J. Dairy Sci. 96:329–334
http://dx.doi.org/10.3168/jds.2012-5944

Comparison of genomic and antimicrobial resistance features of latex agglutination test-positive and latex agglutination test-negative *Staphylococcus aureus* isolates causing bovine mastitis

A. Moser,* R. Stephan,* S. Corti,* and S. Johler*†[1]
*Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland
†Skirball Institute of Biomolecular Medicine, New York University Medical Center, 540 First Avenue, New York 10016

ABSTRACT

The dairy industry suffers massive economic losses due to staphylococcal mastitis in cattle. The Staphaureux latex agglutination test (Oxoid, Basel, Switzerland) was reported to lead to negative results in 54% of bovine *Staphylococcus aureus* strains, and latex-negative strains are thought to be less virulent than Staphaurex latex-positive strains. However, comparative information on virulence and resistance profiles of these 2 groups of *Staph. aureus* is scarce. Our objective was to associate the latex agglutination phenotype of *Staph. aureus* strains isolated from bovine mastitis milk with data on clonal complexes, virulence genes, and antibiotic resistance to (1) determine the virulence profiles of the Staphaureux test positive and Staphaurex test negative groups, and (2) provide data needed to improve treatment of bovine mastitis and to identify potential vaccine targets. Seventy-eight *Staph. aureus* strains isolated from 78 cows on 57 Swiss farms were characterized. Latex agglutination was tested by Staphaureux kit, and resistance profiles were generated by disk diffusion. A DNA microarray was used to assign clonal complexes (CC) and to determine virulence and resistance gene profiles. By the Staphaureux test, 49% of the isolates were latex-positive and 51% were latex-negative. All latex-negative strains were assigned to CC151, whereas latex-positive strains were assigned to various clonal complexes, including CC97 (n = 16), CC8 (n = 10), CC479 (n = 5), CC20 (n = 4), CC7 (n = 1), CC9 (n = 1), and CC45 (n = 1). Although the latex-negative isolates were susceptible to all antimicrobial agents tested, 24% of latex-positive isolates were classified as intermediate with regard to cefalexin-kanamycin and 13% were resistant to both ampicillin and penicillin. Microarray profiles of latex-negative isolates were highly similar, but differed largely from those of latex-positive isolates. Although the latex-negative group lacked several enterotoxin genes and *sak*, it exhibited significantly higher prevalence rates of genes encoding enterotoxin C, toxic shock syndrome toxin, and leukocidins (*lukM/lukF-P83*, *lukD*). Our findings suggest that latex-negative isolates represent a group of closely related strains with specific resistance and virulence gene patterns.
Key words: *Staphylococcus aureus*, bovine mastitis, Staphaurex latex agglutination test, virulence

INTRODUCTION

The dairy industry suffers from massive economic losses due to staphylococcal mastitis in cattle (Wells et al., 1998). The Staphaurex latex agglutination test (Oxoid, Basel, Switzerland) is a diagnostic instrument widely used to confirm putative *Staphylococcus aureus* isolates through detection of characteristic *Staph. aureus* surface proteins. Latex particles coated with human IgG and fibrinogen interact with the bacterial target proteins SpA (staphylococcal protein A), ClfA/B (clumping factor A/B), and FnbA/B (fibronectin-binding protein A/B), mediating a rapid agglutination reaction that is visible to the naked eye. Although the Staphaurex latex agglutination test exhibits high specificity (99.5%) and sensitivity (99.8%) when applied to *Staph. aureus* strains obtained from humans, Stutz et al. (2011) reported that 54% of *Staph. aureus* isolates obtained from cases of bovine mastitis yield negative test results. These false-negative results are due to sequence polymorphisms leading to impaired functionality of one or several of the targeted virulence factors (SpA, ClfA/B, or FnbA/B). Therefore, Staphaurex latex agglutination test (**SLAT**)-negative [SLAT(−)] strains are thought to be less virulent than SLAT-positive [SLAT(+)] strains (Stutz et al., 2011). Although assessing the virulence potential of SLAT(−) strains is of crucial importance to the dairy industry, data on the genomic background and antimicrobial resistance of bovine SLAT(−) isolates are scarce.

