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Original Article

Genotypic characterization of *Staphylococcus aureus* isolated from bovines, humans, and food in Indonesia

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The present study determined the genetic relationships between 41 *Staphyloccocus* (*S.*) *aureus* isolates from bovines, humans, and food using a single enzyme amplified fragment length polymorphism (AFLP) technique. We evaluated the prevalence of staphylococcal enterotoxin (SE) genes and other virulence gene determinants by PCR. The identification of *S. aureus* was based on culturing and biochemical tests, and by amplifying a specific section of the 23S rRNA gene. PCR amplification of the SE genes (*sea*, *seb*, *sec*, *see*, *seg*, *seh*, and *sei*) singly or in combination was observed. Most isolates of bovine origin harbored *hla* (84%) and *cap*5 (74%), while most isolates from humans harbored *hla* (73%), *cap*8 (91%), and *fnb*A (100%). Strains from food sources were positive for *hla* (100%), *cap*5 (100%), and *cap*8 (64%) unlike isolates from humans or bovines. A single enzyme AFLP analysis revealed a correlation between AFLP clusters of some strains and the source of the isolates The genotypic results of the present study might help to better understand the distribution of prevalent *S. aureus* clones among humans, bovines, and food and will help control *S. aureus* infections in Indonesia.

Keywords: amplified fragment length polymorphism, enterotoxin, genetic determinant, *Staphylococcus aureus*

Introduction

Staphylococcus (*S.*) *aureus* is recognized worldwide as a major pathogen that causes food poisoning and various infections in animals and humans. The primary habitat of *S. aureus* is the nasal passage of humans, and on the skin and hair of warm-blooded animals. Hence, the sources of this organism found on food are mostly human or animal [24]. In dairy cows, *S. aureus* causes subclinical intramammary infections [7]. The main reservoir of *S. aureus* is an infected quarter of udder, and transmission between cows usually occurs during milking [7]. *S. aureus* is also an important food-borne pathogen because of its ability to produce a wide range of extracellular toxin proteins and virulence factors that contribute to the pathogenicity of the organism.

Molecular typing of *S. aureus* and determining the clonal relationships between isolates has proved useful for epidemiological studies. One of the most recent and promising of these genotyping techniques is amplified fragment length polymorphism (AFLP) analysis [4]. The objective of the present study was to determine the genetic relationship of *S. aureus* isolated from humans, bovines, and food. The *S. aureus* strains we isolated were also analyzed to evaluate the distributions of genes encoding enterotoxin and other virulence factors.

Materials and Methods

Bacterial isolates

Samples of *S. aureus* were obtained from the mastitic milk of 19 dairy cattle from 19 farms located in Central and West Java, Indonesia. Eleven samples of *S. aureus* from humans were obtained from skin infections of patients at Sardjito Hospital, Indonesia. The strains were kindly provided by Dr. Hera Nirwati, Microbiology Laboratory, Faculty of Medicine, Gadjah Mada University, Indonesia. In addition, 11 samples of *S. aureus* were obtained from cooked foods in Central, West, and East Java, Indonesia. The foods were collected from several supermarkets stored in refrigerator. The origins of the strains are summarized in Table 1.

The strains were identified as *S. aureus* by their properties in culture, Gram staining, and biochemical tests. The biochemical tests included ones that measured catalase, coagulase, and clumping factor reactions as described previously [7]. The catalase test was done by placing a drop of hydrogen peroxide on a microscope slide. A small amount of bacterial isolate was added to hydrogen

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Table 1. *Staphylococcus aureus* strains investigated in the present study

Source	Isolated	Province	City	Isolate code
Bovine	Milk	1*	A†	BY5, BY7
			B	Y5, Y7, SU2, SU5, SU10
			C	SU16, SU24, SU25, SU28, SU34, SU39, SU47
		2	D	Jaed2, Mjl2, P1, I2, I4
Human	Skin wound (Hospital)	1	B	169, 179, 198, 199, 255, 262, 274, 870, 937, 979, 1091
Food	Milk package	1	B	SY1, SR1, SI1
	Fermented milk product	1	B	YK1
	Sosis	1	B	SS2
	Meat ball	1	B	B3
	Rolade	1	B	RO
	Cake	1	B	RY6
	Cake	2	D	BW4
	Cake	2	D	BL2
	Cheese	3	E	K1

