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Agnesia Endang Tri Hastuti Wahyuni, M. H. Wibowo, Franky, B. B. Ajiguno
SURFACE CHARACTER OF COAGULASE-POSITIVE AND COAGULASE-NEGATIVE OF SATPHYLOCCUS AUREUS ISOLATED FROM ORIGIN GOAT’S MILK THAT CONTRIBUTE IN ADHESION TO MAMMARY EPITHELIAL CELLS

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Introduction

Once a bacterium reaches a host surface, it must be adherence to host cell to colonize them. This is particularly important in areas such as the mouth, small intestine, udder and bladder where mucosal surfaces are washed by fluids. In these areas, only bacteria that can adhere to mucosal surfaces will be able to stay in the side. Staphylococcus aureus is an important animal and human pathogen responsible for diverse types of severe infection. In animal, S.aureus is a frequent cause of mastitis both in cows and goats. The mayor pathogen, S.aureus is generally coagulase-positive although coagulase-negative strain of S.aureus do occur. However, pathogenesis of the infection has not been completely define. In the pathogenesis of subclinical mastitis the adherence process is very important in the initiation step of bacteria colonization on the mammary cell surface. Surface character such as hydrophobic interaction, protein A and protein hemagglutinin are generally regarded as being an important virulence factor that responsible in the pathogenesis. This study aimed to characterize S.aureus coagulase positive and coagulase negative S.aureus that play a role in adhesion ability (associated protein A, hemagglutination ability, and the nature of hydrophobicity) to udder epithelial cells.

Materials and Methods

Research carried out using 21 cultures both coagulase-positive and coagulase-negative S.aureus that was isolates from of origin goat’s milk. Reidentification of 21 cultures start with the colony morphology observed on blood agar plate (BAP), Gram staining, catalase test, coagulase test and mannitol fermentation tests with Mannitol Salt Agar (MSA). The production of protein A with serum soft agar test (SSA), the ability of hemagglutination with hemagglutination test using sheep blood concentration of 0.5%, 1%, 1.5%, 2%, the nature of hydrophobicity used ammonium sulfate concentration of 1.2 M; 1.6 M, 2.0 M, 2.4 M, 3.2 M. Adhesion ability of the suspension performed by labeling bacteria with fluorescein isothiocyanate (FITC) and then mixed with udder epithelial cells and observed using a microscope fluorosense.

Results and Discussion

Table 1. Adhesion of S. aureus isolat that were labelled with FITC to epithelial cell

<table>
<thead>
<tr>
<th>No.</th>
<th>Code Isolate</th>
<th>Coagulase</th>
<th>SSA</th>
<th>HA</th>
<th>SAT</th>
<th>Mean Adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PH A1x</td>
<td>+</td>
<td>compac</td>
<td>+</td>
<td>+</td>
<td>14.3</td>
</tr>
<tr>
<td>2.</td>
<td>PH B2y</td>
<td>-</td>
<td>diffus</td>
<td>-</td>
<td>+</td>
<td>12.65</td>
</tr>
<tr>
<td>3.</td>
<td>PH B6y</td>
<td>+</td>
<td>diffus</td>
<td>+</td>
<td>-</td>
<td>8.85</td>
</tr>
<tr>
<td>4.</td>
<td>PH B10y</td>
<td>-</td>
<td>compac</td>
<td>-</td>
<td>+</td>
<td>19.4</td>
</tr>
<tr>
<td>5.</td>
<td>KNH A6y</td>
<td>-</td>
<td>compac</td>
<td>+</td>
<td>-</td>
<td>11.5</td>
</tr>
<tr>
<td>6.</td>
<td>KHH B5x</td>
<td>+</td>
<td>compac</td>
<td>+</td>
<td>+</td>
<td>12.9</td>
</tr>
<tr>
<td>7.</td>
<td>Irr A5x</td>
<td>+</td>
<td>diffus</td>
<td>-</td>
<td>-</td>
<td>14.45</td>
</tr>
</tbody>
</table>

+ HA : have hemagglutinin ; - HA : didn't have hemagglutinin
+ SAT : hydrophobic ; - SAT : hydrophilic

Seven of isolates S.aureus were adhesion test, four isolates is A1x PH, PH B6y, KHH A5x B5x and IRR is coagulase-positive S.aureus. All four have a number of consecutive adhesion of 14.3, 8.85, 12.9 and 14.45 with an average adhesion figure 12.63. While the remaining three isolates B2y PH, PH B10y and KNH A6y a coagulase-negative S.aureus adhesion which has consecutive numbers of 12.65, 19.4 and 11.5. Average number of coagulase-negative S.aureus adhesion is 14.52. When compared between

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the two, *S. aureus*, coagulase-positive have a lower adhesion rate. This is supported that *S. aureus* has a capable coagulate clotting process similar to the activation protrombin into thrombin and thrombin subsequently needed to change fibrinogen into fibrin. This fibrin that coats the surface of bacterial cells and protects *S. aureus* from phagocytosis activity. *S. aureus* with coagulase production tend to be resistant to phagocytosis and has a low level of adhesion. This is because the fibrin layer covering the surface *S. aureus* cover the expression of other surface proteins to bind to host cell receptors that have hampered this adhesion. Similar results found in *S. aureus* isolated from dairy cow milk Akmil Magetang, where *S. aureus* coagulase-positive have a lower level of adhesion than the *S. aureus*, coagulase-negative. However, if significance is measured using T-test with signifikasi 0.05 level, indicating that the level of adhesion between the isolates of *S. aureus* and coagulase-positive *S. aureus*, coagulase-negative that comes from goat's milk does not differ significantly (> 0.05). This was likely influenced by several factors other than coagulase. The presence of other surface proteins such as protein A, FnBP and also the surface hydrophobicity of bacteria is a factor that helped determine the level of bacterial adhesion to host cells.

Character of both coagulase-positive *S. aureus* and coagulase-negative, which tend to have high protein A marked most compact form colonies on the SSA media, and are hydrophobic hemaglutinated of erythrocytes have a high level of adhesion. While *S. aureus*, which tend to have low-protein A, which is marked largely formed diffuse colonies in SSA medium, slightly hemaglutinated of erythrocytes, and the hydrophilic portion has a low level of adhesion. Influence of the presence of coagulase coagulase-positive *S. aureus* showed a lower level than the adhesion of *S. aureus*, coagulase-negative, although not significantly different.

![Figure 1. Adhesion of *S. aureus* to epithelial cells of udder](image)

**Figure 1. Adhesion of *S. aureus* to epithelial cells of udder**

A: *S. aureus*  B: epithelial cells of udder

**Conclusion**

*Staphylococcus aureus* both coagulase-positive *S. aureus* and coagulase-negative *S. aureus*, indicating that the presence of protein A, haemagglutinin and a hydrophobic had higher adhesion in epithelial cells. Coagulase-positive of *S. aureus* has a lower adhesion capacity than coagulase-negative *S. aureus*. The presence of protein A, the haemagglutinin, coagulase and bacterial cell surface hydrophobicity influences the level of adhesion to host cells.

**References**


