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Detection of meca gene from methicillin resistant Staphylococcus aureus isolates of north sumatera

Gabriella Septiani Nasution1,2, Dwi Suryanto1*, R Lia Kusumawati3,4

1Department of Biology, University of Sumatera Utara, Jln. Bioteknologi No. 1, Kampus USU, Medan, 20155
2Medan Health Polytechnique, Ministry of Health, Jln. Jamin Ginting Km. 13.5, Lau Cih, Medan
3Department of Microbiology, Faculty of Medicine, University of Sumatera Utara, Jln. Universitas No. 1, Kampus USU, Medan 20155
4Clinical Research Unit, University of Sumatera Utara Hospital, Jln. Dr. T. Mansyur No. 66, Kampus USU, Medan 20154

*Email: dwisuryanto@usu.ac.id

Abstract. Methicillin Resistant Staphylococcus aureus (MRSA) is a major pathogen associated with hospital-acquired infections (nosocomial infections). MRSA is a type of S. aureus resistant to the sub-group of beta-lactam antibiotics such as penicillin, cephalosporin, monobactam, and carbapenem. MRSA is resistant because of genetic changes caused by exposure to irrational antibiotic therapy. This study aimed to detect meca gene in North Sumatra isolates of MRSA and to determine the pattern of antibiotic resistance in S. aureus isolates classified as MRSA by Vitek 2 Compact in the Central Public Hospital Haji Adam Malik, Medan. Samples were 40 isolates of S. aureus classified as MRSA obtained from clinical microbiology specimens. DNA isolation of the isolates was conducted by a method of freeze-thaw cycling. Amplification of meca gene was done by PCR technique using specific primer for the gene. PCR products were visualized using mini-gel electrophoresis. The results showed that all MRSA isolates showed to have 533 bp band of meca. Antibiotics test of Vitek 2 Compact showed that despite all isolates were resistant to beta-lactam antibiotics groups; the isolates showed multidrug resistant to other common antibiotics, such as aminoglycosides, macrolides, and fluoroquinolones. However, they were still sensitive to vancomycin (82.5% isolates), linezolid (97.5% isolates), and tigecycline (100% isolates).

Keywords: antibiotic resistant, beta-lactam, detection, nosocomial infections, PCR

1. Introduction
Methicillin Resistant Staphylococcus aureus (MRSA) has become one of the worldwide health problems and is a major pathogen associated infections acquired in the hospital (nosocomial infections) [1]. MRSA is a type of S. aureus that is resistant to the sub-group of beta-lactam antibiotics such as penicillin, cephalosporin, monobactam and carbapenem [2]. This resistant caused by genetic changes because of irrational antibiotic therapy exposure.

MRSA strains were first reported in 1961 in the United Kingdom (UK) [3]. Prevalence of MRSA in hospitals in different parts of the world diverse, ranging between 2-70%, 20% in average [4,5,6]. The
percentage of strains of MRSA is relatively high in Asia, such as 60% in Taiwan reached, 20% in China, 70% in Hong Kong, 5% in Philippines, and 60% in Singapore. In Indonesia in 2006, MRSA prevalence is 23.5% [7]. A study in Dr. Soetomo Hospital, Surabaya showed that of 643 patients there were 52 MRSA (8%) [8]. In contrary, a study in Central Public Hospital Haji Adam Malik Medan showed quite higher prevalence of MRSA, in which in January-June 2015 there were 56 isolates (67%), in July-December 2015 there were 48 isolates (57%), and January-June 2016, 58 isolates (45%) were MRSA.

Genotypes examination for MRSA resistance has been conducted to know antibiotics resistance gene such as mecA [9]. The gold standard to determine MRSA genotypes is to detect conserved genes (fixed/preserved) constantly found in mecA gene, which is within range of a particular chromosome in Staphylococcal Cassette Chromosome (SCCmec) [10]. MRSA resistance is due to the mutant protein of penicillin-binding protein 2a (PBP2a or PBP2’) encoded by mecA gene. PBP is a group of enzymes in the cell membrane of S. aureus that catalyzes the trans-peptidation for peptidoglycan chain (cross-linkage) formation. The affinity of PBP2a is so low that MRSA stays alive in high concentration of antimicrobial exposure [11]. Amplification of mecA can be done by using polymerase chain reaction (PCR), which is the gold standard for the detection of mecA [12]. No information on the distribution of mecA on MRSA in North Sumatra is available. Therefore, a study on this gene is urgent.

