VIRULANCE FACTOR OF *Staphylococcus sp*. ISOLATED FROM SUBCLINICAL MASTITIS IN ETTAWA GRADE GOAT'S MILK IN SLEMAN REGENCY -YOGYAKARTA

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ABSTRAK

Stapphylococcus sp., merupakan bakteri penyebab mastitis subklinis pada kambing peranakan Ettawa (PE). Tujuan dari penelitian ini adalah menentukan faktor virulensi *Stapphylococcus sp*., yang diisolasi dari susu kambing PE mastitis subklinis di Kabupaten Sleman, Yogyakarta. Sebanyak 7 isolat *Staphylococcus sp*., yang diisolasi dari susu kambing PE mastitis subklinis ditentukan faktor virulensi hemolisin, *clumping factor*, dan koagulase. Hemolisin ditentukan dengan *culture Staphylococcus sp*., pada media *plate* agar darah dan diinkubasikan pada suhu 37°C selama 24 jam. *Clumping factor* ditentukan melalui pencampuran biakan *Stapphylococcus sp*. dengan plasma kelinci dalam gelas obyek. Koagulase ditentukan dengan mencampurkan biakan *Stapphylococcus sp*., dengan plasma kelinci dalam tabung reaksi dan diamati terbentuknya gel. Haemolitik tipe ß diproduksi oleh 5 isolat *Stapphylococcus sp*., sedangkan 2 isolat *Stapphylococcus sp*., tidak bersifat hemolitik. *Clumping factor* dan koagulase hanya diproduksi oleh 2 isolat *Stapphylococcus sp.* Penelitian ini menunjukkan bahwa tidak semua isolat *Stapphylococcus sp*., penyebab mastitis subklinis pada kambing PE memiliki faktor virulensi.

Kata kunci: faktor virulensi, identifikasi, Stapphylococcus sp.

ABSTRACT

Stapphylococcus sp., is bacteria that caused subclinical mastitis in Ettawa Grade (EG) goat. The purpose of this study was to determine virulance factor *Stapphylococcus sp*., which was isolated from subclinical mastitis EG goat's milk in Sleman regency, Yogyakarta. A total of 7 isolate *Stapphylococcus sp.*, were isolated from subclinical mastitis EG goat's milk were determinated by several virulance factors such as haemolysin, clumping factor, and coagulase. Haemolysin was determinated by culture in blood agar plate and incubated in the temperature of 37°C for 24 hours. Clumping factor was determinated by mixing the rabbit plasma with *Stapphylococcus sp.*, in the glass objects. Coagulase was determinated by mixing the rabbit plasma and broth culture of *Stapphylococcus sp*. After incubated in the temperature of 37°C for 24 hours in tube, then the gel formation was observed. Haemolytic type ß was yielded from 5 isolate *Stapphylococcus sp*., whereas 2 isolates were not haemolytic. Clumping factor and coagulase were produced from 2 isolate *Stapphylococcus sp*. This study showed that not all of *Stapphylococcus sp*., isolate causing subclinical mastitis in EG goat have virulance factor.

Keywords: virulance factor, identification, Stapphylococcus sp.

INTRODUCTION

Sleman Regency is one of areas rearing Ettawa Grade (EG) goat. In addition to its milk, EG goat is reared for hobbies. Recently, people consume goat milk for therapeutic purpose and strengthening their vitality. Therefore, the demand of goat milk in Sleman increases. However, subclinal mastitis still the obstacle due to its ability to reduce milk production. Subclinical mastitis reduces the production of goat milk for about 37% (Fthenakis and Jones, 1990). Koop *et*

al. (2010) stated that subclinical mastitis in goat results in economic loss because milk production decreases until 60%.

Subclinical mastitis is an inflammation found in the udder with no clinical symptoms. Subclinical mastitis is characterized by the increasing number of total somatic cell in milk without inflammation of the udder, and when tested by using California Mastitis Test (CMT), agglutination occurs (Marogna *et al.,* 2012). The average of prevalence of subclinical mastitis found in goat ranch was approximately 20% to 35% (Contreras *et al.,* 2003). In 2007, Contreras *et al.,* (2007) reported that the prevalence of subclinical mastitis in goat was approximately 5% - 30%, and staphylococci negative coagulase was the dominant agent. Prevalence of staphylococci negative coagulase in goat with subclinical mastitis was approximately 44.7% to 95.9% (Contreras *et al.*, 2007; White and Hinckley, 1999). In Israel, the prevalence of subclinical mastitis in goat was 35% to 71% with negative coagulase of *S. aureus* as the dominant agent (Leitner *et al.,* 2004). Report of Suwito *et al.* (2012) showed that subclinical mastitis in EG goat from Sleman district was 35% resulted from *Stapphylococcus sp*.