*1: Central Java, 2: West Java, 3: East Java; †A: Boyolali, B: Yogyakarta, C: Surakarta, D: Sumedang, E: Surabaya.

peroxide, bubbles of oxygen was observed for catalase-positive. The coagulase test was performed by cultivation of the bacteria in the tube coagulase test (Bactident-Coagulase; Merck, Germany). The presence of coagulation was observed at 6 and 24th hours. The clumping factor test was observed by the presence of agglutination reaction of the bacteria with rabbit plasma on a microscope slide. Furthermore, the strains were identified by PCR amplification of the thermonuclease *nuc* gene [6] and 23S rRNA gene [25]. The reaction mixture (30 μL) contained 1 μL primer 1 (10 pmol), 1 μL primer 2 (10 pmol; Invitrogen, USA), 14 μL PCR mix containing Taq DNA polymerase, $MgCl_2$, and dNTPs (Roche, Germany), 5 μL of DNA template, and 9 μL distilled water. The DNA of the isolates was prepared with the QIAamp DNA mini kit (Qiagen, Germany) as described by the manufacturer. The amplification of the genes was carried out with a thermal cycler (Mastercycler; Eppendorf, Germany). The oligonucleotide primers and the thermal cycler programs are shown in Table 2.

DNA isolation and purification

A QIAmp DNA mini kit (Qiagen, Germany) was used to purify the DNA from *S. aureus* according to the manufacturer's protocol. The bacterial strains were cultivated on blood agar base (Oxoid, Germany) containing 5% defibrinated sheep blood for 24 h at 37°C. A total of 5 ~ 10 *S. aureus* colonies were suspended with 180 μL TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 8]) containing 5 μL lysostaphin (1.8 U/μL; Sigma, USA) in 2-mL microfuge tubes. The suspension was incubated for 1 h at 37°C, and 25 μL of proteinase K (14.8 mg/mL; Sigma, USA) and 200 μL of AL buffer (containing reagents AL1 and AL2; Qiagen, Germany) were then added. The suspensions were incubated for 30 min at 56°C, and then for 10 min at 95°C before being spun at 6,000 × g for a few seconds. A total of 420 μL ethanol was added to each sample and placed in a spin QIAmp column. After centrifugation at 6,000 × g for 1 min, the spin columns were placed in a clean collection tube and the sample was washed twice with 500 μL of AW buffer (Qiagen, Germany). After the second wash and a centrifugation at 6,000 × g for 3 min, the QIAamp spin columns were placed in a clean 2-mL microfuge tube, and the DNA was eluted twice with 200 μL and 100 μL of AE buffer (Qiagen, Germany). DNA was stored at −20°C.

Genotype characterization

A PCR method was used to identify the genetic determinants of various virulence factors. The oligonucleotide primers used amplified the genes encoding clumping factor (*clfA*) [23]; coagulase (*coa*) [11]; staphylococcal enterotoxins (SE) (*sea*) [26], (*seb*, *sec*, *sed*, and *see*) [13], (*seg*, *seh*, and *sei*) [12], (*sej*) [16]; exfoliative toxin A (*eta*) and B (*etb*) [13]; fibronectin binding protein A (*fnb*A) and B (*fnb*B) [14]; alpha- (*hla*) and beta-hemolysin (*hlb*) [5]; and capsular polysaccharide 5 (*cap*5) and 8 (*cap*8) [17]. The sequences of the primers and PCR conditions are shown in Table 2.

