The molecular changing mechanism of Ampicillin-Sulbactam resistant Staphylococcus aureus towards Methicillin resistant Staphylococcus aureus

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ABSTRACT
The aim of this study was to determine the molecular changing of S.aureus, which is resistant to Ampicillin-Sulbactam and then become resistant to Methicillin as a result of improper dosage. The study was conducted by isolating Ampicillin-Sulbactam resistant and Methicillin Resistant S.aureus (MRSA), afterwards an amplification process was performed by PCR (Polymerase Chain Reaction.) to isolate the beta lactamase enzyme regulator and PBP 2a genes. The result of this research showed that there were a deletion of few amino acids from the regulator gene, and a suspicion that the DNA sequence had been substituted from PBP 2 gene into PBP 2a (gen mec). This process had formed MRSA.

Key words: S.aureus, betalactamase gene, PBP 2a gene

INTRODUCTION
Until now, infection is still a prevalent problem especially in developing countries. One of dental caused infections is periapical abscess. Prolonged infection will cause jaw osteomyelitis with Staphylococcus aureus (S. aureus) as the main bacteria.

In constant efforts to cope with infection caused by S. aureus, several antibiotics had been used, among others the Betalactam group. However, the present condition revealed many bacteria are resistant to Betalactam. To overcome the resistance, Betalactam is mixed with Betalactamase inhibitor, in this case Ampicillin with Sulbactam complying to antibiotic-consumption-strict-rules of correct doses, correct indication, and correct bacteria. Should this rules be violated, therapy becomes difficult and expensive, and also escalating resistance.

Based on a research by Satari1 from 94 clinical isolates, 79% was Ampicillin resistant, 53% was Ampicillin-Sulbactam resistant and out of this 53% resistance, 38% was resistant towards Methicillin causing bacteria resistance to many antibiotics.

From an empirical 7 years study (1986-1993) conducted at the Microbiology Department of School of Medicine, University of Indonesia, it was revealed that S.aureus pattern of resistance had changed to multi resistance.2 Lyon and Skuray3 had proven that S.aureus was resistant to 20 antibiotics.

According to on Brooks,4 since 1961 the multiresistant S. aureus has caused health problems particularly at health centers that can be fatal. The multiresistant S. aureus was later known as resistant to Methicillin S. aureus (MRSA). In molecular process, the forming of MRSA was initiated by a mutation of PBP 2 which was a transpeptidase enzyme functioning in forming a murein bag of peptidoglican, to become PBP 2a which had low affinity towards Betalactam antibiotic group. The aim of this research was to molecularly analyze the influence of S.aureus resistance pattern against Ampicillin-Sulbactam to form MRSA. This research gave scientific contribution to understand the resistibility process at molecular stage.

MATERIALS AND METHOD
The sample was taken from isolate S. aureus which was resistant against Ampicillin-Sulbactam and Methicillin, complying with the criteria as follows: 1) producing Betalactamase enzyme; 2) producing total DNA isolation; 3) PCR fragment gene regulator technique (blaI and blaRI using 2 primary pairs) and fragment (PBP 2a (gen mec) using 1 primary pair) could be well isolated.

The primary which was used to isolate Beta lactamase enzyme regulator gene (blaRI and blaI) was designed by Okamoto, Okubuta and Inoue.5
Satari: Mechanism of staphylococcus aureus

Forward blaZ: primary sense
Reverse blaRI: primary antisense
blaI: 
P1: 5′ACTCTTTGGCATGTGAACTG 3′
P3: 5′ GGACAAATCTATCGGCTTCT 3′
P4: 5′ TGAGTTGAGTCGAGTATAG 3′
P5: 5′ CATAACATCCCAATTCAGCCA 3′
P6: 5′ AACTTTTCAATGTTCCCTCC 3′

The primary which was used to isolate gen mec was designed by Murakami, Minamide.  
Forward : 5′ AAAATCGATGTTAAAGGTTGGC 3′
Reverse : 5′ AGTTCTGCAGTACCAGGATTTGC 3′

The research material was isolate S. aureus which was resistant to Ampicillin-Sulbactam and Methicillin. It was obtained from the Microbiology Clinic of Hasan Sadikin General Hospital/School of Medicine Padjadjaran University and School of Medicine Diponegoro University.

