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RESEARCH ARTICLE
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Analysis of Mec-A Gene on Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

The prevalence of MRSA in various hospitals in the world ranges from 2-70% with an average of 20% .In recent decades there has been an increasing prevalence of S. aureus and MRSA in the world. Population-based studies in North America and Europe indicate the prevalence of S. aureus is between 18-30%. The overall prevalence of MRSA in Asia has reached 70%, while publications and prevalence of MRSA in Indonesia are still very limited and very difficult to obtain. The prevalence of MRSA in Atmajaya Hospital, Jakarta, reached 47%. Yuwono also reported in 2010 MRSA at Dr. RSUP. Moh. Hoesin Palembang reached 46%. Amplification were carried out under PCR conditions as a result of optimization, namely the results of PCR optimization were carried out for 30 cycles with the following thermal conditions. Denaturation phase at 94ºC for 30 seconds, annealing at 52.0ºC for 30 seconds, and extension at 72ºC for 1 minute. Amplification results showed that of the 30 samples only 90% (27 samples) were detected to have the Mec-A gene, 10% (3 samples) were negative.

Keywords: Mec-A; gene; Methicillin Resistant *Staphylococcus aureus*

INTRODUCTION

The prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in various hospitals in the world ranges from 2-70% with an average of 20% [(1)]. In recent decades there has been an increasing prevalence of S. aureus and MRSA in the world. Population-based studies in North America and Europe indicate the prevalence of S. aureus is between 18-30% [(1)]. According to Wahid (2007), the overall prevalence of MRSA in Asia has reached 70%, while publications and prevalence of MRSA in Indonesia are still very limited and very difficult to obtain. Noviana reported that in 2003 the prevalence of MRSA in Atmajaya Hospital, Jakarta, reached 47%. Yuwono also reported in 2010 MRSA at Dr. RSUP. Moh. Hoesin Palembang reached 46%.

The cause of MRSA is the presence of a mecA resistance gene that is owned by these bacteria. This gene is located in the SCCmec chromosome of S. aureus and can be detected by polymerase chain reaction (PCR) method. This gene encodes a specific transpeptidase that causes bacteria to be resistant to methicillin and also produces Penicillin Binding Protein 2a (PBP-2a) [(1,2)]. The study of Pournajaf et al., (2014) in Tehran, Iran compared the Oxacillin Disk sensitivity test with the identification of the mecA gene using PCR. The results obtained were 133 (47.6%) of 292 isolates of S. aureus were resistant to oxacillin and as many as 126 (45.1%) isolates had the mecA gene with an amplicon of 533 bp. This shows that the oxacillin testand the identification of the mecA gene have similar results. Oxacillin is used because it is chemically in the same group as methicillin, it is more stable, the test results between methicillin and oxacillin are the same and at this time methicillin is no longer produced commercially[(3)].This study aims to detect the presence of the MecA gene in MRSA bacteria, so that the distribution of the MecA gene in MRSA bacteria can be known. [(8)]

METHODS

Isolation and Identification of *S. aureus*

MRSA isolates from the Microbiology Laboratory of RSUD DR Soetomo were re-cultured. The isolates were inoculated on BAP (Blood Agar Plate) medium with an incubation temperature of 37oC for 24 hours. In colonies that hemolyzed completely suspected of S. aureus, Gram staining was performed to ensure that the

bacteria were cocus-shaped and gram-positive. The colonies were then planted in mannitol salt agar (MSA) Oxoid TM and Nutrient Agar Slanted with an incubation temperature of 37oC for 24 hours.

Isolation and Extraction of *S. aureus* Bacterial DNA

Polymorphism analysis begins with the isolation and extraction of DNA from S. aureus bacteria using the spin column method

DNA Quantification

DNA quantification was carried out to determine the level of purity and concentration of DNA obtained from the isolation stage. Quantification was carried out using a Thermo Sciencetific TM 2000 nanodrop spectrophotometer. The samples were measured Optical density (OD) or absorbance at wavelengths of 260 nm and 280 nm.

Amplification of Mec-A Gene, *S. aureus* Methicillin Resistance Gene using PCR Technique

The mecA gene was amplified using TGGCTATCGTGTCACAATCG forward primer, reverse CTGGAACTTGTTGAGCAGAG with 304 bp product. The primers were obtained from research from Saha et al. (2008) and based on NCBI GeneBank data: KC243783.1, the S. aureus mecA gene region can be seen in appendix 8. The positive control for the coA gene used was the S. aureus strain TN/CN/1/12 mecA[9,10]

RESULTS

The character of S. aureus bacteria refers to Bergey's Manual of Systematic Bacteriology Volume 3. Observations of the characterization of S. aureus bacteria above were carried out using conventional methods[8]. Based on the results of this bacteriological examination, it was known that from 30 samples of bacterial isolates were S. aureus bacteria.