AFLP analysis

An AFLP analysis was performed according to Boerema *et al*. [4]. Genomic DNA (5 μL) was digested overnight (16 h) at 37°C with 10 U of *Hin*dIII (Invitrogen, USA) and 5 mM spermidine trihydrochloride (Sigma, USA) added to a

Table 2. Oligonucleotide primers and PCR programs used for amplifying the genes encoding 23S rRNA along with various other staphylococcal proteins including toxins and adhesive molecules

Target gene	Sequence (5′-3′)	PCR Program
23S rRNA	ACG GAG TTA CAA AGG ACG AC	1*
	AGC TCA GCC TTA ACG AGT AC	
nuc	GCG ATT GAT GGT GAT ACG GTT	2
	ACG CAA GCC TTG ACG AAC TAA AGC	
coa	ATA GAG ATG CTG GTA CAG G	3
	GCT TCC GAT TGT TCG ATG C	
*clf*A	GGC TTC AGT GCT TGT AGG	4
	TTT TCA GGG TCA ATA TAA GC	
sea	AAA GTC CCG ATC AAT TTA TGG CTA	6
	GTA ATT AAC CGA AGG TTC TGT AGA	
seb	TCG CAT CAA ACT GAC AAA CG	6
	GCA GGT ACT CTA TAA GTG CC	
sec	GAC ATA AAA GCT AGG AAT TT	6
	AAA TCG GAT TAA CAT TAT CC	
sed	CTA GTT TGG TAA TAT CTC CT	6
	TAA TGC TAT ATC TTA TAG GG	
see	TAG ATA AGG TTA AAA CAA GC	6
	TAA CTT ACC GTG GAC CCT TC	
seg	AAT TAT GTG AAT GCT CAA CCC GAT C	6
	AAA CTT ATA TGG AAC AAA AGG TAC TAG TTC	
seh	CAA TCA CAT CAT ATG CGA AAG CAG	6
	CAT CTA CCC AAA CAT TAG CAC C	
sei	CTC AAG GTG ATA TTG GTG TAG G	6
	AAA AAA CTT ACA GGC AGT CCA TCT C	
sej	CAT CAG AAC TGT TGT TCC GCT AG	7
	CTG AAT TTT ACC ATC AAA GGT AC	
eta	CTA GTG CAT TTG TTA TTC AA	6
	TGC ATT GAC ACC ATA GTA CT	
etb	ACG GCT ATA TAC ATT CAA TT	6
	TCC ATC GAT AAT ATA CCT AA	
*fnb*A	GCG GAG ATC AAA GAC AA	8
	CCA TCT ATA GCT GTG TGG	
*fnb*B	GGA GAA GGA ATT AAG GCG	8
	GCC GTC GCC TTG AGC GT	
hla	GGT TTA GCC TGG CCT TC	9
	CAT CAC GAA CTC GTT CG	
hlb	GCC AAA GCC GAA TCT AAG	10
	GCG ATA TAC ATC CCA TGG C	
*cap*5	ATG ACG ATG AGG ATA GCG	11
	CTC GGA TAA CAC CTG TTG C	
*cap*8	ATG ACG ATG AGG ATA GCG	12
	CAC CTA ACA TAA GGC AAG	

*1: 37 cycles of 94°C for 40 sec, 64°C for 60 sec, and 72°C for 75 sec; 2: 37 cycles of 94°C for 60 sec, 55°C for 30 sec, and 72°C for 30 sec; 3: 30 cycles of 94°C for 60 sec, 58°C for 60 sec, and 72°C for 60 sec; 4: 35 cycles of 94°C for 60 sec, 57°C for 60 sec, and 72°C for 60 sec; 5: 30 cycles of 94°C for 60 sec, 60°C for 60 sec, and 72°C for 60 sec; 6: 30 cycles of 94°C for 120 sec, 55°C for 120 sec, and 72°C for 60 sec; 7: 30 cycles of 94°C for 60 sec, 62°C for 60 sec, and 72°C for 60 sec; 8: 30 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 60 sec; 9: 20 cycles of 94°C for 10 sec, 53°C for 10 sec, and 72°C for 30 sec; 10: 20 cycles of 94°C for 10 sec, 62°C for 10 sec, and 72°C for 30 sec; 11: 20 cycles of 94°C for 15 sec, 57°C for 15 sec, and 72°C for 30 sec; 12: 20 cycles of 94°C for 15 sec, 52°C for 15 sec, and 72°C for 30 sec.