Received July 15, 2012.
Accepted September 18, 2012.
[1]Corresponding author: sophia.johler@uzh.ch

Although antibiotic treatment is widely used to fight bovine mastitis, its merits are controversial. Use of antimicrobial agents is not only economically questionable and favors the development of antibiotic resistance, but it is also unsuitable in addressing intracellular persistence of the organism (Steeneveld et al., 2011; Fluit, 2012; Saini et al., 2012). Therefore, increased efforts are now focused on the development of vaccines. Recent studies postulate extended characterization of the genetic background of bovine mastitis isolates to enable identification of proteins crucial for colonization and infection that could serve as biomarkers in the identification of vaccine targets (Fluit, 2012; Klein et al., 2012).

The objective of this study was to link the latex agglutination phenotype of *Staph. aureus* strains isolated from bovine mastitis milk with data on clonal complexes, virulence genes, and antibiotic resistance to (1) determine the virulence profiles of the SLAT(+) and SLAT(−) groups, and (2) provide data needed to improve treatment of bovine mastitis and to identify potential vaccine targets.

MATERIALS AND METHODS

Bacterial Isolates, DNA Extraction, and Presumptive Species Identification

Seventy-eight *Staph. aureus* strains were isolated from bovine mastitis milk samples collected from different cows on 57 Swiss farms between March 2011 and February 2012. Putative *Staph. aureus* isolates were identified by streaking samples onto rabbit plasma fibrinogen plates (Oxoid), which were subsequently incubated at 37°C and examined for coagulase activity after 48 h. A single typical *Staph. aureus* colony from each plate was transferred to blood agar and incubated overnight at 37°C. Then, DNA was isolated using kits supplied by Qiagen (Hilden, Germany), according to the manufacturer's instructions. The concentration of nucleic acids was measured by using a Nanodrop ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Staphaureux Latex Agglutination Test and Genotyping

The Staphaurex test kit (Oxoid) was used according to the manufacturer's instructions to determine latex agglutination.

The presence of 284 genes and allelic variants was assessed using StaphyType ArrayStrips (Clondiag Chip Technologies, Jena, Germany) following the manufacturer's instructions. Multiplex linear DNA amplification and microarray hybridization allowed for identification of species markers, genes conferring resistance to antimicrobial agents, and virulence determinants such as genes encoding enterotoxins, toxic shock syndrome toxin, leukocidins, hemolysins, and adhesins. The microarray also enables assignment of strains to clonal complexes and *agr* types. The DNA microarray profiles were converted to sequence-like strings, as described elsewhere, to allow for visualization by SplitsTree4 (http://www.splitstree.org/), a software package designed to compute unrooted phylogenetic networks from molecular sequence data (Wattinger et al., 2012).

Susceptibility Testing

Disk diffusion was used to classify isolates as susceptible, intermediate, or resistant depending on respective zone diameters following Clinical and Laboratory Standards Institute (**CLSI**) standard protocols (CLSI, 2008). All antimicrobial agents were chosen with regard to their relevance in mastitis therapy. Antibiotic agents tested included ampicillin (30 μg), amoxicillin (20 μg) with clavulanic acid (10 μg), cephalothin (30 μg), ceftiofur (30 μg), erythromycin (15 μg), cefoxitin (30 μg), gentamicin 10 (μg), kanamycin (30 μg), kanamycin-cefalexin (30 μg-15 μg), penicillin (10 IU), and penicillin-novobiocin (10 IU-30 μg). Mueller-Hinton agar and disks containing ceftiofur and penicillin-novobiocin were provided by Oxoid, and disks containing cefalexin-kanamycin (Ubrolexin) were provided by Boehringer Ingelheim (Basel, Switzerland). All other disks containing antibiotic agents were obtained from Becton Dickinson (Basel, Switzerland). Reference strain *Staph. aureus* ATCC 25923 was used as a quality control.

Statistical Analysis

The distribution of specific genes among latex-positive and latex-negative isolates was compared based on the hybridization results of the DNA microarray. SPSS Statistics 19 (SPSS Inc., Chicago, IL) was used to run the Pearson χ^2 test, identifying significant associations between the latex phenotype and the presence of the examined genes. Results were considered to be statistically significant for P-values <0.05.

RESULTS

Species Confirmation and Exclusion Criteria

All isolates were confirmed to represent *Staph. aureus* using the species markers of the DNA microarray. To avoid bias, the sample collection was screened for identical isolates by comparison of all features tested, including microarray profiles and resistance patterns, and all isolates were found to be unique.