Determination of Haemaglutinin and Gene Encoding Fibronectin Binding Proteins Staphylococcus aureus Isolated from Dairy Milk Cows

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Abstract

Staphylococcus aureus is a major pathogen causing clinical and subclinical mastitis in dairy milk cows. The mastitis has immense economical impacts, where it reduces of the quantity and quality of milk production. The aims of the research were to analyse haemaglutinin and gene encoding fibronectin binding proteins. Nineteen Staphylococcus aureus isolates used in the present study were isolated from dairy milk cows from Yogyakarta, Solo, Boyolali and Sumedang. The haemaglutinin of Staphylococcus aureus were determined based on haemaglutination reaction to erythrocytes of rabbit. Detection of gene encoding fibronectin binding proteins could be performed with specific primers using polymerase chain reaction (PCR). The results of studies showed that most of Staphylococcus aureus (78,95%) expressed haemaglutinin based on their ability to aglutinate rabbit erythrocytes. Analysis of gene encoding fibronectin binding proteins of Staphylococcus aureus revealed gene fnbA with size of approximately 1300 bp for 57,89% isolates, gene fnbB with size of approximately 900 bp for 31,58% isolates and both of gene fnbA and fnbB could be detected for 31,58% isolates. The characters of Staphylococcus aureus based on haemaglutinin, gene fnbA and fnbB of the present study could be used as an information to control of S. aureus infection in dairy herds.

Keywords : Staphylococcus aureus, haemaglutinin, gene encoding fibronectin binding protein, milk cow

Introduction

Staphylococcus aureus is world wide recognized as a major pathogen causing subclinical intramammary infections in dairy cows. Intramammary infections can cause high economic loss especially because of decreasing up to 10-25% of the total milk cows production (Han et al., 2000). Staphylococcus aureus from the environment available to contaminate fresh milk during milking of cows or during milk processing (Morandi et al., 2007).

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The ability of S. aureus to infect the hosts are supported by their ability to produce toxins, enzymes and surface proteins of the bacteria. Adhesin or haemaglutinin is a protein that play role in adhesion of S. aureus to host cells. The existence of S. aureus adhesin could be determined by haemaglutination reaction and amplification of genes encoding fibronectin binding protein. In dairy cattle, the adhesion process of S. aureus to the host cells correlates with the pathogenesis of mastitis. Fibronectin binding proteins (FnBP) are important factors in course of adhesion of S. aureus to milk cow glands. This proteins are encoded by fnbA and fnbB genes (Nasev et al., 2004; Pohlman-Dietze et al., 2000)

The control of S. aureus infection is recently developed based on character
of pathogenicity which is vary between countries. In Indonesia, the mapping of the characters and the phylogenetic nature of the S. aureus population have not been well done, thus the S. aureus infection can not be controled completely. The aims of this study were to characterize S. aureus phenotypic and genotypically through the detection of haemaglutinin and the existence of genes encoding fibronectin binding proteins among S. aureus isolated from dairy milk cows from various origins.

Materials and Methods

Bacterial Isolates

Nineteen Staphylococcus aureus isolates used in the present study were isolated from dairy milk cows from Yogyakarta, Solo, Boyolali and Sumedang. The identification of S. aureus were performed based on Gram staining, fermentation of mannitol salt agar (MSA), catalase and coagulase assays. Molecular identification of the S. aureus 23S rRNA genes was conducted by PCR using oligonucleotide species specific primers (Table 1).

Haemaglutinin and Fibronectin Binding Protein Assays

The haemaglutinin of S. aureus was determined based on haemaglutinating reaction to erythrocytes of rabbit. The genes encoding fibronectin binding proteins were determined by using PCR with specific primers (Table 1).

Preparation of DNA

The bacterial DNA was prepared with the QIAamp tissue kit as described by the manufacturer (Qiagen). After cultivation of the isolates for 24 h at 37°C on blood agar plates, 5-10 colonies of the bacteria were suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8)) containing 5 µl lysostaphin (1.8 U/µl; Sigma). After 1 h incubation at 37°C, 25 µl of proteinase K (14.8 mg/mL; Sigma) and 200 µl of buffer AL (containing reagents AL1 and AL2) were added. The suspension was incubated for 30 min at 56°C and for 10 min at 95°C, and after a spin for a few sec an amount of 420 µl ethanol was added to each sample and placed in a spin column. After centrifugation for 1 min, the QIAamp spin columns were placed in a clean collection tube and the samples were washed twice with 500 µl of buffer AW (Qiagen). After a sec wash and a centrifugation for 3 min, the QIAamp spin columns were placed in a clean 2 mL microfuge tube and the DNA was twice eluted with 200 µl and 100 µl of buffer AE, respectively. The DNA could be stored at -20°C. Amplification reactions with PCR were performed by program and specific primer according to the references for 23S rRNA and fnbA and fnbB genes. PCR products were then separated by gel electrophoresis in a 1.5% w/v agarose (Seakem) gel run in 0.5 × TBE buffer. The 1 kb Plus DNA ladder (Invitrogen) was used as a size marker. Resulting bands were visualized with cyber safe by UV transillumination.

