DETECTION OF MecC GENE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES AT JAMBI CITY HOSPITALS

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ABSTRACT

Background: Methicillin Resistant Staphylococcus aureus (MRSA) has a low-affinity to penicillin-binding protein (PBP) in B1subclass, and is often known as PBP2a or PBP2, characterized by the presence of mecA gene. Further research found MRSA isolates that did not contain the mecA gene. They have mecA homolog gene (mecALGA251) which has similarity with the classic mecA gene, then named the mecC gene. The discovery of MRSA mecC in humans still low in prevalence.

Objective: This study aimed to detect mecC gene in MRSA isolates at hospitals in Jambi.

Method: This study was a descriptive study conducted in the Biomedical and Biomolecular Laboratory of Faculty of Medicine and Health Sciences, Jambi University.

Result: A total of 117 clinical specimens in the forms of purulent wound swabs of the inpatients at hospitals in Jambi City has been cultured on the MSA agar. Then yellowish samples (43 isolates) were screened for susceptibity of cefoxitin (30 µg) by disc / diffusion test and the presence of mecA, femB and mecC genes by conventional PCR. There were 22 (51.16%) samples contained mecA, 2 (4.65%) samples had also femB gen, 1 (2.32%) samples contained femB gene only and none contained the mecC gene.

Conclusion: There was no mecC gene had been found in MRSA isolates in Jambi.

Keywords: *PCR, MRSA, MecC, MecA, FemB*

INTRODUCTION

Methicillin-resistant*Staphylococcus aureus* (MRSA) is a bacterium that causes major health problems in health facilities or society. This bacterium causes diseases that are dangerous and difficult to treat, so prevention is an essential measure. MRSA is a strain of *Staphylococcus aureus* bacteria with phenotypic resistance characteristics to beta-lactam antibiotics, or called low-affinity penicillin-binding protein (PBP) in the B1 subclass and often known as PBP2a or PBP2.[1,2] PBP2a is characterized by the mecA gene that is located in conjunction with its regulators, mecR1 and mecI, located inside a mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec).[1,3]

Studies also proved that PBP2a, the mecC gene, was stated to encode PBP2c, which was different from PBP2a in terms of its bond characteristics with beta-lactam and its activity that depends on temperature; this PBP2c activity would decrease at 37°C. PBP2c has four times stronger bonds to oxacillin than PBP2a, so

MRSA strains containing the MecC gene have low MIC beta-lactam characteristics and high expression of oxacillin resistance.[4] This high resistance property makes it more difficult for sufferers to get proper treatment.

The mecC gene in MRSA isolates was firstly found in cow's milk with mastitis in the United Kingdom (UK), and the MRSA isolate showed a negative result of the mecA gene. The results of the sequencing analysis of the isolate genome showed that the isolate carried the mecA homolog gene (mecALGA251) with an approximately 69% similarity to the classic mecA gene; this gene also encodes a protein with an approximately 63% similarity to PBP2a; this gene was later named mecC gene in 2012.[5] MRSA mecC has been found in various host species, including livestock and wild animals in several European countries. As in humans, this animal isolate is also associated with clone complex 130 (CC130) and ST425. This strain can attack a broader range of hosts in the tropics.[6–8]

Several studies have shown that MRSA mecC is associated with infection in cow's milk. It indicates an infection transmission from animals to humans, especially farmers with direct contact with their livestock.[9] [6,10] This is supported by studies using whole-genome sequencing, which have reported zoonotic transmission of MRSA mecC from cattle to humans.[9] In addition, MRSA that contains mecC gene is also found in sheep, which is also the reservoir hosts.[11] MRSA mecC is still rarely found in humans, but there have also been significantly increasing cases in Denmark.[11] Therefore, more extensive research is needed on its prevalence in humans. Until recently, no research has been done on MRSA mecC either in Jambi Province or in other regions in Indonesia, so the existing research is only limited to the findings of MRSA mecA. This study aimed to detect the presence of MRSA mecC in a clinical sample of patients treated at hospitals in Jambi Province.

METHOD

This study was a descriptive study conducted in the Biomedical and Biomolecular Laboratory of Faculty of Medicine and Health Sciences, Jambi University from March to September 2020. The sample in this study was clinical sample in the form of wound swabs, blood, urine, drainage fluid. The number of respondents for sampling was 117. After the swabs were carried out, the bacteria on the cotton swabs were planted on nutrient agar and incubated for 24 hours. After growing in nutrient agar media, the colonies were planted on Mannitol Salt Agar (MSA). Then 43 yellowish colonies screened for susceptibity of cefoxitin (30 µg) by disc diffusion test. The results would show the diameter of the inhibition zone which was adjusted to the Clinical and Laboratory Standards International (CLSI).[12] The data obtained were presented in the form of a descriptive table.

