Tris HCl), 5 µl of 25 mM $MgCl_2$, 3 µl of 10 mM deoxynucleoside triphosphate (dNTP) mix, 1 µl of 20 µM each 16S rRNA and *mecA* primers, 0,4 µl of 20 µM each *nuc* primer and 2U of Taq DNA polymerase. The buffers and enzymes used in this assay were obtained from Fermentas Inc. (Canada). The oligonucleotide primers were synthesized from Bio Basic Inc. (Canada). The amplification of DNA was performed as follows: 94ºC for 5 min of initial denaturation; 30 cycles of 94ºC for 45 s, 68ºC for 45 s and 72ºC for 90 s; and a final extantion at 72ºC for 10 min. Amplicons were loaded onto 1.5% Agarose Gel containing 1 µg/ml ethidium bromide. The 756-bp (16S rRNA), 310-bp (*mecA*) and 279-bp (*nuc*) amplified DNA fragments were seperated by agarose gel electrophoresis and visualized under UV-light.

Phenotypic detection of methicillin resistance

Disc diffusion sensitivity testing of *S. aureus* isolates was performed with 5 µg oxacillin discs. On Mueller Hinton Agar,

according to NCCLS recommendation, oxacillin complete inhibition zone diameter of ≤12 mm were considered resistant, those with inhibition zone of ≥13 mm were susceptible.

Slime production assay

Slime production assay was performed by cultivation of *S. aureus* strains on Congo Red Agar (CRA) plates containing 0.8 g of Congo Red dye and 36 g saccharose (49). Strains were inoculated on CRA plates and incubated for 24-72 h at 37ºC. Slime producing strains and non-slime producing strains constitutes rough black colonies and red colonies on CRA, respectively.

PCR detection of *icaA* and *icaD* genes

For genotypic determination of slime production, the PCR targetting *icaA* and *icaD* genes were performed (47). The primers for *icaA* and *icaD* genes were designated from published sequence of the *ica* locus in GenBank and synthesized from Bio Basic Inc. For the amplifying of *icaA*, AF (5'-CCT AAC TAA CGA AAG GTA G-3') and AR (5'-AAG ATA TAG CGA TAA GTG C -3') primers and of *icaD* gene, DF (5'-AAA CGT AAG AGA GGT GG-3') and DR (5'-GGC AAT ATG ATC AAG ATA-3') primers were used. The PCR with *icaA* and *icaD* genes were amplified a products of 1315-bp and 381-bp, respectively.

Ten microliters of the rapid extracted DNA was used as a template in a 50 µl PCR mixture, containing 1X PCR buffer (50 mm KCl, 20 mM Tris HCl), 5 µl of 25 mM $MgCl_2$, 5 µl of 10 mM deoxynucleoside triphosphate (dNTP) mix, 1 µl of 20 µM each primers and 1U of Taq DNA polymerase. The buffers and enzymes used in the assay were obtained from Fermentas Inc. The amplification of DNA was performed as follows: 92ºC for 5 min of initial denaturation; 30 cycles of 92ºC for 1 min, 49ºC for 1 min and 72ºC for 1 min; and a final extention at 72ºC for 7 min. Amplicons were loaded onto 1.5% Agarose Gel containing 1 µg/ml ethidium bromide. The presence and molecular weight of the amplified DNA fragments were confirmed by agarose gel electrophoresis and visualized under UV-light.

RESULTS

Initial characterization of bacterial strains

The isolated bacteria from bovine mastitis were identified by conventional methods. All of the 161 strains were found gram positive and catalase positive cocci. They were identified as staphylococci. Among these staphylococci, 59 strains were rabbit plasma-coagulase positive and they were considered as *S. aureus*. Confirmations of the strains were done using triplex-PCR.

Agar disc diffusion test for detection MRSA

Thirteen of 59 (22.0%) *S. aureus* isolates tested in this study gave a zone of 12 mm or more and were considered as methicillin resistant (Table 1).

Slime production assay

Rough-black colonies on CRA plates were considered to be slime producing strains and red colonies were evaluated as non-slime producing strains. Among the 59 *S. aureus* strains tested, 22 (37.2%) were found to produce black colonies within 24-48 h. After 72 h, no more strains produced black colonies (Fig. 1).