<table>
<thead>
<tr>
<th>Gene</th>
<th>5’ primer sequence (5’→3’ )</th>
<th>3’ primer sequence (3’→5’ )</th>
<th>Program*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23S rRNA</td>
<td>AGC GAG TTA</td>
<td>CAA AGC TCA GCC TTA</td>
<td>1</td>
<td>Straub et al., 1999</td>
</tr>
<tr>
<td>fnbA</td>
<td>GCG GAG ATC</td>
<td>AAA CCA TCT ATA GCT</td>
<td>2</td>
<td>Signas et al., 1989</td>
</tr>
<tr>
<td>fnbB</td>
<td>GGA GAA ATT GCC GTG TGG</td>
<td>GGA GCG AAG GCG</td>
<td>2</td>
<td>Jonsson et al., 1991</td>
</tr>
</tbody>
</table>

*1: 37 cycles (94°C. 40 s, 64°C. 60 s, 72°C. 75 s); 2: 30 cycles (94°C. 30 s, 50°C. 30 s, 72°C. 60 s)
Results and Discussions

Based on biochemical and molecular identification showed that all 19 isolates used in this study were *S. aureus*. All *S. aureus* were Gram positive, fermented mannitol salt agar (MSA), catalase and coagulase positive. The genotypic identification showed that 23S rRNA gene could be amplified by PCR from all *S. aureus* isolates. This target genes allowed a rapid identification of this species with high sensitivity and specificity (Salasia et al., 2004b).

Haemaglutinin of *S. aureus* was determined based on haemaglutinating reaction. Haemaglutination by *S. aureus* relate to mastitis pathogenesis in cows. It was reported that more than 75% of *S. aureus* strains causing mastitis mediated by haemaglutination. Haemaglutination also related to *S. aureus* adherence in human epithelial cells (Rupp et al., 1995).

The results of haemaglutinating reaction indicated that most of *S. aureus* could aglutinate erythrocytes of rabbit (15 isolates/78.95%). Haemaglutinin play an important role on adhesion, at the preliminary colonization of the bacteria on the surface of intramammary epithelial cells. *Staphylococcus aureus* with haemaglutinin positive were more adhesive to intramammary epithelial cells than haemaglutinin negative *S. aureus*. The role of haemaglutinin on adhesion was predicted as an important factor for *S. aureus* virulence (Wahyuni et al., 2005).

Some *S. aureus* strains used in the study showed no reaction with erythrocytes of rabbit. Wahyuni (2002) reported that *S. aureus* isolates which have no haemaglutination activity, might be have haemaglutinin but its existence were covered by capsule on the surface of bacteria. Abrar (2001) reported that polysacharide capsules of *S. aureus* have an important role of subclinical mastitis of cows. This capsules could cover the expression of haemaglutinin. The results of haemaglutinating reaction *S. aureus* isolated from dairy milk cows from various origins are presented in Table 2.

The microbial adherence to cells and extracellular matrix are considered as an essential step in the process of colonization and infection of *S. aureus* (Nashev et al., 2004). Fibronectin binding protein play a role towards adhesion of *S. aureus* to the epithelial cells and milk gland cells of cows (Pohlmann-Dietze et al., 2000; Salasia et al., 2004a). Binding towards fibronectin are mediated by two protein FnBPA and FnBPB which are encoded by genes fnbA and fnbB respectively (Nashev et al., 2004). Ligand binding region of fibronectin binding proteins (FnBPA and FnBPB) are located in C-terminal D repeats (Hartford, 1999).

Amplification of fnbA and fnbB genes in the recent study showed the amplicons as informed at the previous study by Nashev et al. (2004). The fnbA gene has an amplicon with size of approximately 1300 bp (Figure 1) and 900 bp for fnbB gene (Figure 2).

![Figure 1. Amplification of fnbA gene of S. aureus with size of approximately 1300 bp. M (marker DNA, Fermentas), K- (negative control), SU39, SU34 (isolates from dairy milk cows from Solo), SU28, SU25, SU24 (isolates from dairy milk cows from Yogyakarta), K+ (positive control).](image1)

![Figure 2. Amplification of fnbB gene of S. aureus with size of approximately 900 bp. M (marker DNA, Fermentas), K- (negative control), SU39, SU34 (isolates from dairy milk cows from Solo), SU28, SU25, SU24 (isolates from dairy milk cows from Yogyakarta), K+ (positive control).](image2)
Staphylococcus aureus isolated from dairy milk cows from various origins could be detected fnbA gene for 11 isolates (57.89%), included six isolates from Yogyakarta (SU2, SU5, SU28, Y5, Y7), one isolate from Solo (SU47), 2 isolates from Boyolali (BY5, BY7) and three isolates from Sumedang (I4, Jaed2, P1). Staphylococcus aureus strains used in the study revealed fnbB gene for 6 isolates (31.58%), included 4 isolates from Yogyakarta (SU2, SU28, Y5, Y7) and 2 isolates from Boyolali (BY5, BY7). Combination of fnbA and fnbB genes of Staphylococcus aureus could be detected for 31.58% isolates (Table 2).

The characterisation of S. aureus isolated from dairy milk cows from various origins based on phenotypic and genotypic are presented in Table 2. Group I showed that S. aureus strains SU2 and SU28 had the same phenotypic and genotypic characters. These strains were haemaglutination positive and revealed both genes encoding fibronectin binding proteins (fnbA and fnbB). S. aureus strains SU47 and SU5 showed also the same phenotypic and genotypic characters, and these strains were positive haemaglutination and positive of the gene fnbA, but negative gene fnbB. Staphylococcus aureus of group II showed the same character of phenotypic- and genotypically, positive on haemaglutination reaction, but negative of both gene encoding fibronectin binding proteins (fnbA and fnbB). The group III of S. aureus showed various characters of phenotypic- and genotypically. However, group IV of S. aureus showed that all strains were positive haemaglutination reaction and had both genes encoding fibronectin binding proteins (fnbA and fnbB). S. aureus strains used in the recent study which have the same character phenotypic- and genotypically character could be grouped into the same cluster.

The results of haemaglutination reaction and the existence of genes encoding fibronectin binding proteins (fnbA and fnbB) of S. aureus isolates from milk cows from various origins in Indonesia might use as a basic epidemiology study and control of S. aureus infections in herds.

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### References