Virulance factor *Stapphylococcus sp.,* plays the important role in the pathogenesis of subclinical mastitis in goat. *Stapphylococcus sp.,* has several virulance factors such as surface type antigen, degradation enzym, enterotoxin, leucocidin, and haemolysin (Peacock *et al.,* 2002). Clumping factor is one of virulance factors surface antigen from *Stapphylococcus sp*. Clumping factor and coagulase are capable of forming coagulation of rabbit plasma, but the reaction mechanism is different. In addition to clumping factor and coagulase, haemolytic also serves as important virulance factor as it performs haemolytic erythrocytes. *Stapphylococcus sp*., produces two haemolytic factors, namely α and ß. Haemolytic type α is produced from 20% to 50%, whereas haemolytic type ß is approximately 75% to100% (Dinges *et al.,* 2000).

The purpose of this study was to determine virulance factor *Stapphylococcus sp*., from subclinical mastitis EG goat's milk.

MATERIALS AND METHODS

Research Materials

The materials were 7 isolates of *Stapphylococcus sp.* which were isolated from subclinical mastitis EG goat milk in Sleman regency.

Re-identification of *Stapphylococcus sp*

Re-identification *Stapphylococcus sp.,* was performed by observing the morphology of the colony in the blood agar plates (BAP) (BAP; Oxoid Ltd., Basingstoke, UK), Gram staining, catalase test, coagulase test, and mannitol fermentation test (Green *et al.,* 2004).

Haemolytic Test

The ability of *Stapphylococcus sp.,* in the haemolytic factor was tested by culturing in the blood agar plate (BAP) (BAP; Oxoid Ltd., Basingstoke, United Kingdom) and then incubated at 37°C for 24 hours. Types of haemolytic were clasified into three categories, those were α, β, and non haemolytic (Barrow and Feltham, 1993).

Clumping Factor

Detection of clumping factor was determined by slide agglutination test. One colony of *Stapphylococcus sp.*, was taken from BAP media using sterile *Ose,* and mixed with aquades sterile until homogen suspension *Stapphylococcus sp*. These suspension of *Stapphylococcus sp*. were added with two drops of rabbit plasma stirred by a sterile toothpick and observed the occurrence of the agglutination. Clumping factor positive occurs when the agglutination may be formed from the suspension of *Stapphylococcus sp*., and rabbit plasma. It would be negative if suspension of *Stapphylococcus sp*., remains homogen (Barrow and Feltham, 1993).

Coagulase

The presence of enzym coagulase was detected by tube coagulation test. Culture of S*tapphylococcus sp.* in 5 ml brain heart infusion medium (BHI; Oxoid Ltd., Basingstoke, UK) was incubated in 37°C for 24 h and then mixed with rabbit plasma in tube reaction with same volume and put in a water bath with a temperature of 37° C. The formation gel was observed in 15 min, 30 min, and 60 min. Positive coagulase will be found if the suspension of *Stapphylococcus sp.* and rabbit plasma forms gel, but the coagulase will consider negative if this mixing remains homogen (Barrow and Feltham, 1993).

Data Analysis

Virulance factor Staphylococci data was

analyzed descriptively (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Re-identification of 7 *Staphylococcus sp*., isolate were presented in Table 1. The 7 isolates *Staphylococus sp*., was identified as *S. intermedius* (3 isolates), *S. hyicus* (2 isolates), *S. aureus* (1 isolate) and *S. epidermidis* (1 isolate). Contreras *et al.* (1995) reported that 49 isolates of *Staphylococcus sp*., were isolated from subclinical mastitis goat consisted of *S. caprae* was 22%, *S. epidermidis* was 20%, *S. chromogenes* was 12%, and *S. aureus* was 6%. Similar research was also conducted by Sanchez *et al.* (1999) that subclinical mastitis in goats resulted from *S. aureus* was 19.7%, *S. epidermidis* was 12.7%, *S. hyicus* was 4.2%, while *S. intermedius* was negative.