Triplex PCR

Triplex PCR was used to discriminate *S. aureus* strains among all our mastitis isolates and to simultaneously detect methicillin resistant strains. This assay was targetted the *16S rRNA*, *nuc* and *mecA* genes. Fragments of expected sizes were 756, 279 and 310 bp for the *16S rRNA*, *mecA* and *nuc* genes, respectively (Fig. 2). All the 59 *S. aureus* strains identified phenotypically were found to possess both *16S rRNA* and *nuc* genes and were confirmed as *S. aureus*. Strains were characterized in this PCR analysis targetting *mecA* gene simultaneously for methicillin resistance. Among the 13 *S. aureus* strains found as methicillin resistant phenotypically, 4 (30.7%) were positive for *mecA* gene. Also; the phenotypically methicillin sensitive 46 *S. aureus* strains were found as negative for *mecA* gene.

PCR detection of *icaA* and *icaD* genes

Among all *S. aureus* isolates, 15 of 59 (25.4%) were positive for both *icaA* and *icaD* genes and only 8 of them (53.3%) were slime positive on CRA. 16 of 59 (27.1%) strains were positive for on *icaA* and 38 of them (64.4%) were positive for *icaD* gene (Fig. 1, Table 1 and 2).

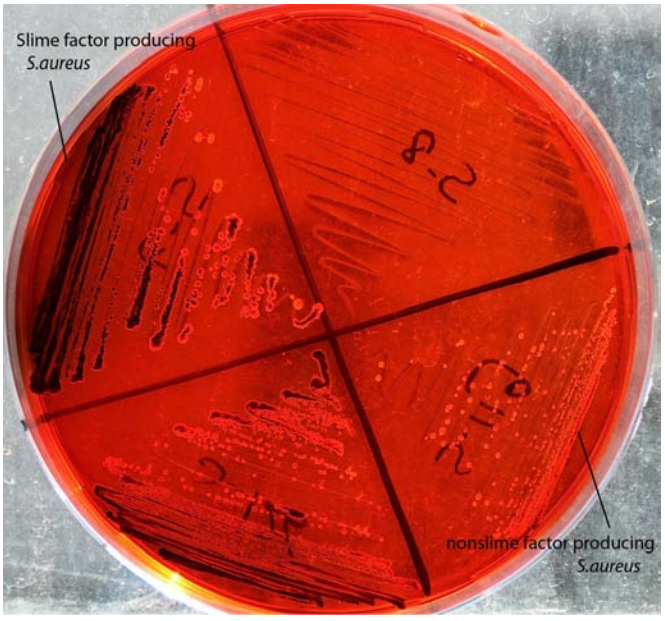

Figure 1. Slime Factor Production of *S.aureus* on Congo Red Agar.

Table 1. Cross-evaluation of slime factor production and methicillin resistance of *S.aureus* strains analyzed phenotypically and genotypically.

		Slime factor production on CRA (n)		*icaA* (n)		*icaD* (n)		*icaA* and *icaD* (n)	
		P	N	P	N	P	N	P	N
Methicillin resistance with oxacillin disk (n)	R	3	10	4	9	7	6	4	9
	S	19	27	12	34	31	15	11	35
mecA (n)	P	0	4	2	2	2	2	2	2
	N	22	33	14	41	36	19	13	42

P: Positive, N: Negative, R: Resistant, S: Sensitive.

Table 2. Distribution of *S.aureus* strains according to their phenotypic and genotypic slime factor production characteristics.

		icaA		*icaD*		*icaA* and *icaD*	
		Positive (n)	Negative (n)	Positive (n)	Negative (n)	Positive (n)	Negative (n)
Slime production on CRA	Positive (n)	8	14	15	7	8	7
	Negative (n)	8	29	23	14	7	13