2. Methods
2.1. Bacterial isolates and phenotypic characterization
This research was conducted in the central public hospital of Haji Adam Malik Medan. S. aureus isolates were obtained from clinical microbiology specimens sent to Hospital Diagnostic Laboratory Installation H. Adam Malik, Medan from March to May 2017. A total of 40 Vitek 2 Compact classified MRSA were used in this study.

2.2. DNA isolation
MRSA was sub-cultured on blood agar and incubated at 37°C for 18-24 hours. The cell was broken by a freeze-thaw method as previously described [13]. The freeze-thawed solution was spin at 13.000 rpm for 5 minutes. The supernatant was separated from cell debris, and subjected to DNA purification check.

2.3. Amplification and detection of mecA gene using PCR technique
mecA gene amplification was performed by PCR technique using specific primer mecA-F: AAA AAA GGT GGT ATC GAT TGG C and mecA-R: AGT TCT GCA GTA CCG GAT TTG C [14] [15]. Amplification was done in PCR solution of 12.5µl GoTaq® Green Master Mix, 1µl of forward and reverse primer each, 8.5µl nuclease-free water, and 2µl bacterial DNA. Thermocycling reaction was conducted for denaturation at 95°C for 3 minutes, annealing for 55°C for 30 seconds, extension at 72°C at 60 seconds, and extended extension at 72°C for 6 minutes. The reaction was done for 30 cycles. PCR product was visualized in mini gel electrophoresis and documented in UV Reader/Gel Documentation System. MRSA ATCC43300 and MSSA ATCC25923 were used for positive and negative control, respectively.

3. Results and Discussion
3.1. Bacterial isolates and phenotypic characterization
A total of 40 isolates MRSA were collected from March-May 2017 isolated from various clinical specimens of Diagnostic Laboratory Installation. Staphylococcus aureus is a common type of bacteria found in the skin, nasal cavity healthy person or patients, and in pus. The prevalence of MRSA in the sample was between 2.5-40%. Most MRSA was isolated from pus (40%), sputum (22.5%), and blood (17.5%)(Table 1). A study carried out by Islam et al. (2011) isolated MRSA mostly from pus (7.5%) [16]. Pournajat et al. (2014) found that most MRSA was isolated from pus (29%) [15].
Table 1. Prevalence of MRSA from various clinical specimens

<table>
<thead>
<tr>
<th>No</th>
<th>Clinical specimens</th>
<th>Total sample (n)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brain fluid</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>Tissue</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Urine</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>Pleural fluid</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Swab</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>6</td>
<td>Blood</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>7</td>
<td>Sputum</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>8</td>
<td>Pus</td>
<td>16</td>
<td>40</td>
</tr>
</tbody>
</table>

Phenotype test of antibiotic resistant using Vitek 2 Compact showed that all isolates were resistant to cefoxitin. Data from Clinical and Laboratory Standards Institute (CLSI) 2015 showed that isolates resisted to cefoxitin were also resistant to 52 other types of antibiotics. Cefoxitin including second-generation Cephalosporin is a potent inducer of mecA regulatory system that is used extensively as a marker for the detection of the mecA gene [17]. The percentage for antimicrobial sensitivity can be seen in Table 2 below.

Table 2. Antimicrobial sensitivity of MRSA isolates

<table>
<thead>
<tr>
<th>No.</th>
<th>Antimicrobial Agents</th>
<th>Antibiotic sensitivity of 40 isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>1</td>
<td>Amoxicillin</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Ampicillin/Sulbactam</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Cefalexin</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Cefazolin</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Cefuroxime</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Ciprofloxacin</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>Clindamycin</td>
<td>52.5</td>
</tr>
<tr>
<td>8</td>
<td>Erythromycin</td>
<td>47.5</td>
</tr>
<tr>
<td>9</td>
<td>Gentamycin</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>Levofoxacin</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Tetracycline</td>
<td>37.5</td>
</tr>
<tr>
<td>12</td>
<td>Vancomycin</td>
<td>82.5</td>
</tr>
<tr>
<td>13</td>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>72.5</td>
</tr>
<tr>
<td>14</td>
<td>Moxifloxacin</td>
<td>2.5</td>
</tr>
<tr>
<td>15</td>
<td>Linezolid</td>
<td>97.5</td>
</tr>
<tr>
<td>16</td>
<td>Tigecycline</td>
<td>100</td>
</tr>
</tbody>
</table>

It was shown that all isolates were resistant to beta-lactam antibiotics of amoxicillin, ampicillin/sulbactam, and cephalosporins group such as cefalexin, cefazolin, and cefuroxime. High resistant was also showed to ciprofloxacin (97.5%), gentamycin (87.5%), and levofoxacin (97.5%). In contrary, the isolates were still sensitive to vancomycin (82.5%), trimethoprim/sulfamethoxazole (72.5%), linezolid (97%), and tigecycline (100%). For antibiotics treatment, these antibiotics may still be used.