The chemical substance to totally isolate DNA: Tris HCL 50mM containing EDTA 5mM, NaCl 50mM, lysostapin 20µg/ml and Rnase 200µg/ml. The total DNA isolation was performed with Tokue and Shoji technique.

The chemical substance for PCR technique were: buffer lysis, PCR kit core system (Promega), Primary (P1-P6) and primary mec. BlaRI and blaI isolation with PCR technique using Primary (P1-P6) under PCR condition i.e.: 1) denaturation process 94° C for 4 minutes, 2) 94° C denaturation cycle stage in 30 seconds, 3) 50° C primary attachment stage in 30 seconds, and 4) 72° polymerisation stage in 2 minutes. Polymerase chain reaction (PCR) was done in 40 cycles with 72° stabilizing stage.

The chemical substance for sequencing technique was ABI PRIMS Dye Terminator Cycle Sequencing Ready Kit (Perkin Elmer Corporation). The equipments for microbiologic examinations were Eppendorf tube, 25°C and -4°C ultra centrifugation, vortex mixter, E-C minicell, PCR machine, transilluminator with ultraviolet, polaroid, DNA sequencing ABI Prims 377.

RESULTS

Amplification result of blaZ, blaRI and blaI genes using 4 primary pairs can be seen on figure 1.

Figure 1. Electrophoresis PCR product utilizing P1-P6 to amplify blaZ, bla RI and blal gene fragments.
Hinfl (pUC19) was the marker used to give restriction results sized 1419,517,396,214 and 65 pb.

Figure A: isolate using primary P1 - P3 → 878pb
Figure B: isolate using primary P1 - P4 → 1426pb
Figure C: isolate using primary P1 - P5 → 1579pb
Figure D: isolate using primary P1 - P6 → 1686pb

Primary P1–P6 with primary internal P3, P4, P5 were used in this research. P1–P3 had 878pb, amplifying blaZ which was a Betalactamase enzyme structure gene. P1–P4 had 1426pb, amplifying blaZ and part of blaRI. P1–P5 had 1579pb, amplifying blaZ, blaRI and part of blaI. P1–P6 had 1686pb, amplifying blaZ, bla R1 and blaI.

To study the deletion of regulator gene, which later on was suspected being fusion with gen mec, a sensitivity test was performed towards S. aureus Ampicillin-Sulbactam resistant to Meticillin. Thirty eight percent of samples were resistant to Meticillin. After amplifying gen mec a, the result was an amplification with 517pb length.

Hyperproduction took place evoked by nucleotides changes on regulator genes where they transmitted (blaRI) and received signal (blaI). Wiederman and Peter8 stated that should a regulator gene be inactive as a result of several nucleotides deletion, then the production of Betalactamase enzyme multiplied enormously.

According to Okamoto2 and Murray9 a deletion of 150 nucleotides on the tip of the regulator gene could vanish its regulating function, a change of production characteristic occurred, that was from inductive to constitutive production manifolding Betalactamase output in 50–100 times. While Lewis, Curnok and Dyke10 clarified that MRSA was formed due to the fusion between the regulator gene and the structural PBP2 gene which afterward expressed a PBP 2a gene with a low affinity towards Ampicillin-Sulbactam. The shaping of PBP 2a was the reason for the resistibility of S. aureus against several antibiotics, this was later known on as MRSA.

The conclusion of the research revealed that the usage of antibiotics in particular of Ampicillin-Sulbactam mixture, should stick to certain regulations to avoid resistance. The resistance against Ampicillin-Sulbactam, phenotypically was caused by a hyperproduction of Betalactamase enzyme. Genotypically, the hyperproduction was due to the deletion of several nucleotides of regulator genes. It was assumed that the vanished nucleotide fusioned with PBP 2 becoming PBP 2a. The fusion happened because the regulator gene with PBP 2 gene had formed a tertiary DNA structure Kernodle.11

REFERENCES