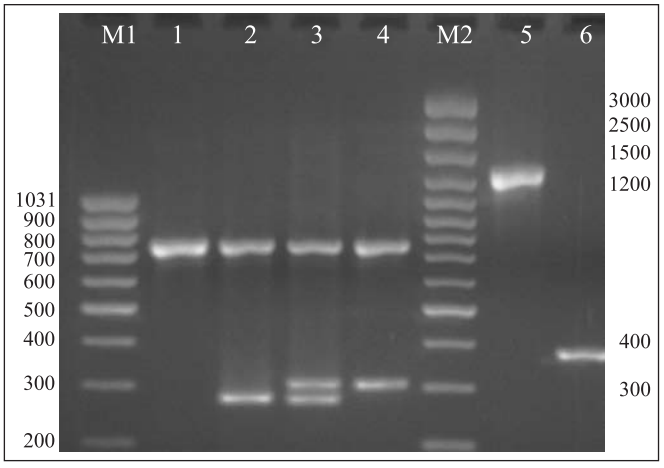

Figure 2. The results of triplex-PCR for confirmation of the identification of *S. aureus* and determination of methicillin resistance; single PCR for *icaA* and *icaD* genes.
M1: Marker (80-1031 bp); M2: Marker (100-3000 bp); 1: *Staphylococcus* spp., 16S rRNA (756 bp) pozitive; 2: *S. aureus*, 16S rRNA (756 bp) and *nuc* (279 bp) pozitive; 3: MRSA, 16S rRNA (756 bp), *nuc* (279 bp) and *mecA* (310 bp) pozitive; 4:Methicilline resistant *Staphylococcus* spp., 16S rRNA (756 bp) and *mecA* (310 bp) pozitive; 5: *icaA* (1315 bp); 6: *icaD* (381 bp).

DISCUSSION

Bovine mastitis is the most costly disease to the dairy industry worldwide as well as in Turkey. *Staphylococcus aureus* is a frequent cause of bovine mastitis and several tecniques are used to characterize the bovine *S. aureus* strains in veterinary microbiology. Rapid detection of methicillin resistance together with the identification of *S. aureus* is necessary for therapeutic and epidemiological purposes (16,36). *S. aureus nuc* gene encodes the TNase producing by these bacteria. *S. aureus* TNase has species-specific sequences and amplification of the *nuc* gene has potential for the rapid diagnosis of *S. aureus* infections (11,16). The primary tool for controlling staphylococcal mastitis is antimicrobial therapy. Therefore antimicrobial susceptibility tests are so important that the results of these help guide the veterinarian in selecting the most appropriate antimicrobial agent for treatment of mastitis caused by *S. aureus* (19). Many studies are avaliable on antibiotic resistance of *Staphylococcus aureus* (24,29,33,39). Methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-resistant *S. aureus* strains has also been reported in some cases in veterinary medicine (9,31). MRSA show an intrinsic resistance to penicillinase-resistant beta lactam antibiotics. This resistance is based on "*mecA*" gene encoding PBP2a, an inducible and an altered PBP that has low affinity for binding β-lactam antibiotics. *mecA* gene is located on the chromosome of MRSA and methicillin resistance does not due

to destruction of the antibiotic by beta-lactamase. MRSA strains show also a heterogeneous character with the level of resistance varying according to the culture conditions and β-lactam antibiotic being used. Because of this heterogeneous resistance, the detection of MRSA by phenotypic methods becomes problematic. Although laboratory conditions has been changed (e.g. additional NaCl to medium, prolonged incubation, incubation at low temperatures, higher inoculum) to enhance the phenotypic expression, all strains can not be classified. Furthermore, the conditions that are used to enhance expression of methicillin resistance also can cause the susceptibility test results for susceptible strains to shift toward or above the breakpoint for resistance (25). The multidrug-resistant (methicillin and other more common antibiotics such as oxacillin, penicillin and amoxicillin) phenotype of MRSA strains and their intrinsic beta-lactam resistance make them difficult and costly to treat (12,28,41). Methicillin and oxacillin are not used in veterinary medicine in Turkey except for cloxacillin used in mastitis cases (45). Considering the multiple antibiotic resistance, the rapid and correct detection of MRSA strains must be performed to select appropriate antibiotic regimens. However selected methods detecting MRSA strains must be useful, reliable, simple and rapid (27). Several phenotypic methods such as oxacillin disk diffusion test, agar plate screen, the microbroth dilution and the *E*-tests have been used to detect the MRSA isolates (5). Phenotypic-based identification and susceptibility testing methods are time consuming and most have inherent limitations (16). However, polymerase chain reaction (PCR)-based methods have shown to be a rapid and reliable approach for the identification and genotypic characterization of these organisms. *mecA*-based PCR methods has accepted as "gold standard" (5,12,40).