Table 3. Summary of the genotypic properties of the *Staphylococcus aureus* strains isolated from bovines, humans,and food based on the AFLP analysis along with the distribution of the staphylococcal enterotoxin genes and various virulence determinants

No.	Code	AFLP	Toxin gene	*coa*	*eta*	*etb*	*hla*	*hlb*	*cap*5	*cap*8	*fnb*A	*fnb*B
Bovine isolates												
1	BY5	A	−	4*	−	−	+	−	+	−	+	+
2	BY7	A	−	4	−	−	+	+	+	−	+	+
3	SU47	C	*se* (*b,i*)	3	+	−	+	−	+	−	+	−
4	SU34	D	*sec*	1	−	−	+	−	+	−	−	+
5	SU39	D	*se* (*c,e*)	2	+	−	+	−	+	−	−	+
6	SU25	E	−	2	+	−	+	−	+	+	−	+
7	Y5	G	*se* (*c,g,i*)	2	−	−	+	−	+	+	+	+
8	Jaed2	I	*se* (*a,b,h,i*)	3	+	−	+	−	−	ND	+	−
9	Mjl2	I	*se* (*a,b,h,i*)	3	−	−	−	−	−	ND	−	−
10	SU28	J	*se* (*b,c,i*)	3	+	−	+	+	+	−	+	−
11	P1	L	*se* (*a,b,h,i*)	2	+	−	−	−	−	ND	+	−
12	SU2	O	*seh*	2	+	−	+	−	+	+	+	−
13	Y7	O	*se* (*g,i*)	2	−	−	+	+	+	−	+	+
14	I2	Q	*se* (*a,b,h,j*)	ND	−	−	−	−	−	ND	−	−
15	I4	Q	*se* (*a,b,h,i*)	3	+	−	+	−	−	ND	+	−
16	SU5	R	*sec*	2	+	−	+	−	+	+	+	+
17	SU16	S	*se* (*c,e,i*)	3	−	−	+	−	+	+	−	+
18	SU24	S	−	2	+	−	+	−	+	+	−	+
19	SU10	V	*sec*	3	−	−	+	−	+	+	−	+
n=19					10	0	16	3	14	7	11	11
					53%	0	84%	16%	74%	50%	58%	58%
Human isolates												
1	179	F	*see*	3	−	−	+	+	−	+	+	−
2	198	F	*se* (*e,h*)	3	−	−	+	−	−	+	+	−
3	255	G	*se* (*g,i*)	3	−	−	−	+	−	+	+	−
4	169	L	*seh*	2	+	−	+	−	−	+	+	−
5	274	N	*se* (*b,g,h,i*)	2	−	−	−	+	+	−	+	−
6	937	R	*se* (*a,h*)	2	+	−	+	−	−	+	+	−
7	870	T	*se* (*a,h*)	2	−	−	+	+	+	+	+	−
8	199	U	*see*	3	−	−	+	+	−	+	+	−
9	262	V	*se* (*a,h*)	2	−	−	−	−	−	+	+	−
10	979	W	−	3	+	−	+	+	+	+	+	+
11	1091	W	−	1	+	−	+	−	−	+	+	+
n=11					4	0	8	6	3	10	11	2
					36%	0	73%	55%	27%	91%	100%	18%
Food isolates												
1	SY1	B	*se* (*b,c*)	2	−	−	+	−	+	+	−	−
2	K1	H	*se* (*b,c*)	1	+	−	+	−	+	+	−	−
3	SR1	H	*se* (*c,e*)	2	−	−	+	−	+	+	−	−
4	SI1	J	*sec*	3	−	−	+	−	+	−	−	−
5	BW4	M	*see*	3	+	−	+	+	+	+	+	+
6	YK1	M	*se* (*b,c*)	2	−	−	+	−	+	−	−	+
7	BL2	N	*sec*	2	−	−	+	−	+	+	+	−
8	SS2	P	*se* (*b,c*)	3	−	−	+	−	+	+	+	−
9	B3	P	*sec*	3	−	−	+	−	+	−	+	+
10	RY6	U	*se* (*c,g*)	3	+	−	+	+	+	−	+	−
11	RO	X	−	4	−	−	+	−	+	+	−	−
n=11					3	0	11	2	11	7	5	3
					27%	0	100%	18%	100%	64%	45%	27%

AFLP: amplified fragment length polymorphism. *Size of the coa genes: 1 (540 bp), 2 (600 bp), 3 (680 bp), 4 (850 bp); −: negative, ND: not detected, n: number of strains.