Figure 1. Results of culture examination and sensitivity test for MRSA bacteria

Isolation of DNA from samples of bacterial isolates was carried out using the Spin Column method. The results of DNA isolation were quantified using a Spectrophotometer-NanoDrop Maestro. The results of quantification of DNA isolation samples obtained DNA concentration of 67.7 ng/µl and an average DNA purity of 1.81. The detailed results are presented in table 2. A good quality sample will have a ratio of absorbance of wavelengths of 260 nm and 280 nm is 1.8-2.0. Samples with a ratio of 260nm/280nm less than 1.8 indicate protein contamination, while samples with a ratio of 260nm/280nm more than 2 indicate RNA contamination [2,3]. Based on the measurement of the concentration and purity of DNA from the results of DNA extraction, the research sample still meets the requirements to continue the process to the next stage, namely the DNA amplification stage using the RT-PCR technique (table 2).

The next step after DNA quantification is to amplify the mecA gene. The mecA gene is the gene responsible for resistance to beta-lactam antibiotics such as methicillin. This stage was carried out with the aim of genetically screening MRSA for the presence of the mecA gene in research samples using the RT-PCR method. This amplification was carried out under PCR conditions as a result of optimization, namely the results of PCR optimization were carried out for 30 cycles with the following thermal conditions. Denaturation phase at94oC for 30 seconds, annealing at 52.0oC for 30 seconds, and extension at 72ºC for 1 minute. Amplification results showed

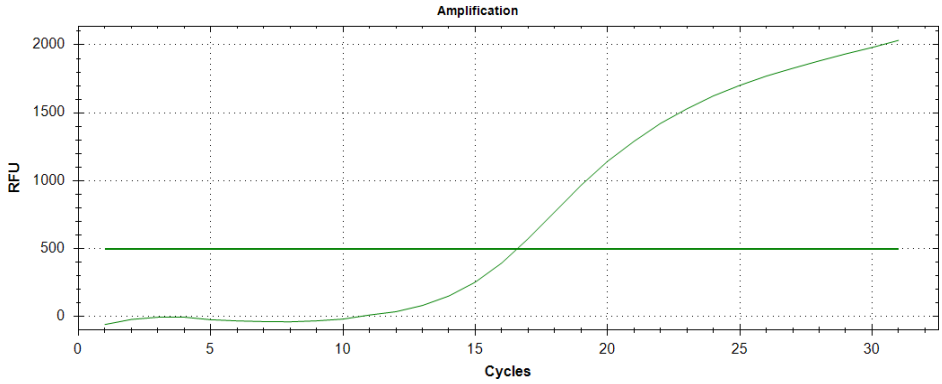
that of the 30 samples only 90% (27 samples) were detected to have the Mec A gene, 10% (3 samples) were negative. The detailed amplification results can be seen in table 3 and the graph.

Table 2. Results of DNA quantification of samples

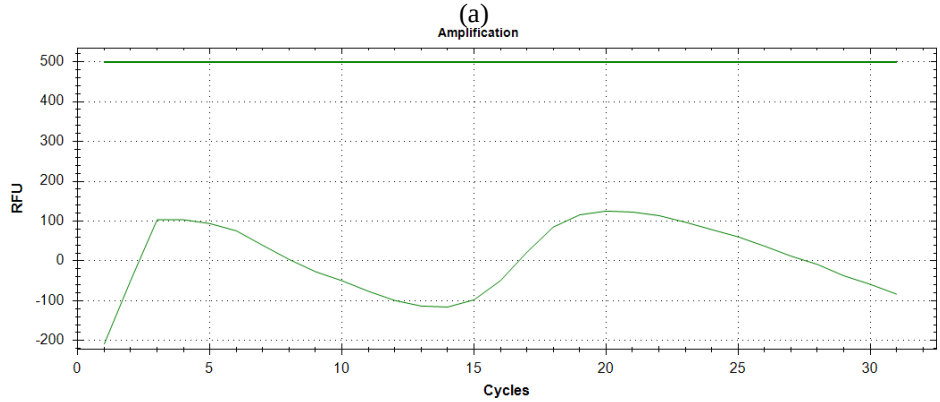
No	Sample	A260/ A280	Conc (ng/UL)
1.	Positive control	1.954	13.25
2.	43301	1.840	18.91
3.	43302	1.877	27.8
4.	43303	1.842	57.17
5.	43304	1.543	349.90
6.	43305	1.881	18.8
7.	43306	1.882	19.6
8.	43307	1.900	18.09
9.	43308	1.908	16.21
10.	43309	1.833	26.9
11.	433010	1.838	14.12
12.	99251	1.842	21.30
13.	99252	1.915	21.27
14.	99253	1.253	188.19
15.	99254	1.842	57.17
16.	99255	1.858	66.21
17.	99256	2.142	59.97
18.	99257	1.998	18.02
19.	99258	1.059	136.88
20.	99259	1.950	85.54
21.	992510	1.990	68.4
22.	100451	1.983	17.8
23.	100452	1.013	395.01
24.	100453	1.858	66.21
25.	100454	1.999	25.32
26.	100455	1.980	96.9
27.	100456	1.980	23.21
28.	100457	1.808	21.92
29.	100458	1.844	18.21
30.	100459	1.842	57.17
31.	1004510	1.934	19.41

