ANALYSIS OF PROTEIN A GENE POLYMORPHISM IN STAPHYLOCOCCUS AUREUS ISOLATES FROM BOVINE MASTITIS

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Abstract

One hundred and three isolates of Staphylococcus aureus from bovine mastitis were investigated by PCR for gene polymorphism in the X region of the protein A. On the basis of the size of corresponding PCR products (100-320 bp), 2-11 repeats were supposed to be present in the genes investigated. The isolates showed 8, 9, 10 and 11 repeats most frequently (20.39%, 15.53%, 14.56% and 16.50%, respectively). Seven or less repeats were found only in 33% of the isolates. Somatic cell count in milk samples from quarters infected with strains which harboured more than 7 repeats was significantly higher than from quarters infected with strains which harboured 7 or less repeats (6 244 ± 7 829 x 10³/ml vs. 2 803 ± 2 415 x 10³/ml).

Key wards: cows, mastitis, Staphylococcus aureus, protein A gene, polymorphism, PCR.

Material and Methods

One hundred and three S. aureus strains isolated from cows belonging to 19 herds from 9 different districts in Poland were tested. All the isolates were identified on the basis of colony morphology and positive coagulase tube test. Coagulase-positive staphylococcal isolates were confirmed as S. aureus using a commercial biotyping system (api STAPH, bioMerieux, Inc., Hazelwood, MO) and by using the PCR to amplify the S. aureus-specific nuc gene as described previously (11). The strains were stored in brain heart infusion (BHI) broth with 20% of glycerol at -20°C. The working cultures of the isolates were prepared in BHI broth at 37°C for 18 h. Chromosomal DNA from the S. aureus strains isolated from milk samples was extracted as described by Dalla Pozza et al. (3) with some modification (10). The repetitive region within the protein A gene was amplified by using oligonucleotide primers with the following DNA
sequences: SPAX1 (5’ CAA GCA CCA AAA GAG GAA-3’) and SPAX2 (5’CAC CAG GTT TAA CGA CAT-3’) (5). The PCR amplification was carried out in 0.5 ml tubes in the reaction final volume of 50 µl. The PCR mixture consisted of 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton®X-100, 200 µM (each) deoxynucleotide triphosphate, 0.2 µM of the respective primers, and 0.025u/µl of Taq DNA polymerase (all the reagents from Promega Corporation, Madison). The amplification was performed with an automated thermal cycler T-1 (Biometra). The PCR cycles consisted of pre-heating at 94°C for 4 min, denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The amplification was performed for 35 cycles with a final extension step at 72°C for 5 min. The PCR products were analysed by electrophoresis through a 1.5% agarose gel containing 0.5 mg of ethidium bromide per ml (all the reagents from Promega), visualised and photographed with Image Master VDS (Pharmacia Biotech). The 50 bp DNA step ladder (Promega) served as a size standard for the calculation of the size of spa amplicons. The number of repetitive units present in the genes' variable region was estimated by comparisons with molecular weight markers. SCC in milk was measured with a Fossomatic Cell Counter (Foss-Electric Denmark).

The samples were divided into 2 groups with regard to the number of 24 bp repeats elements in the X region of the spa gene of S. aureus strain infected quarter: I-number of repeats ≤7 and II - number of repeats >7. SCC in milk samples of the two groups were compared by an independent t-test.

**Results**

According to the results, of cultural and biochemical analysis as well as of amplification of the nuc gene, all 103 isolates used in the present investigation were identified as S. aureus. Amplification of the X region of the spa gene yielded a single amplicon for each isolate included in this study. Ten differently sized amplicons of approximately 100-320 bp were observed (Fig. 1).

On the basis of the size of corresponding PCR products 2-11 repeats were supposed to be present in the region X of each of the spa gene investigated. The distribution of the different size spa amplicons is shown in Table 1. Patterns which showed 8, 9, 10 and 11 repeats in the amplified region were observed most frequently (20.39%, 15.53%, 14.56% and 15.50%, respectively). The percentage of strains with 2 to 7 repeat units (group I) was twice lower than those from group 2 (33.01% and 66.99%, respectively).

SCC in mastitic milk from quarters infected with S. aureus strains from the group II was significantly higher than that from quarters infected with strains from the group I (6 244 ± 6 029 x 10³/ml and 2 803 ± 2 415 x 10³/ml, respectively). The difference was significant (P= 0.024).

![Fig. 1. Analysis of the X region of the spa gene by PCR. The numbers of 24 bp repeats are indicated above each band, line M - DNA weight standard (50 bp DNA step ladder).](image-url)
Table 1
Distribution of the number of 24 bp repeats in the X region of the protein A gene among 103 Staphylococcus aureus strains isolated from bovine mastitis

<table>
<thead>
<tr>
<th>Number of repeats</th>
<th>Number of strains</th>
<th>Percentage of strains</th>
<th>Percentage of strains</th>
<th>SCC/ml x 10^3 (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>1.94</td>
<td>33.01</td>
<td>2803 ± 2415*</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>10.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>5.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>20.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>15.53</td>
<td>66.99</td>
<td>6244 ± 7829*</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>14.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17</td>
<td>16.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P=0.024

Discussion

Similarly as in studies by Pozza et al. (3) and Lange et al. (13) amplification of the X region spa gene yielded a single amplicon for each isolate tested. We found that 66.99% of S. aureus isolates had more than 7 repeat elements compared with 33.01% of S. aureus with equal or fewer than 7 repeats in their X region of the spa gene, and these values are similar to previous published findings (3). The functional capabilities of bovine neutrophils are a major factor which determines the establishment of new intramammary infections. Aarestrup et al. (1) observed statistically significant difference between phagocytosis and killing of S. aureus strains belonging to the most common and rare coagulase genotypes. The results suggest that the prevalent types have some characteristics which enable them to evade host defence mechanisms and establish intramammary infections more successfully than the less prevalent types. In Poland, S. aureus strains with >7 repeat elements seem to be more prevalent than strains with ≤7 repeats.

The number of somatic cells in milk is closely associated with inflammation and the udder health. Milk SCC determination is universally accepted as a measure of mammary gland inflammation (19). According to our results S. aureus strains with repeats more than 7 in the X region were more transmissible and caused increased SCC significantly greater than strains with 2 to 7 repeats (P=0.024). It will be of interest to evaluate differences in the capabilities of S. aureus strains with >7 and ≤7 number of 24 bp repeats in the X region of the spa gene to evade neutrophil phagocytosis and killing. Study by Zecconi and Piccinini (24) showed that SCC in milk is higher in quarter infected with S. aureus strain with more than 7 repeats, but only with cna (collagen adhesin gene) negative strains. Middleton et al. (17), on the basis of SCC and N-acetyl-B-D-glucosaminidase activity did not find any differences between pathogenicity of different strains of S. aureus. Other authors have suggested that virulence may be the strain dependent and therefore, targeting cows infected with virulent and possibly predominant strains of S. aureus in mastitis control programmes may lead to advances in the control and prevention of this dangerous mastitis pathogen (9, 23). Smith et al. (20) reported an outbreak of mastitis caused by a single strain of S. aureus in the Washington State University dairy herd. Unlike other strains of S. aureus isolated from cows in the herd, the outbreak strain spread easily from cow to cow.

The results of the present study support the hypothesis that strains of S. aureus causing bovine mastitis vary with regard to their pathogenicity.

References