Morphology of *Stapphylococcus sp*., isolates was cocci (Figure 1A) and a positive catalase test

Table 1. Identification of *Stapphylococcus sp*. from Subclinical Mastitis of Ettawa Grade (EG) Goat in Sleman Regency

Test	Isolate						
	A	B	C	D	E	F	G
Morphology	*coccus*	*coccus*	*coccus*	*coccus*	*coccus*	*coccus*	*coccus*
Catalase	+	+	+	+	+	+	+
Manitol	+	–	+	+	–	–	+
Voges-Proskauer (VP)	+	–	–	–	+	–	–

A: *Stapphylococcus aureus*; C,D&G: *Stapphylococcus intermedius*; E: *Stapphylococcus epidermidis*; B&F: *Stapphylococcus hyicus*

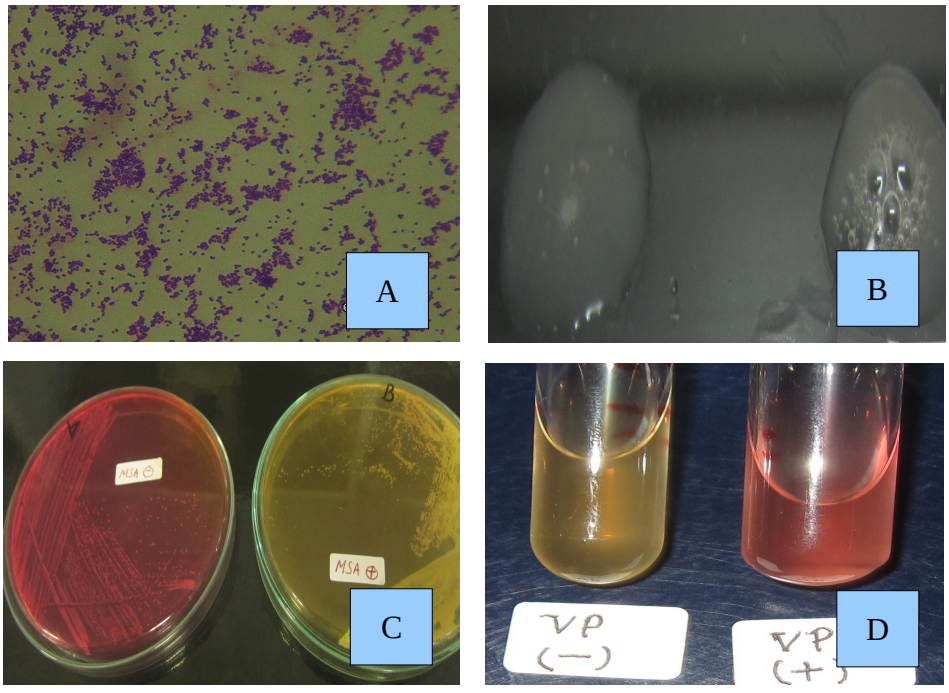

Figure 1. Morfology and Biochemical Characteristics of *Stapphylococcus sp*
A: morphology *Stapphylococcus sp*; B: catalase test;
C: *Stapphylococcus sp* in MSA; D: voges-proskauer test

with oxygen bubble formation (Figure 1B). In the mannitol salt agar (MSA) medium (MSA; Oxoid Ltd., Oxoid Ltd., Basingstoke, UK), *Staphylococcus sp*., had two kinds of mannitol fermentation in which yellow coloni was mannitol fementation and red coloni was not mannitol fermentation.(Figure 1C). Mannitol is one of carbohydrate medium used to differentiate the biochemical properties among *Stapphylococcus sp*., isolate (Barrow and Feltham, 1993).

Stapphylococcus aureus and *S. intermedius* have characteristic of fermented mannitol, whereas the others were not fermented mannitol. The result of voges-proskauer (vp) test was only positive in *S. aureus* and *S. epidermidis* (Figure 1D). The purpose of v-p test is to determine the ability of *S. aureus* to produce *acetyl methyl carbinol* (acetoin) from glucose metabolism. *Stapphylococcus aureus* may produce acetoin as the result of glucose fermentation which differentiates it with other *Staphylococcus sp* (Quinn *et al*., 2002).

Virulance factor from each *Stapphylococcus sp.*, isolate is presented in Table 2. Table 2 shows that virulance factor of type ß haemolytic may be found in most *Stapphylococcus sp*., isolated from subclinical mastitis EG goat's milk. Dinges *et al.* (2000) stated that most *Stapphylococcus sp*. isolate had haemolytic type ß about 75% to 100%. The type ß haemolytic from *S. aureus*, *S. hyicus*, *S. intermedius, S. epidermidis* isolates are shown in Figure 2.

Haemolysin is virulance factor in *Stapphylococcus sp.*, which works by performing haemolysis in red blood cells (Dinges *et al.*, 2000). Haemolysin was not produced from any isolates of *S. intermedius* and *S. hyicus* (Table 2), so these isolates were less pathogen. Andresen (2005) reported that *S. hyicus* isolated from pig with exudative epidermidis was pathogen with the exfoliative toxin, and so it was the *S. hyicus* from mastitis cow in Japan. *Stapphylococcus hyicus* was grouped into two types, those were pathogens and less pathogens. The pathogen *Stapphylococcus hyicus* causes exudative epidermitis in pigs (Wegener *et al.*, 1993).