Table 3. Results of Mec-A gene amplification in MRSA bacteria

Well	Flour	Sample	Ct	Note
A01	SYBR	43301	15.42	Positive
A02	SYBR	43302	N/A	Negative
A03	SYBR	43303	14.59	Positive
A04	SYBR	43304	15.00	Positive
A05	SYBR	43305	17.69	Positive
A06	SYBR	43306	16.30	Positive
A07	SYBR	43307	15.60	Positive
A08	SYBR	43308	15.48	Positive
A09	SYBR	43309	16.40	Positive
A10	SYBR	433010	24.52	Positive
A11	SYBR	PC	16.58	Positive
A12	SYBR	99251	N/A	Negative
C01	SYBR	99252	16.43	Positive
C02	SYBR	99253	21.30	Positive
C03	SYBR	99254	14.61	Positive
C04	SYBR	99255	15.03	Positive
C05	SYBR	99256	14.68	Positive
C06	SYBR	99257	18.93	Positive
C07	SYBR	99258	16.27	Positive
C08	SYBR	99259	16.52	Positive
H01	SYBR	992510	15.56	Positive
H02	SYBR	1004510	18.29	Positive
H03	SYBR	100451	17.14	Positive
H04	SYBR	100452	17.96	Positive
H05	SYBR	100453	15.05	Positive
H06	SYBR	100454	15.37	Positive
H07	SYBR	100455	15.04	Positive
H08	SYBR	100456	16.58	Positive
H09	SYBR	1004510	N/A	Negative
H10	SYBR	100457	15.24	Positive
H11	SYBR	100459	15.96	Positive



(a)



(b)

Figure 2. Figure 2. (a) MecA gene positive sample yield curve (b) MecA gene negative sample yield curve

DISCUSSION

Resistance is defined as the absence of inhibition of bacterial growth by systemic administration of antibiotics with normal doses or minimal inhibitory levels. Meanwhile, multiple drug resistance is defined as resistance to two or more drugs or drug classifications[7,8]. While cross resistance is the resistance of a drug followed by another drug that has never been described. Resistance occurs when bacteria change in one way or another causing a drop or loss of effectiveness used to prevent or treat infection. Bacteria that are able to survive and multiply, pose more danger. The sensitivity of bacteria to antibiotics is determined by the minimal inhibitory level that can stop the growth of bacteria [4,5].

The emergence of resistance to an antibiotic occurs based on one or more of the following mechanisms:

1. Bacteria synthesize an enzyme inactivating or destroying antibiotics. For example, Staphylococcus resistant to penicillin G produces beta-lactamase, which destroys the drug. Other beta-lactamases are produced by Gram-negative rods.
2. Bacteria change their permeability to drugs, eg tetracycline, accumulate in susceptible bacteria but not in resistant bacteria.
3. Bacteria develop an altered target structure for the drug. For example, chromosomal resistance to aminoglycosides is related to the loss or alteration of a specific protein in the 30s subunit of the bacterial ribosome that acts as a receptor on the susceptible emeryth.
4. Bacteria develop altered metabolic pathways that are directly inhibited by the drug. For example, somebacteria that are resistant to sulfonamides do not require extracellular PABA, but like mammalian cells can use folic acid that has been formed.
5. Bacteria develop altered enzymes that can still perform their metabolic functions but are less affected by drugs than enzymes in susceptible bacteria.

The results showed that from 30 samples there were 3 samples that were negative. This shows that not all MRSA bacteria tested are methicillin sensitive but when the MecA gene is detected, the results are negative. It may be suspected that there is a mutation in the MecA gene region so that it is necessary to carry out a sequence analysis.

CONCLUSION

Based on the results of this study, it was concluded that from 30 samples of MRSA there were 27 samples that were positive for the MecA gene and 3 samples were negative.

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