Identification of methicillin resistant staphylococcus aureus (mrsa) using cefoxitin disc diffusion test and dupleks polymerase chain reaction in Jambi city hospitals

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Identification of methicillin resistant staphylococcus aureus (mrsa) using cefoxitin disc diffusion test and dupleks polymerase chain reaction in Jambi city hospitals

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Abstract. Methicillin Resistant Staphylococcus aureus (MRSA) remains the important health problems because the frequency of isolate MRSA tends to increase in the world. Cefoxitin disc diffusion test and detection of MecA gene using PCR were used in MRSA identification. FemB gen is used for spesies SA confirmation. FemB encodes enzyme in cross-linking peptidoglycan in SA. This research aimed to identified MRSA from clinical samples of hospitalized patients in Jambi city using cefoxitin disc diffusion test and dupleks PCR. This research has been conducted in Biomedic Laboratory of Medical Faculty of Jambi University during periode June – November 2017. Swab of 76 festering wounds were grown on Mannitol Salt Agar medium which incubate at 30°C 18–24 h. The Yellowish colony confirmed with gram staining and tested using Staphytect plus Test DR 850 M. Identified of MRSA using cefoxitin disc diffusion test and dupleks PCR. This research reported cefoxitin-RSA was detected in 41,67% of SA, MecA gene was detected in 45,83% of SA. MecA gene was detected in 100% of cefoxitin-RSA and 7,14% of cefoxitin-SSA. FemB gene was detected in 8,33% of cefoxitin-RSA, and 64,28% of cefoxitin-SSA. Detection of MecA and FemB genes by PCR for identification of MRSA is sensitif and fast.

1. Introduction

In the recent years, incidence of infection disease was incereased, include infection diseases caused by *Staphylococcus aureus* (SA). SA is a human pathogen bacterial that causing nosocomial infection. It also cause morbidity and mortality [1-4]. Some SA was resistant to methycillin antibiotics and other betalactam antibiotics such as penicillin, sefalosporin, monobactam and carbapenem, which called *Methicillin Resistant Staphylococcus aureus* (MRSA). The resistancy of MRSA not only to the betalactam antibiotics but also to the non-betalactam antibiotics such as macrolide (erythromicin), protein synthetize inhibitor (tetrasiklin, chloramfenicol) and quinolon [5-7].

The resistency of MRSA caused by irrational antibiotic treatment. Many mechanisme of resistancy in the hospitals are: (1) resistant organisme exposure, (2) spontan mutation or genetic transfer of sensitive strain, (3) resistence expression of bacterial in population, (4) spreading of resistant organisme by cross transmission mechanisme [8].

Many methods identification of MRSA has been developed, such as bacterial sensitivity test to antibiotics (dilution test, lateks aglutination test and disc diffusion test) and molecular diagnosis (PCR). Todays, cefoxitin disc diffusion test often used rather than oxacillin, because there was no false positive [9]. Detection of MecA gene using PCR become an indicator of MRSA. Further epidemiological studies revealed that mecA genes are associated with methicillin resistance [10,11]. FemB gen is used for spesies SA confirmation [12]. FemB encodes enzyme in cross-linking peptidoglycan in SA.

There was no research about MRSA as a infection in Jambi city. While some research reported about incidence of MRSA in many hospitals in Indonesia. Therefore, it was being important to identified MRSA from clinical specimens of hospitalized patient in the Jamby city hospitals. Aim of this research is to identified MRSA incidence from clinical specimens in Jambi city hospitals using cefoxitin disc diffusion test and dupleks PCR.

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2. Methods

This research was a descriptive experimental research, it has been conducted in Biomedic and Biomolecular Laboratory of Medical Faculty Jamby University on June – November 2017. A total of of 76 clinical specimens were employed in this study. These clinical specimens were taken from festering wound by swab technique. The swab was grown on *Mannitol Salt Agar* (MSA) 30 ºC 18 – 24 h, the yellowish colony confirmed with Gram staining methode. Coccus positive Gram bacteria was tested using Staphytect plus Test DR 850 M (Oxoid) to detected clumping factor, protein A and polysacharide capsule type 5 and 8. Positive aglutinated samples was tested using cefoxitin disc diffusion test on Mueller Hinton Agar Medium and dupleks PCR. Dupleks PCR was done by following steps:

2.1 Preparation of bacterial DNA samples and mixed PCR

A 4 µl of bacterial suspension (0,5 Mc Farland) from fresh colony culture 30 ºC 18 – 24 h which grown in Mannitol Salt Agar Medium were mixed with 21 µl PCR mix contained Go Taq green master mix (Promega) 15 µl, 2 µl primer MecA and 3 µl primer FemB. Based on the nucleotide sequences of mecA and femB genes, the oligonucleotides listed in Table 1 were synthesized and employed as PCR primers. This research used *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 as positive and negative control.

