CHARACTERIZATION OF METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) ISOLATED FROM HUMAN AND DIARY COWS

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ABSTRACT

Staphylococcus aureus is the causative agent of clinical or subclinical mastitis in dairy cows. *Staphylococcus aureus* cause also pathogenic diseases in human. Several hospitals reported the increase of methicillin resistant *S. aureus* (MRSA) frequency, because this srtain was resistant to several antibiotics. The aim of research was to characterize *S. aureus*, distribution of resistant factor to methicillin and several other antibiotics.

The isolates of *S.aureus* originated from dairy cows (11 isolates) and from patients of Sarjito hospital (10 isolates). Bacterial identification based on the growth in PAD media, Gram staining, mannitol salt agar (MS) fermentation, catalase, coagulase, clumping factor tests and the resistancy/sensitivity to methicillin, penicillin G, ampicillin, oxacillin, tetracyclin, gentamycin and erythromycin. It was also identified to 23S rRNA and *mec*A genes for the resistancy screening to methicillin (MRSA) by using polymerise chain reaction (PCR).

All isolates from milk and human used in this study were *S.aureus* based on phenotypic and genotypic identifications. *Staphylococcus aureus* were resistant to penicillin group (20-100%), and to tetracyclin, gentamycin and erythromicin (9-40%). By PCR amplification could be observed for 90% methicillin resistant (*mec*A) genes for human isoates and 45% for milk isolates. The resistancy of *S.aureus* to methicillin in the present study might help to understand the distribution of methicillin resistant *S. aureus* (MRSA) among human and bovine isolates and might help to control *S. aureus* infecions in Indonesia.

Key words : *Staphylococcus aureus*, methicillin resistant, *mec*A

INTRODUCTION

Staphylococcus aureus is the causative agent of clinical or subclinical mastitis in dairy cows. *Staphylococcus aureus* cause also pathogenic diseases in human. Several hospitals reported the increase of methicillin resistant *S. aureus* (MRSA) frequency, because this srtain was resistant to several antibiotics. The primary reservoir of *Staphylococcus aureus* is located in the infected quarter of glandula mammae, the transmission occurs among cattle during milking process

(Akineden *et al*., 2001). Intra-mammae infection can cause a high economical loss because of the decrease of milking production between 10 – 25% from total dairy milk production (Han *et al.*, 2000).. *Staphylococcus aureus* is the primary pathogen to human that it cause several diseases with clinical signs such as impetigo, scalded skin syndrome, pneumonia, osteomyelitis, pioarthrosis, endocarditis, metastasis, food poison, toxic shock syndrome (TSS), meningitis and sepsis (Marrack & Kappler, 1990; Dinges *et al*., 2000; Omoe *et al*., 2002). Toxic shock syndrome oxin-1 (TSST-1) produced by *Staphylococcus aureus* is a toxin, especially, it is related to menstruation case in woman. Several strain *Staphylococcus aureus* can also produce one or two exfoliatif ETA or ETB that it has connection with impetigo disease or scalded skin syndrome (Lee *et al*., 1987; Marrack & Kappler, 1990; Akineden *et al*., 2001). Staphylococcal infection is often known as nosokomial infection in the hospital that is caused from lesion of post surgery, infection during hemodialisis, bacteriaemia and pneumonia (Na'was *et al*., 1997).

The primary problem to solve *Staphylococcus aureus* infection is the antibiotic resistancy. Several strains of *Staphylococcus aureus* has been reported resistant to almost all commercial antibiotics (Todar, 2002). It is reported from several hospitals that it occured the frequency increase of methicillin resistant *Staphylococcus aureus* (MRSA) and it is usually resistant to multiple antibiotics (Na'was *et al.*, 1997; Todar, 2005).

MATERIALS AND METHODS

*Staphylococcus aureus*was isolated from several dairy cattle farm in DIY and Central Java (11 isolates). *Staphylococcus aureus* from human was isolated from patient of Sarjito hospital which was collected from Laboratorium Microbiology, Faculty of Medicine, GMV (10 isolates).

Samples from milk of cattle and skin of human was selected in blood agar plate, THB, Gram stain, annitol salt agar, Catalase, Koagulase tests. Clumping factor, Biochemistry test (Bruchler *et al*, 1994). Hemolysa production used agar plate of sheep blood (Skalka *et al*, 1979).

Antibiotic sensitivity test used. Müller – Hinton agar to Methicillin (Met), Tetracyclin(TE), Penicillin G(P), Ampicillin (AMP), Erythromycin(E), Gentamisin(GM), Oxacillin (OX). The inhibition zone was evaluated based on, Anonim (1998). The diameter zone of inhibition was compared with the standart of Kirby Baner (Jang *et al*, 1978).