All isolates were examined by PCR to detect the presence of mecA , femB and mecC gene. DNA templates were taken from the colony that was diluted with sterile NaCl until reaching 0.5 McFarland turbidity. MecA gene detection was performed using

forward primers (5'- GTA GAA ATG ACT GAA CGT CCG ATA A-3') and reverse primers (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'). While femB gene detection was performed using forward primers (5'-TTA CAG AGT TAA CTG TTA CC-3') and reverse primers (5'-ATA CAA ATC CAG CAC GCT CT-3').[13] PCR reactions consisted of 10 µl of mastermix (Go Taq green, Promega), 0.5 µl of each mecA primer, 1.5 µl of each femB primer and 5 µl of DNA template with a total volume of 25 µl. Amplification was performed in a thermocycler machine starting with an initial denaturation at 94°C for 5 minutes followed by 30 cycles with denaturation temperature at 94°C for 45 seconds, annealing temperature at 50°C for 45 seconds, extension temperature at 72°C for 90 seconds, followed by final extension at 72°C for 10 minutes and ended at 4°C.[14]

Another PCR was done to detect mecC gene in Staphylococcus aureus isolates that did not contain the mecA gene. MecC gene detection was carried out using forward primers (5'- GCT CCT AAT GCT AAT GCA -3') and reverse primers (5'- TAA GCA ATA ATG ACT ACC-3).[15] MecC gene amplification was performed with annealing temperature at 50°C for 40 seconds and an extension temperature at 72°C for 40 second.[16] Visualization of the PCR band results was carried out by electrophoresis on 0.8% agarose gel, 80 volts, 400 milliampere for 20 minutes, stained with Cybr safe DNA staining 7 mg/mL and visualized with ultraviolet light on Gel Documentation.

RESULTS

The sample in this study was 117 sterile cotton swabs from three referral hospitals in Jambi City, that are Raden Mattaher Hospital, dr. Bratanata Hospital, and Kambang Hospital. Most research subjects were male (64.1%) and in the adult age group (88%) **(Table 1)**.

Swab samples were cultured on nutrient agar and incubated for 24 hours. After the growing in nutrient agar media, the colonies were recultured on Mannitol Salt Agar (MSA) media. A total of 43 (36.75%) yellowish samples grew on MSA media. Gram staining performed on all yellowish samples on MSA media yielded Gram-positive results. Those 43 samples were screened for sensitivity susceptibity of cefoxitin and the presence of mecA, femB and mecC. MRSA and MSSA identification results can be seen in **Table 2.**

There were 22 (51.16%) samples contained mecA, all of them were cefoxitin resistant. Among of cefoxitin resistant samples, 2 (4.65%) samples had also femB gen. One (2.32%) samples contained femB gene only and 20 (46.51%) none contained any gene of, mecA, femB or mecC. All of them were cefoxitin sensitive (**Figure 1).**

Table 1. Characteristics of Research Subjects

Characteristics	Frequency (n)	Percentage (%)
Gender		
Male	75	64.10
Female	42	35.90
Age Group		
Children (<18 years)	16	13.68
Adults (18-65 years)	95	81.20
Elderly (>65 years)	6	5.13

Table 2. Identification of MRSA and MSSA with Cefoxitin Disc Diffusion Test and PCR

PCR Result	Disc Diffusion Test	
	Cefoxitin sensitive	Cefoxitin resistant
MecA (-), FemB (-)	20	0
MecA (+), FemB (-)	0	20
MecA (-), FemB (+)	1	0
MecA (+), FemB (+)	0	2

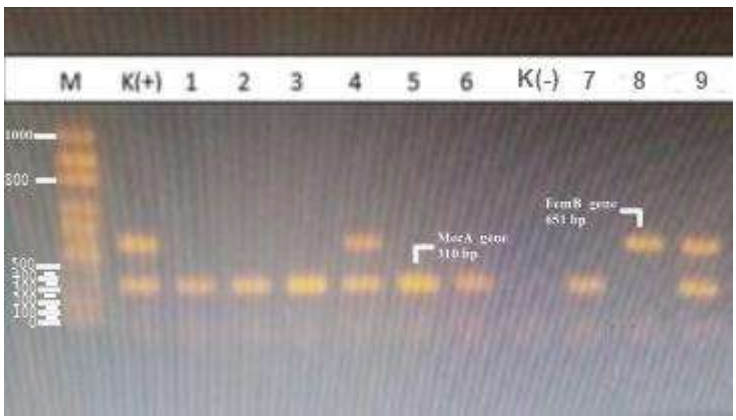

Figure 1. Agarose Gel Electrophoresis of PCR Product **Amplified from Meca and Femb Genes (310 And 651 Bp). M DNA marker; K(+) positive control** (*S. aureus* ATCC 43300); K(-) negative control (*S. aureus* ATCC 25923); **Lane 1-3, 5-7 shows MRCNS; Lane 4,9 shows MRSA; Lane 8 shows MSSA.**

DISCUSSION

From the results of the study in Table 2, it can be identified that MRSA bacteria based on their sensitivity to cefoxitin as many as 22 of 43 samples of *Staphylococcus aureus* (51.16%) isolates contained mecA gene, but no mecC gene was found in these isolates. This situation shows that the incidence of infection caused by MRSA bacteria is quite high.

However, several other studies in various countries gave different results.

Research in Denmark, found 12 isolates containing the MecC gene from 203 isolates of Staphylococcus aureus originating from humans[7] Another study in Slovenia, which involved 395 samples of S. aureus isolates in humans, 385 samples contained mecA gene and 6 samples contained mecC gene.[17] This shows the importance of a large sample size because mecC prevalence is still very low.

CONCLUSIONS

As many as 51.16% of the Staphylococcus aureus isolates from clinical samples contain mecA gene, whereas mecC gene has not been found in S. aureus isolates in Jambi.

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