final volume of 20 μL. The DNA of the isolates was prepared with the QIAamp DNA mini kit (Qiagen, Germany) as described by the manufacturer's protocol. The 5 μL of digested DNA was added to a ligation reaction containing 0.2 μg of each adapter oligonucleotide (ADH-1 ACG GTA TGC GAC AG and ADH-2 AGC TCT GTC GCA TAC CGT GAG) (Invitrogen, USA) and 1 U of T4 DNA ligase (Invitrogen, USA) in a final volume of 20 μL, and incubated for 4 h at room temperature (approximately 20°C). The ligated DNA samples were heated for 10 min at 80°C to inactivate the T4 ligase and then diluted 1 : 5 in sterile water.

PCR were performed in a total volume of 50 μL containing 2.5 μL of template DNA, 200 μM of dNTPs (Roche, Germany), 1 μL of HI-X primer (GGT ATG CGA CAG AGC TTX, where X = A, T, G or C; 100 pmol/μL; Invitrogen, USA), and 1 μL (5 U) of *Taq* DNA polymerase (Roche, Germany) in 1 × PCR buffer provided by the manufacturer (Roche, Germany). Each HI-X primer was used in four separate PCR reactions. Amplification was performed in a thermal cycler (Eppendorf, Germany). After initial denaturation for 4 min at 94°C, target gene fragments were amplified for 33 cycles. Each cycle consisted of a denaturation step for 1 min at 94°C, an annealing step for 1 min at 60°C, and an extension for 2.5 min at 72°C. Each adapter oligonucleotide incorporated an additional base pair in the restriction site in order to eliminate it after the ligation reaction. The PCR products were separated by gel electrophoresis in a 1.5% (w/v) agarose gel (Roth, Germany) in 0.5 × TBE buffer (containing a mixture of Tris base, boric acid and EDTA). A 1-kb Plus DNA ladder (Invitrogen, USA) was used as a size marker. The resulting bands were visualized using ethidium bromide staining under UV transillumination. The gel images were subsequently evaluated visually. AFLP patterns were analyzed using BioNumerics software (ver. 6.01; Applied Maths, Belgium). Dendrograms were obtained with the average linkage method (unweight pair group average method, UGPMA) using Dice coefficient.

Results

According to the results of cultural and biochemical properties, along with amplification of the *nuc* and 23S rRNA specific to *S. aureus*, all 41 isolates examined in the present investigation were identified as *S. aureus*. All 41 cultures investigated were Gram positive, positive for catalase, coagulase, and clumping factor reaction on microscope slides. PCR amplification of the clumping factor gene (*clf*A) revealed that all isolates had a single amplicon approximately 1,000 bp in size (Table 3). Amplification of the *coa* gene revealed four different PCR products of 540, 600, 680, and 850 bp for 1, 8, 7, and 2 of the *S. aureus* isolated from bovines, and for 1, 4, 5, and 1 of the food isolates, respectively. Three different PCR products with sizes of 540, 600, and 680 bp were found for 1, 5, and 5 of the *S. aureus* strains isolated from human, respectively. The distribution of each isolate for *coa* gene of *S. aureus* is shown in Table 3.

By PCR amplification, one or more staphylococcal enterotoxin gene was observed either singly (*sec*, *see*, and *seh*), or in a combination of two genes [*se*(*a*,*h*), *se*(*e*,*h*), *se*(*g*,*i*), *se*(*c*,*g*), *se*(*b*,*c*), and *se*(*c*,*e*)], three genes [*se*(*b*,*c*,*i*), *se*(*c*,*e*,*i*), *se*(*c*,*g*,*i*)], or four genes [*se*(*a*,*b*,*h*,*i*), Fig. 1; *se*(*a*, *b*,*h*,*j*), and *se*(*b*,*g*,*h*,*i*)]. No strain harbored *sed*, *sej*, or *etb*. The *eta* gene was found in 10 out of the 19 *S. aureus* strains isolated from bovines, four of the 11 strains collected from humans, and three of the 11 strains from food. The *hla* gene was found almost in all *S. aureus* isolates including 16 isolates from bovines, eight isolates from humans, and all 11 isolates from food. The *hlb* gene was observed in three isolates from bovines, six isolates from humans, and two isolates from food. It was noted that most of the *S. aureus* isolated from bovines harbored the gene *cap*5 (14 isolates) while most strains isolated from humans contained the *cap*8 (10 isolates) and *fnb*A (11 isolates) genes. Both *cap*5 (11 isolates) and *cap*8 genes (7 isolates) were predominantly found in strains from food. Distribution of the various genes among the *S. aureus* cultures investigated in the present study is shown in Table 3.