Samples from cattle and human were rRNA extracted using Qiamp tissue kit (Qiagen, Germany), then were amplified in 23S region with species primers.

rRNA extraction product was then digested with CoU Hind III (invitrogen) and 5 µM spermidine trihydrochloride (Sigma – Aldrich, USA) for 16 hours, 5 µl of digested DNA was ligated with 0,2 µg adapter oligonucleotide (ADH-1 ACG GTA TGC GAC AG and ADH-2 AGC TCT GTC GCA TAC CGT GAG), IU T4 DNA ligase (invitrogen) until 20 µl of volume, was incubated at 20^0C for 4 hours. They were heated at 80^0C for 10 minutes for inactivation of T4 ligase then soluted in 1/5 sterile water. The amplification reaction used 50 µl of total volume containing 2,5 µl of template DNA, 200 µM of dNTPs, 1 µl of primer HI-X (GGT ATG CGA CAG AGC TTX (X=A, T, G or C) (100 pmol/µl), and 1 µl (5U) of Taq DNA polymerase in 1x PCR buffer.

Each HI-X primer (derivative) was used in 4 different PCR reaction. The product of PCR was examined with gel electrophoresis 1,5 % W/V agarose.

RESULT AND DISCUSSION

The result of of sensitivity test to several antibiotics showed that all antibiotics produced the inhibition zone to *S. aureus* with diameter variation (Table 1).

Table 1. Inhibition zone diameters of several antibiotics to *Staphylococcus aureus.*

No	Isolate code	Isolate origin	Inhibition zone diameter (mm)						
			MET 5	TE 30	P 10	AMP 10	GM 10	E 15	OX 5
Dairy milk origin									
1.	SU 2	Milk, yogya	6	18	6	13	13	6	6
2.	SU 10	Milk, yogya	23	25	23	22	20	22	17

3.	SU 16	Milk, yogya	17	28	30	25	20	25	10
4.	SU 24	Milk, yogya	12	20	8	7	20	20	6
5.	SU 25	Milk, yogya	13	18	8	10	20	20	6
6.	SU 28	Milk, yogya	20	20	8	7	22	28	6
7.	SU 34	Milk, Solo	17	22	23	21	20	25	14
8.	SU 39	Milk, Solo	12	17	7	7	18	20	6
9.	BY 5	Milk, Boyolali	17	25	7	6	17	20	18
10.	BY 7	Milk, Boyolali	20	20	7	8	15	20	13
11.	Y 7	Milk, Yogya	20	21	6	6	15	20	6
Human skin infection									
12.	169	Human skin, Yogya	22	20	7	7	17	25	7
13	179	Human skin, Yogya	20	25	7	7	17	20	15
14.	198	Human skin, Yogya	22	8	9	9	15	20	20
15.	199	Human skin, Yogya	6	6	6	15	12	6	6
16.	255	Human skin, Yogya	20	25	10	10	16	20	15
17.	262	Human skin, Yogya	25	20	9	9	20	6	6
18.	274	Human skin, Yogya	28	30	12	10	12	20	25
19.	870	Human skin, Yogya	20	16	16	30	17	15	24
20.	979	Human skin, Yogya	22	20	20	7	20	27	8
21.	1091	Human skin, Yogya	7	7	7	7	7	7	7

All inhibition zones were then compared with standar zones of Kirby-Bauer (Jang *et al*.,1978),the result could be seen in Table 2

Table 2. Sensitivity of isolates *S.aureus* from dairy cows and human skin to several antibiotics.