In the present study, *S. aureus* strains originated from bovine mastitis were identified phenotypically by some conventional tests and also were characterized genotypically by a triplex PCR targetting *16srRNA* (staphylococcus genus-spesific), *nuc* (*S. aureus* species-spesific) and *mecA* (a determinant of methicillin resistance*)*. In this study, all 59 *S. aureus* isolates identified by conventional tests were confirmed as *S. aureus* by this PCR genotypically and at the same time, methicillin resistance of these strains were detected. While 13 of 59 (22.0%) *S. aureus* isolates were found to be methicillin resistant in oxacillin disc diffusion method, only 4 of 59 (6.7%) *S. aureus* isolates were found to be MRSA in triplex PCR. Similarly, some investigators (5,46) have reported the discrepant results between disc diffusion methods and PCR for detection of methicillin resistance. It is reported that conventional susceptibility tests such as agar disc diffusion and broth dilution methods may not give reliable results in detecting MRSA because of heterogenic expression of resistance (46). In this study, MRSA lacking *mecA* gene are classified as false resistant by the oxacillin disc diffusion method and it was considered that it may due to another resistance mechanism such as hyperproduction of beta-lactamase.

Extracellular polysaccharides, slime factor, are considered to be significant virulence factors for some staphylococci (20). Slime layer surrounding the *S. aureus* strains help in adherence and colonization of these microorganisms on the mammary gland epithelium. It is reported that slime factor production in *S. aureus* isolates from mastitis cause antibiotic resistance which is due to the decreased diffusion of antibiotics through the biofilm matrix and decreased metabolic activity of bacteria (4,36). Several phenotypic methods such as standard tube method, Christensen's method, Congo Red Agar and microdilution methods are used to detect slime production (2). In the present study, slime producing *S. aureus* isolates were detected on Congo Red Agar (CRA) plates in vitro. CRA plate test has been reported as a simple, rapid, sensitive, and reproducible and advantageous in those colonies remain viable on the medium and also more specific than standard tube test (23,37,48). We found that only 22 of 59 (37.2%) *S. aureus* isolates were slime-producing on Congo Red Agar. Slime-producing *S. aureus* isolates from different clinical origins such as wound infection (49), catheter-associated infections (6), bovine mastitis (47) has been detected in vitro by using Congo Red Agar plates as 52%, 60.8% and 91.4%, respectively. Knobloch et al (30) have reported that the phenotype on CRA was found to be an unreliable indicator of slime-forming capacity among clinical isolates of *S. aureus*. Therefore, although CRA methods may be easier to perform than a molecular analysis of the genes implicated in biofilm production and could be performed easily in a diagnostic laboratory, it may be a poor method for determining the slime-producing capacity of clinical isolates in the diagnostic laboratory (21).

Recently developed molecular methods provided a direct evidence of the genetic basis of slime production complementary to the CRA test. Slime synthesis is controlled by the *ica* (intercellular adhesion) operon (7). The *ica* locus consists *ica A, D, B, C* genes which encode the proteins mediating the synthesis polysaccharide intercellular adhesin and capsular polysaccharide/adhesin (PS/A) in *S. epidermidis* and *S. aureus*, respectively (49). *N-acetylglucosaminyltransferase* that synthesizes the polysaccharide intercellular adhesin from *UDP-N-acetylglucosamine* encoded by the *icaA* particularly. However sole expression of *icaA* induces only low enzymatic activity, coexpression of *icaA* with *icaD* leads to a significant increase in activity and is related to phenotypic expression of the capsular polysaccharide (6). In the operon, coexpression of *icaA* and *icaD* is required for full slime synthesis. In this study slime production of *S. aureus* isolates were detected by PCR targetting *icaA* and *icaD*. In PCR analysis, not all isolates, only 15 of 59 were positive for both *icaA* and *icaD* genes and only 8 of them were slime positive in CRA. 16 of 59 strains were positive for *icaA* and 38 of them were positive for *icaD* gene. This result was contrast to Arciola *et al.* (6) who have reported that all strains which were positive for *icaA* were also positive for *icaD*. Further