To evaluate the relationships between the *S. aureus* strains isolated from humans, bovines, and food, AFLP was performed with primer G (with a G base at the 3′ end) using a single specific restriction enzyme. AFLP types were defined on the basis of 5 to 12 bands in the gel. This allowed us to differentiate 23 AFLP types (A to X). A dendrogram was generated with the AFLP data using

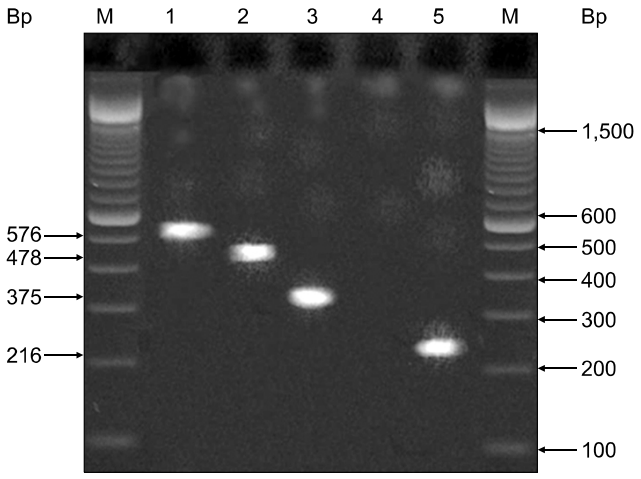

Fig. 1. Agarose gel electrophoresis of *se* gene PCR products including *sei* (Lane 1, 576 bp), *seh* (Lane 2, 478 bp), *seb* (Lane 3, 375 bp), a negative enterotoxine strain (Lane 4), and *sea* (Lane 5, 216 bp). M: marker = 100 bp DNA ladder.

BioNumerics software with the Dice coefficient and cluster analysis with UPGMA (Fig. 2). Using a cutoff value of 0.25, all *S. aureus* patterns could be grouped into nine clusters (I, II, III, IV, V, VI, VII, VIII, and IX). Results of this analysis presented in Fig. 2 revealed a correlation between some AFLP strain clusters and the origins of the strains. Pattern types A, B, C, D, and E formed one cluster (I), and six out of seven of the strains associated with this cluster were of bovine origin. Similarly, three out of four strains from the second cluster (II) formed by patterns F and G were of human origin while one strain was derived from food. A third cluster (III) was formed by pattern H and originated from food. By cutting point of 0.25, the other strains could be grouped into smaller clusters.

Discussion

According to phenotypic and genotypic properties, all 41 isolates investigated in the present study were identified as *S. aureus*. Molecular identification and characterization