No	Isolate code	Isolate origin	Resistance (R), intermediate (I), Sensitivity (S)						
			MET	TE	P	AMP	GM	E	OX
Dairy milk origin									
1.	SU 2	Milk, Yogya	R	I	R	I	I	R	R
2.	SU 10	Milk, Yogya	S	S	I	S	S	S	S
3.	SU 16	Milk, Yogya	S	S	S	S	S	S	R
4.	SU 24	Milk, Yogya	I	S	R	R	S	S	R
5.	SU 25	Milk, Yogya	I	I	R	R	S	S	R
6.	SU 28	Milk, Yogya	S	S	R	R	S	S	R
7.	SU 34	Milk, Solo	S	S	I	S	S	S	S
8.	SU 39	Milk, Solo	I	I	R	R	S	S	R
9.	BY 5	Milk, Boyolali	S	S	R	R	S	S	S
10.	BY 7	Milk, Boyolali	S	S	R	R	S	S	S
11.	Y 7	Milk, Yogya	S	S	R	R	S	S	R
Human skin infection									
12.	169	Human skin, Yogya	S	S	R	R	S	S	R
13	179	Human skin, Yogya	S	S	R	R	S	S	S
14.	198	Human skin, Yogya	S	R	R	R	S	S	S
15.	199	Human skin, Yogya	R	R	R	S	R	R	R
16.	255	Human skin, Yogya	S	S	R	R	S	S	S
17.	262	Human skin, Yogya	S	S	R	R	S	R	R
18.	274	Human skin, Yogya	S	S	R	R	R	S	S
19.	870	Human skin, Yogya	S	I	R	S	S	I	S
20.	979	Human skin, Yogya	S	S	R	R	S	S	R
21.	1091	Human skin, Yogya	R	R	R	R	R	R	R

Sensitivity of *S.aureus* to several antibiotics showed that isolates from milk (36,4%) and isolates from human (20%) were resistant to methicillin. Isolates from milk (90,9%) and isolates from human (100%) were resistant to penicillin G (Table 3).

Table 3. Sensitivity percentage of *S. aureus* isolates from dairy cows and human skin to several antibiotics (%).

Antibiotics	Milk Origin Isolate		Human isolate	
	Resistance(R)	Sensitivity(S)	Resistance(R)	Sensitivity(S)
Methicillin	36,4	63,6	20	80
Penicillin G	90,9	9,1	100	0
Ampicillin	72,7	27,3	80	20
Oxacillin	63,6	36,4	50	50
Tetracyclin	27,3	72,7	40	60
Gentamycin	9,1	90,9	30	70
Erythromycin	9,1	90,9	40	60

Based on the presence of gene which had responsible to methicillin, isolates from human positive 9 (90%) had methicillin resistancy (mecA gene), and it was only 1 (10%) was negative. *Staphylococcus aureus* from dairy cows were 5 isolates (45,5%) which was positive contained *mec*A gene (Table 4). *MecA* gene of *S. aureus* from dairy cows and human in this research showed the same result (300 bp).

It was found the derivate of Penicillin (methicillin) that was effective to penicillin resistant of microorganism (Black, 1999). However, it has been reported from many hospitals that the frequency of methicillin resistant *S.aureus* (MRSA) was increase to multiple antibiotics (Na'was *et al*., 1997; Todar, 2002).

The presence of MRSA in dairy cows in Indonesia has not been reported. This research showed that 45,5% of S. aureus isolates from dairy cows had MRSA. The infection of MRSA might be caused by the resistancy of *S. aureus* to penicillin.

Table 4. Amplification result of isolates from dairy cows and human skin in 23S of rRNA gene region and *mec*A gene.

No.	Isolate code	Isolate origin	23S rRNA gene (1250 bp)	*mec*A Gene (+/- 300 bp)	AFLP
1.	SU 2	Milk, Yogya	+	+	I
2.	SU 10	Milk, Yogya	+	+	III
3.	SU 16	Milk, Yogya	+	+	I
4.	SU 24	Milk, Yogya	+	+	I
5.	SU 25	Milk, Yogya	+	+	III
6.	SU 28	Milk, Yogya	+	-	I
7.	SU 34	Milk, Solo	+	-	V
8.	SU 39	Milk, Solo	+	-	V
9.	BY 5	Milk, Boyolali	+	-	I
10.	BY 7	Milk, Boyolali	+	-	I
11.	Y 7	Milk, Yogya	+	-	II
12.	169	Human skin, Yogya	+	+	III
13.	179	Human skin, Yogya	+	+	I
14.	198	Human skin, Yogya	+	+	III
15.	199	Human skin, Yogya	+	+	I
16.	255	Human skin, Yogya	+	+	VI
17.	262	Human skin, Yogya	+	+	V
18.	274	Human skin, Yogya	+	-	VII
19.	870	Human skin, Yogya	+	+	IV
20.	979	Human skin, Yogya	+	+	I
21.	1091	Human skin, Yogya	+	+	VI

CONCLUSION

Isolates from dairy cows (11 isolates) and from human skin (10 isolates) were positive *Staphylococcus aureus* which was resistant to penicillin (20-100%) and to tetracycline, gentamycine, erythromycine (9-40%). Isolates of S.aureus from human (90%) and isolates from dairy cow (45,5%) contained *mec*A gene. Isolates of *Staphylococcus aureus* from human (60%) was similar to isolates from dairy cows (30%)

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