Another study in Indonesia showed similar pattern of antibiotic resistant in which S. aureus of clinical samples was resistant not only to penicillin and methicillin, but also resistant to tetracycline.
(24.5%), oxacillin (2%), gentamicin (1%), erythromycin (5.1%), chloramphenicol (9.2%), and trimethoprim/sulfamethoxazole (7.1%) [18]. However, the percentage of isolates resistant to other antibiotics was relatively lower compared to that of this study. Al Ruaily and Khalil (2011) study in Saudi Arabia showed S. aureus isolates were resistance to cephalosporins (95%), gentamycin (95%), ciprofloxacin (87%), vancomycin (100%), and penicillin (100%) [19]. A similar study of Elhassan et al. (2015) isolated from different clinical specimens in Shendi City, Sudan showed that all isolates of S. aureus were resistance to methicillin, penicillin, and ampicillin [20]. Resistant to gentamycin, ciprofloxacin, and clindamycin were showed in 80% of the isolates, while resistant to trimethoprim/sulfamethoxazole was showed in 74% of the isolates. However, the isolates resistant to linezolid were relatively low (13%). Sudigdoadi (2010) showed that 89% of his isolates were resistant to gentamycin [21].

Beta-lactam antibiotics kill bacteria by inhibiting cell wall synthesis. MRSA resistance to beta-lactam group is due to a protein mutant of penicillin-binding protein 2a (PBP2a or PBP 2') encoded in mecA gene. PBP is a group of enzymes in the cell membrane of S. aureus that catalyzes the transpeptidation for the formation of peptidoglycan chain webbing (cross-linkage). Affinity PBP2a antimicrobial beta-lactam group is so low that MRSA remains alive in a high concentration of antimicrobial exposure in [11].

It was interesting that the isolates showed resistant to vancomycin although only a few. The resistant to vancomycin (VRSA) is associated with changing and resetting bacterial cell wall. In addition, overproduction of Penicillin Binding Protein-2 (PBP-2) is also considered as an important factor for the expression of resistance to vancomycin. It is known that resistance to vancomycin is mediated by specific gene vanA to glycopeptides [22]. Vancomycin resistant isolates is likely due to spontaneous mutations, occur acquisition of resistant factors from elsewhere, or from the surrounding enteric bacterial population [21].

3.2. Detection of mecA gene

MRSA detection is essential for proper patient care and infection control. Examination of MRSA can be done either phenotype or genotype. The gold standard to determine information about the genotype distribution of MRSA is to detect genes conserved constantly found. mecA gene is located within chromosome in a structure called Staphylococcal Cassette Chromosome (SCCmec) encodes mutant PBP2a or PBP2' of 76 kDa [10]. The PCR results of all isolates were shown in Figure 1.
Figure 1. Distribution of mecA gene in isolates of MRSA (533bp product) (M=100 bp, K- = negative control (MSSA ATCC25923), K+ = positive control (MRSA ATCC43300), lane 1-40 = MRSA isolates of clinical samples)

The presence of mecA gene is generally to indicate the potential resistance to beta-lactam group and used as a marker to identify MRSA. In this study, PCR product was shown as 533 bp amplicon in all resistant isolates using a primer designed by Pournajat et al. (2015) [15]. The similar result showed by Sudigdoadi (2010) in which all 45 isolates of MRSA were investigated, certainly has a mecA gene which is found in a 20-100 kb called staphylococcal cassette chromosome (SCCmec) [23][24]. MRSA resistance to methicillin and all beta-lactam group antimicrobial is due to changes in normal penicillin binding protein PBP 2 to PBP 2a. Mutation to PBP 2a showed that the change in the binding site resulted in lower affinity to beta-lactam group [25], therefore if the bacteria are cultured in medium containing a high concentration of beta-lactams, they still survive and grow.

4. Conclusions
Test of antibiotic sensitivity using Vitek 2 Compact of 40 isolates showed that the isolates were not only resistant to beta-lactam antibiotics groups, but also to other antibiotics such as aminoglycosides, macrolides, and fluoroquinolones. Gene detection of mecA showed that all isolates carried the gene.

Acknowledgments
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References