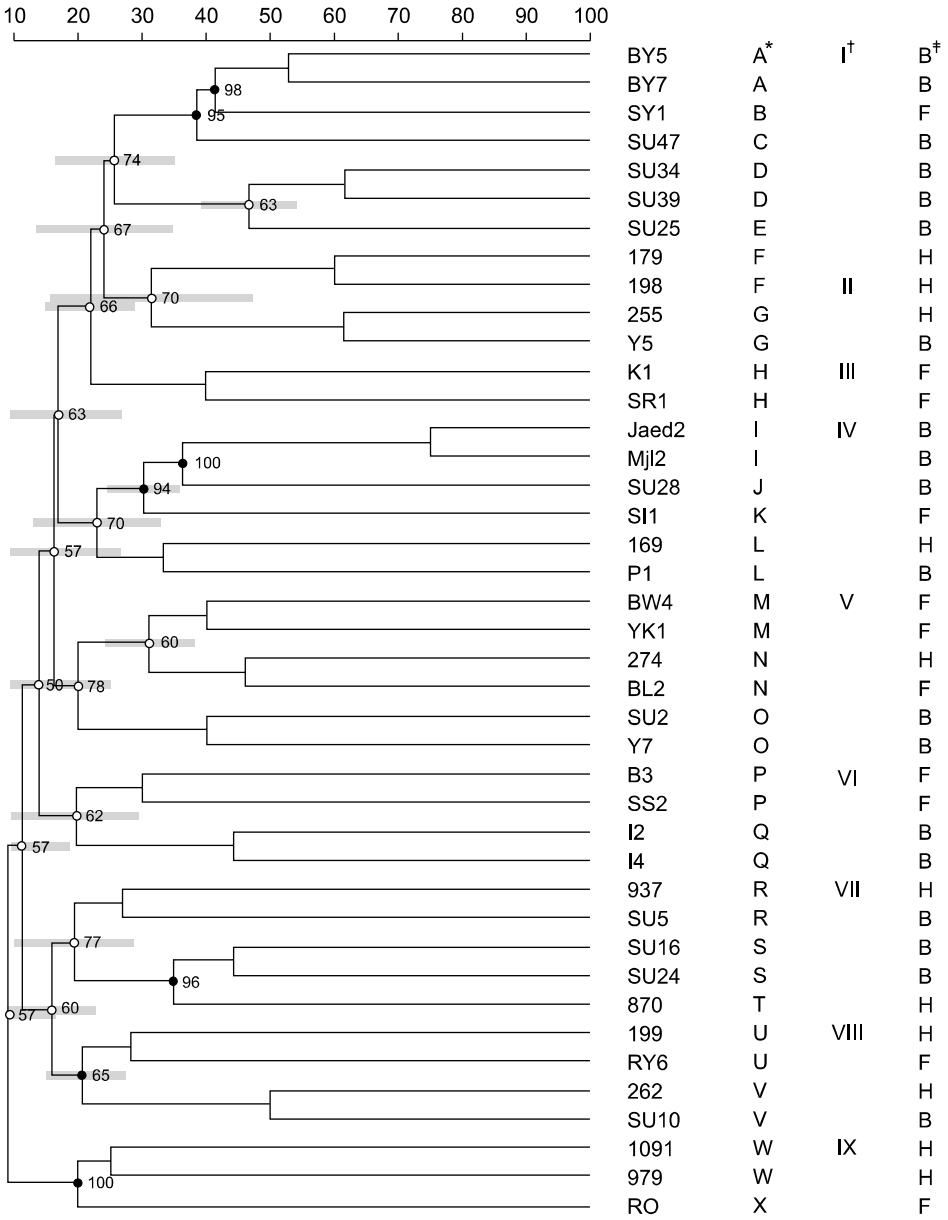

Fig. 2. Dendrogram of amplified fragment length polymorphism (AFLP) patterns showing the relatedness of the 41 *Staphylococcus aureus* strains examined in this study. Degrees of similarity were calculated using BioNumerics software with the Dice coefficient and cluster analysis with UPGMA. *AFLP patterns A to X (23 types). †Clusters of strains identified by a cutoff value of 0.25 (I to IX). ‡Strain designations: B; bovine source, F; food source, H; human source.

were performed by PCR amplification of genes encoding 23S rRNA, clumping factors, nuclease, and coagulase. Gene polymorphisms of the coagulase gene have been commonly used for molecular typing of *S. aureus* [2]. A comparable PCR-based system for identifying *S. aureus* isolated from various sources had already been used by numerous authors [1,2,23,25].