Vasudevan *et al.* (47) have reported that although only 24 of 35 *S. aureus* mastitis isolates produced slime factor in vitro, all of them were found to possess the *ica* locus as well as the *icaA* and *icaD*. Arciola *et al.* (8) have been found that the genes of the *ica* locus appear, in all the clinical isolates analyzed in their study, strictly linked each other, so they are either all present or all absent. Our discrepant findings that *icaA* and *icaD* genes were not be together in some isolates may due to some mutations on *icaA*. Although coexpression of *icaA* and *icaD* is necessary for slime production, it was considered that other genes in *ica* locus play role in controlling slime expression. Fitzpatrick et al (21) have reported that there are *ica*-independent mechanisms of biofilm formation in *S. aureus*. In this study, among the 37 strains which did not produce slime factor on CRA plate *in vitro*, 7 strains were positive for both *icaA* and *icaD* genes. This suggests the possibility that some environmental conditions or the presence of accessory genes can influence the phenotypic behavior on the Congo red agar plate, giving colonies which did not fully express the *ica* genes.

Slime factor may also play a role in antibiotic resistance (18,44) and it is reported that slime-producing strains are more resistant to antibiotics than non slime producing strains (4). Günaydin *et al.* (26) are also reported that there is a correlation between slime producing and multiple antibiotic resistances. In contrast to these studies, Ciftci *et al.* (15) have not found any effect of slime producing on antibiotic resistance in their study and furthermore they have reported that slime producing strains are more susceptible to antibiotics than nonslime producing strains. In this study, we found that only 2 of 59 strains were positive for both methicillin resistance and slime producing, phenotypically. Thus, we suggested that there is no correlation between methicillin resistance and slime producing in our isolates.

In conclusion, it was showed that several cases of mastitis in cows in Turkey were caused by MRSA despite of low percentage. It was considered that the triplex PCR optimized in this study is useful for diagnostic laboratory because of its rapid and reliable characters for detection of methicillin resistance of *S. aureus*. Using the phenotypic and genotypic methods together is required for accurate identification of MRSA. Thus it was found that not all isolates phenotypically slime positive were positive both *icaA* and *icaD* and there were differences in the presence of these two genes. We considered that only PCR targetting *icaA* and *icaD* was not sufficient to detect slime production. Further studies targetting other genes that are present in *ica* locus and studies for investigation of mutations on these genes should be conducted to accurate evaluation of slime production characters of *S. aureus* strains.

ACKNOWLEDGEMENT

This work was supported by OMU-BAP (VET-013).

RESUMO

Detecção de resistência a meticilina e produção do fator *slime* por *Staphylococcus aureus* em mastite bovina

Este estudo objetivou a detecção de *Staphylococcus aureus* resistente a meticilina e produtor do fator *slime* em casos de mastite bovina. Um PCR triplex foi otimizado, com alvo no genes 16SrRNA, *nuc* e *mecA* para detecção de *Staphylococcus* spp, *S. aureus* e resistencia a meticilina, respectivamente. Para detecção das cepas produtoras do fator slime, empregou-se um PCR com alvo nos genes *icaA* e *icaD*. No estudo, 59 cepas foram identificadas como *S. aureus* por testes convencionais e PCR, sendo 13 resistentes a meticilina e quatro positivas para o gene *mecA*. Embora 22 das 59 cepas tenham sido produtoras do fator *slime* em Agar Vermelho Congo, no teste PCR somente 15 foram positivas para os genes *icaA* e *icaD*. Dezesseis e 38 das 59 cepas foram positivas para os genes *icaA* e *icaD*, respectivamente. Somente duas das 59 cepas foram positivas simultaneamente para resistência a meticilina e produção do fator slime, sugerindo falta de correlação entre estas características. Em conclusão, o PCR triplex otimizado neste trabalho mostrou-se ser um método rápido e confiável para detecção de *S.aureus* meticilina resistente. Por outro lado, somente PCR para os genes *icaA* e *icaD* pode não ser suficiente para detectar produção de fator *slime* e outros estudos com alvo em outros genes ica são necessários para um avaliação correta da produção do fator *slime* por S. aureus.

Palavras-chave: *Staphylococcus aureus, icaA, icaD, mecA, slime*, mastite

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