

## Determination of Haemagglutinin and Gene Encoding Fibronectin Binding Proteins *Staphylococcus aureus* Isolated from Dairy Milk Cows

Feny Prabawati Pratomo<sup>1</sup>, Siti Isrina Oktavia Salasia<sup>2\*</sup>, and Syarifudin Tato<sup>3</sup>

<sup>1</sup>Research Center for Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia

<sup>2</sup>Department of Clinical Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

<sup>3</sup>Departement of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

### 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 haemagglutinin 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 haemagglutinin of *S. aureus* were determined based on haemagglutination 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 *S. aureus* (78,95%) expressed haemagglutinin based on their ability to agglutinate rabbit erythrocytes. Analysis of gene encoding fibronectin binding proteins of *S. 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 *S. aureus* based on haemagglutinin, 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*, haemagglutinin, 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).

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 haemagglutinin is a protein that play role in adhesion of *S. aureus* to host cells. The existence of *S. aureus* adhesin could be determined by haemagglutination 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

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#### \*Corresponding author:

Siti Isrina Oktavia Salasia

Department of Clinical Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia,

Email: [isrinasalasia@ugm.ac.id](mailto:isrinasalasia@ugm.ac.id)

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 haemagglutinin 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).

### Haemagglutinin and Fibronectin Binding Protein Assays

The haemagglutinin of *S. aureus* was determined based on haemagglutinating 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 secs 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.

Tabel 1. Primer and PCR programs for amplification of 23SrRNA gene and genes encoding fibronectin binding proteins

Gene	5' primer sequence (5'→3')				3' primer sequence (3'→5')				Program*	Reference
23S rRNA	AGC	GAG	TTA	CAA	AGC	TCA	GCC	TTA	1	Straub <i>et al.</i> , 1999
	AGG	ACG	AC		ACG	AGT	AC			
<i>fnbA</i>	GCG	GAG	ATC	AAA	CCA	TCT	ATA	GCT	2	Signas <i>et al.</i> , 1989
	GAC	AA			GTG	TGG				
<i>fnbB</i>	GGA	GAA	GGA	ATT	GCC	GTC	GCC	TTG	2	Jonsson <i>et al.</i> , 1991
	AAG	GCG			AGC	GT				

\*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).

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

The results of haemagglutinating reaction indicated that most of *S. aureus* could agglutinate erythrocytes of rabbit (15 isolates/78,95%). Haemagglutinin play an important role on adhesion, at the preliminary colonization of the bacteria on the surface of intramammary epithelial cells. *Staphylococcus aureus* with haemagglutinin positive were more adhesive to intramammary epithelial cells than haemagglutinin negative *S. aureus*. The role of haemagglutinin 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 haemagglutination activity, might be have haemagglutinin but its existence were covered by capsule on the surface of bacteria. Abrar (2001) reported that polysaccharide capsules of *S. aureus* have an important role of subclinical mastitis of cows. This capsules could cover the expression of haemagglutinin. The results of haemagglutinating 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).

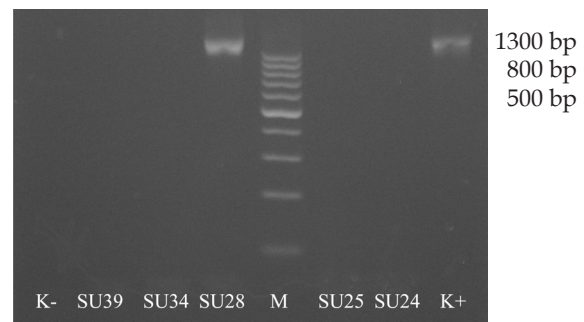


Figure 1. Amplification of *fnbA* gene of *S. aureus* with size of approximately 1300 bp. M (marker DNA, Fermentas), K- (negative control), SU39, SU34 (isolates from dairy milk cows from Solo), SU28, SU25, SU24 (isolates from dairy milk cows from Yogyakarta), K+ (positive control).

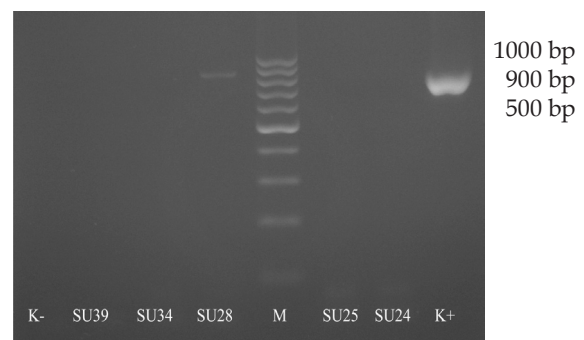


Figure 2. Amplification of *fnbB* gene of *S. aureus* with size of approximately 900 bp. M (marker DNA, Fermentas), K- (negative control), SU39, SU34 (isolates from dairy milk cows from Solo), SU28, SU25, SU24 (isolates from dairy milk cows from Yogyakarta), K+ (positive control).

*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 haemagglutination 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 haemagglutination 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 haemagglutination 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 haemagglutination 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 haemagglutination 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.

Table 2. Haemagglutination reaction and genes encoding fibronectin binding proteins of *Staphylococcus aureus* isolated from dairy milk cows from various origins

No	Code of Isolate	Origin of Isolate	Haema-glutination reaction	<i>fnbA</i> gene	<i>fnbB</i> gene
1	SU28	Yogyakarta	+	+	+
2	SU2	Yogyakarta	+	+	+
3	SU47	Solo	+	+	-
4	SU5	Yogyakarta	+	+	-
5	SU25	Yogyakarta	+	-	-
6	SU16	Yogyakarta	+	-	-
7	SU24	Yogyakarta	+	-	-
8	SU39	Solo	+	-	-
9	SU34	Solo	+	-	-
10	SU10	Yogyakarta	+	-	-
11	I4	Sumedang	-	+	-
12	I2	Sumedang	-	-	-
13	Mjl2	Sumedang	-	-	-
14	Jaed2	Sumedang	+	+	-
15	P1	Sumedang	-	+	-
16	Y7	Yogyakarta	+	+	+
17	BY7	Boyolali	+	+	+
18	BY5	Boyolali	+	+	+
19	Y5	Yogyakarta	+	+	+

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