2.2 Polymerase chain reaction

DNA amplification was performed in 25 µl of reaction mixture as described above. The reactions were allowed to proceed with 30 cycles of denaturation (94 °C, 45 sec), annealing (50 °C, 45 sec), and primer extension (72 °C, 1 min) [13]. The DNA amplification product was analysed by electrophoresis on 0,8% agarose gel with Cybr safe DNA staining and visualized with ultraviolet light in gel documentation.

Table 1. Sequence of oligonucleotide primers [14].

Target Gene	Primer name	Nucleotide sequence (5'-3')	Product lenghth (base pairs)
Mec A gene	Mec A1	GTA GAA ATG ACT GAA CGT CCG ATA A	310
	Mec A2	CCA ATT CCA CAT TGT TTC GGT CTA A	
Fem B gene	Fem B1	TTA CAG AGT TAA CTG TTA CC	651
	Fem B2	ATA CAA ATC CAG CAC GCT CT	

3. Results

A total of 76 clinical samples employed in this study were collected from RSU Raden Mattaher, RS dr. Bratanata, RS Kambang, RS St. Theresia dan RS Mayang Medical Center, which were located in Jambi city. Subject characterize based on sex and age was described on table 2.

Table 2. Subject Characteristic.

Characteristic	**Frequency (n)**	**Percentage (%)**
Sex		
Male	46	60,53
Female	30	39,47
Age		
Child (<18 years old)	10	13,16
Adult (18-65 years old)	61	80,26
Elderly (>65 tahun)	5	6,58

Based on sex, the most subjects were male (60,53%), then female (39,47%). Based on age, the most subjects were adult (80,26%), child (13,16%), and elderly (6,58%).

From 76 swab specimens were grown on MSA medium incubated 30 ºC until 48 h and twenty six yellowish colonies were confirmed by Gram staining methode. A total of 26 Coccus gram positive bacteria were tested using Staphytect plus Test DR 850 M (Oxoid) to detected clumping factor, protein A and polysacharide capsule type 5 and 8. Result of Staphytect test seen on table 3.

Table 3. Staphytect Test

Staphytect Test	Frequency (n)	Percentage (%)
Agglutinated	24	92,31
Not agglutinated	2	7,69
Total	26	100

A total of 24 positive aglutinated samples were tested using cefoxitin disc diffusion test to identify Cefoxitin-RSA and Cefoxitin-SSA. Result of Cefoxitin-RSA (≤21) and Cefoxitin-SSA (≥22) seen on Table 4.

Table 4. Identification of Cefoxitin-RSA and Cefoxitin-SSA

Bacteria	Frequency (n)	Percentage (%)
Cefoxitin-RSA	10	41,67
Cefoxitin-SSA	14	58,33

A total of 24 positive aglutinated samples also tested using dupleks PCR to identify MecA and FemB gene as MRSA indicator. Result of dupleks PCR dupleks seen on Figure 1, Figure 2 and Table 5. Figure 1 and 2 show electrophoretic patterns of the DNA products after PCR. The DNA fragments of 310 and 651 bp were amplified from MecA and FemB genes, respectively. The identification of MecA and FemB Gene by PCR Dupleks is 12.50% for MecA (+)/FemB (+), 33.33% respectively for MecA (+)/FemB (-) and MecA (-)/FemB (+) and 20.83% for MecA (-)/FemB (-).

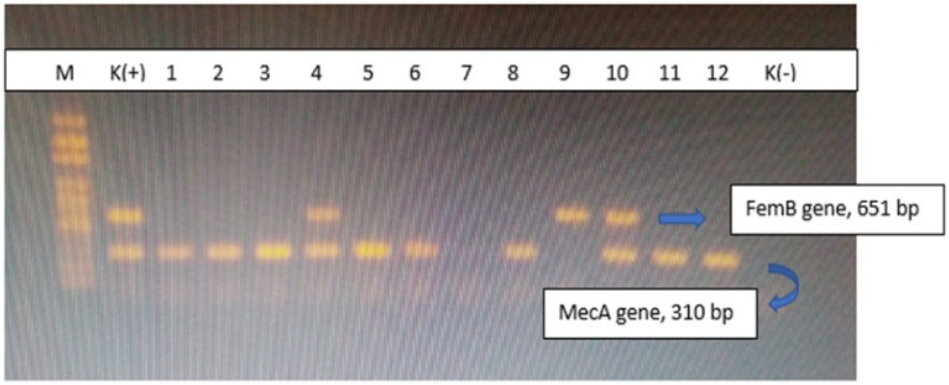

Figure 1. Agarose gel electrophoresis of PCR product amplified from of MecA and FemB genes (310 and 651 bp). M DNA marker; K (+) positive control (*S. aureus* ATCC 43300); K(-) negative control (*S. aureus* ATCC 25923); Lane 1-6, 8, 10-12 are MecA fragment; Lane 4,9,10 are FemB fragment.