According to Nishi *et al*. [19], *S. aureus* genotyping by determining expression patterns of different toxins might be useful for comparing epidemiologically related strains. Evaluating the expression of different toxin genes in the *S. aureus* isolates revealed different combinations of *sea*, *seb*, *sec*, *see*, *seg*, *seh*, *sei* and *sej* gene expression in some of the *S. aureus* strains we investigated. Different combinations of these genes in clinical *S. aureus* isolates have also been described by Zhang *et al*. [27] and Jarraud *et al*. [12]. The *seg* and *sei* genes were present in most strains associated with staphylococcal toxic shock syndrome and staphylococcal scarlet fever [12]; this could be explained by a shared location of these genes on a pathogenicity island [3,15]. The *seh* gene was found in the strains isolated from humans in this study. Staphylococcal enterotoxin H has been described as having the highest affinity ever measured for an enterotoxin specific for MHC class II molecules [18]. The importance of toxin production by *S. aureus* isolated from animals with bovine mastitis associated with udder-associated pathogenesis remains unclear. According to Ferens *et al*. [8], superantigenic enterotoxins seem to induce immunosuppression in dairy animals. In contrast to *eta*, *etb* was not found in this study. Hayakawa *et al*. [10] reported that the production of exfoliative toxins by *S. aureus* isolates from cattle with bovine mastitis seems to be rare.

PCR analysis of genetic determinants has been suggested as an important tool for defining the pathogenicity of infectious *S. aureus*. Various staphylococcal surface determinants related to bacteria adherence to human epithelial cells have been reported [22]. Staphylococcal cell wall teichoic acid, lipoteichoic acid, type 5 and type 8 capsular polysaccharides, and fibronectin binding proteins had been proposed as major ligands [21,22]. Jönsson *et al*. [14] reported that two *S. aureus* fibronectin binding proteins, and the corresponding *fnb*A and *fnb*B genes, show a high degree of sequence similarity. In the present study, *fnb*A was detected in all human isolates. Booth *et al*. [5] observed that 89.7% of clinical isolates of *S. aureus* possess *fnb*A whereas only 20.1% harbor *fnb*B. It was interesting that most of the *S. aureus* isolated from bovines in our study harbored the *hla* (84%) and *cap*5 (74%) genes, and most of strains isolated from humans contained the *hla* (73%), *cap*8 (91%), and *fnb*A (100%) genes. The *hla* (100%) gene and both *cap*5 (100%) and *cap*8 (64%) genes were predominantly found in the *S. aureus* strains isolated from food.

To determine the clonal relationships between the isolates, a single-enzyme AFLP technique was used. Boerema *et al*. [4] reported that single-enzyme AFLP has a lower discriminatory power than pulsed field gel electrophoresis (PFGE), but is a useful typing method for investigating clonal relationships between different *S. aureus* strains. An AFLP procedure is easy to perform, and the results can be obtained within 24 h compared to the approximately 3～4 days required to acquire PFGE results. This makes AFLP more suited for routine use or analyzing a large number of isolates.

In the present study, single-enzyme AFLP analysis revealed a correlation between some AFLP clusters and the origins of the strains. In correlation with the AFLP clusters, some genes seem to be more closely associated with strains from food (*hla* and *cap*5) and humans (*cap*8 and *fnb*A) compared to ones from bovine. This findings indicated that cluster 1 was associated with bovine isolates (*hla* and *cap*5). Surprisingly, one isolate from food belonged to bovine cluster group 1. However, this food isolate (*hla* and *cap*5) was associated to the *S. aureus* of the bovine group. Additionally, cluster 2 was found to primarily contain human isolates (*cap*8 and *fnb*A) with one strain derived from bovine origin. The bovine isolate carried the *cap*8 and *fnb*A genes and was associated to the human isolates. The food isolate in cluster 1 was probably of bovine origin, and the bovine isolate in cluster 2 was probably of human origin. Additional studies are required to further investigate these unique findings.

The food isolates (*hla* and *cap*5) were grouped in cluster 3. The other clusters were formed by a variety of bovine, human, and food isolates. However, the geographical origin of the isolates resulted in a high level of diversity. Griffith *et al*. [9] reported that genetic variability in a population might be caused by genetic mutation, recombination, and movement of an individual or a group of population from one region to another. It is possible that these patterns were influenced by movement of dairy cattle from one area to another. Cross contamination between humans and food might occur via humans with skin lesions or nasal discharge [20], probably while handling food or during food processing.

At present, there is still very little information about the relationship between specific genetic determinants and *S. aureus* isolates from bovine, human and food sources in Indonesia. The results of the present study identified specific virulence traits of *S. aureus* pathogenicity and provided a better understanding about the distribution of prevalent *S. aureus* clones among bovines, food, and humans. This will help to determine the source and transmission routes of infective *S. aureus* strains.

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