Based on the Table 2, isolate A (*S. aureus*) and E (*S. epidermidis*) had virulance factors, clumping factor and coagulase. Positive clumping factor and coagulase are shown in Figures 2B and 2D. Clumping factor is type of surface antigen which plays important role in the process of attachment in the mammary gland epithelial cells (McDevitt *et al.*, 1992).

Surface antigen consists of clumping factor A (ClfA), clumping factor B (ClfB), fibronectin protein A, protein collagen, elastin proteins, sialoprotein, protein A (IgG-binding protein), synthesis CP5 enzyme, synthesis CP8 enzyme, and intracellular adhesion protein A. *Staphylococcus sp*., produces coagulase enzyme which can coagulate the rabbit plasma (Dinges *et al.*, 2000). In *Staphylococcus sp*., mechanism of clumping factor is similiar to coagulase, but different in the pathogenesis. The role of coagulase in the infection endocarditis caused by *S. aureus* is to facilitate procoagulant in the bonding activity with fibrinogen (Boden and Flok, 1989).

CONCLUSION

Stapphylococcus aureus, S. epidermidis, S. intermedius, and *S. hyicus* from Ettawa Grade (EG) goat's milk subclinical mastitis have virulance factor with haemolytic ß, but *S. aureus* and *S. epidermidis* only have clumping factor and

Table 2. Virulance Factor *Stapphylococcus sp*. Isolated from Subclinical Mastitis of Ettawa Grade (EG) Goat in Sleman regency

Virulance Factor	Isolate						
	A	B	C	D	E	F	G
Haemolytic	ß	ß	ß	−	ß	−	ß
Clumping factor	+	−	−	−	+	−	−
Coagulase	+	−	−	−	+	−	−

A: Stapphylococcus aureus; C, D & G: Stapphylococcus intermedius; B & F: *Stapphylococcus hyicus*; E: *Stapphylococcus epidermidis*

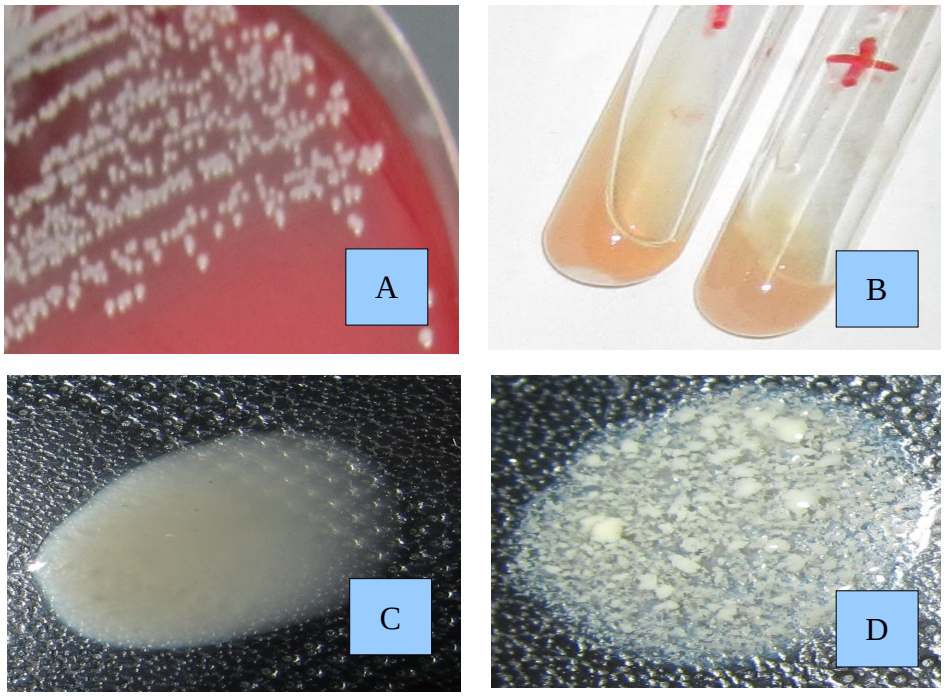

Figure 2. Virulance factor from *Stapphylococcus sp*
A: Type ß haemolytic; B: Catalase test;
C: Clumping factor −; D: Clumping factor +

coagulase. Further research to examine the characterization of genotifik *Stapphylococcus sp*. from subclinical mastitis goat's milk is essential to perform.

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