Mec A gene was detected in 100% of cefoxitin-RSA and in 7,14% of cefoxitin-SSA. Detection rates of FemB gene in *S. aureus* was 8,33% of cefoxitin-RSA and 37,5% of cefoxitin-SSA (table 6).

In two samples (8,33%) of cefoxitin-RSA and one samples (7,14%) of cefoxitin-SSA, both of the genes were detected. Eight samples of cefoxitin-RSA were FemB negative. In eight samples of cefoxitin-SSA, FemB gene were detected, but five samples others were not.

4. Discussions

In this study, from 76 clinical specimen, the most have festering wound is adult (80,26%) and male (60,53%). A total of 26 spesimen have yellowish colony on MSA medium, MSA medium is a selective and differential medium for SA identification. SA will give a yellowish colony dan yellow zone in MSA medium because SA can fermetate mannitol and acid pH made yellow colour from phenol red indicator.

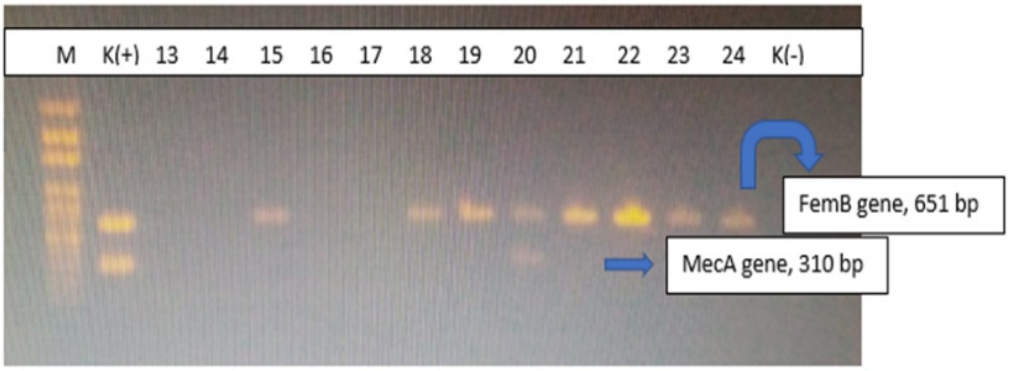

Figure 2. Agarose gel electrophoresis of PCR product amplified from of MecA and FemB genes (310 and 651 bp). M DNA marker fragments; K(+) positive control (*S. aureus* ATCC 43300); K(-) negative control (*S. aureus* ATCC 25923); Lane 20 is MecA fragment; Lane 15,18-24 are FemB fragment.

Table 5. Identification of MecA and FemB Gene by PCR Dupleks

Indicator	Frequency (n)	Percentage (%)
MecA +, FemB +	3	12.50
MecA +, FemB -	8	33.33
MecA -, FemB +	8	33.33
MecA -, FemB -	5	20.83

Table 6. Detection Pattern of MecA and FemB Gene in *S. aureus*

S. aureus	PCR		Jumlah isolat
	MecA	FemB	
Cefoxitin-RSA	+	+	2
	+	-	1
Cefoxitin-SSA	+	+	1
	-	-	5
	-	+	8

Staphytect plus was done in 26 specimen and 92,31% spesimen were agglutinated. Staphytect plus is A latex slide agglutination test for the differentiation of Staphylococcus aureus by detection of clumping factor, Protein A and certain polysaccharides found in Methicillin Resistant Staphylococcus aureus (MRSA) from those staphylococci that do not possess these properties. It means that 7,69% specimen was not MRSA.

A total of 24 positive aglutinated samples were tested using cefoxitin disc diffusion test and the result is Cefoxitin-RSA(≤21) and Cefoxitin-SSA(≥22), based on Clinical and Laboratory Standards Institute 2017. Cefoxitin-RSA was detected in 41,67% of *S. aureus*, and 58,33% of cefoxitin-SSA.

5. Conclusion

The identification of MecA and FemB Gene by PCR Dupleks with DNA fragments of 310 bp for MecA gene and 651 bp for FemB gene. This research found that 3 specimen yielded both MecA and FemB product which indicating the presence of MRSA; 8 spesimen yielded a femB product but no mecA product, as expected for MSSA; 8 specimen yielded a mecA product but no femB product, indicating R-CNS; and 5 specimen with PCR negative. However, it could be noted that MecA genes were detected in all of MRSA, while FemB genes were detected in almost *S. aureus*, with or without MecA genes. Thus, our data confirmed the usefulness of the simultaneous detection of MecA and FemB genes by PCR for identification of MRSA. Further analysis of the distribution of these genes in *S. aureus* still needed. Evaluation and monitoring of clinical use of antibiotic is needed to control and prevent the increasing of MRSA isolate.

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