Sensitivity Test Cefoxitin (Second Generation of Cephalosporin) and Cefepime (Fourth Generation of Cephalosporin) Towards Methicillin Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

MRSA is known as multidrug resistant bacteria that are resistant towards many antibiotics. These bacteria are also considered as the bacteria that cause sepsis which may lead to death. To manage sepsis condition, an antibiotic agent that is still sensitive towards MRSA is needed. There are some antibiotics that are still presumably sensitive towards MRSA including, among others, Cefoxitin and Cefepime.

The objective of this study is to analyze the sensitivity of MRSA towards Cefoxitin and Cefepime in order to get scientific information on the effectiveness of the two antibiotics to be used in the management of sepsis patients.

This study is a laboratory experimental study with 40 isolates of methicillin resistant Staphylococcus aureus collected from several hospitals in Bandung to define MRSA bacteria. The method used for sensitivity testing is the agar diffusion method. The result of the sensitivity test is the clear area around the antibiotic disc which is then measured. The Cefoxitin sensitive MRSA bacteria have an inhibition area of >18 mm while the resistant bacteria have an inhibition area of < 14 mm. The same is also true for Cefepime sensitive bacteria.

In this study, 1 isolate is resistance, 33 isolates present Cefoxitin sensitive MRSA, i.e. 82.5%, while 100% of them are sensitive towards Cefepime.

It is concluded that the growth of MRSA can be considered as being inhibited by Cefoxitin, which is the second generation of Cephalosporin.

Keywords: Cefepime, Cefoxitin, MRSA

Introduction

Staphylococcus aureus is a bacterium that may cause an infection due to the enzymes and toxins produced, which is also a factor for pathogenicity. Currently, there are a lot *Staphylococcus aureus* bacteria that are resistant towards several antibiotics, making these bacteria known as the bacteria that causes nosocomial infections. In general, these beta-lactam resistant bacteria are known as the MRSA (Methicilin Resistant *Staphylococcus aureus).* Originally, methicillin antibiotics is use for inhibition the growth of *Staphylococcus aureus* bacteria that are resistant towards penicillin/beta-lactam.[1] However, due to the inappropriate use of methicillin, the bacteria become resistant towards this agent.

Molecularly, the MRSA is caused by a gene mutation at the penicillin-binding protein (PBP) 2 which is mutated to 2a, making the binding site of the PBP 2a, which is the antibiotic target, is not recognized anymore by the methicillin. This PBP2 is known as the transpeptidase enzyme, a beta-lactam antibiotic target. The beta-lactam antibiotic has a similar structure to PBP2 in the form of transpeptidase. When the induction by beta-lactam antibiotics happens, a bound between proteins that catalyze the final stage of peptidoglycan synthesis with antibiotics will be created leading to the disturbance in D-alanyl D-alanine breakage that causes disorder in the peptide bridge development which prevents the development of murein sacs. This failure will eventually cause

bacterial lysis. The mutation of PBP2 to PBP2a makes this binding site unrecognizable for beta-lactam antibiotics, in this case methicillin/oxacillin, which then leads to bacterial resistance.[2,3]

This PBP 1-3 is an enzyme that catalyzes disaccharide unit polymerization in the glycan chain known as transglycolase which also catalyzes cross-binding at the pentapeptide side known as transpeptidase. Transpeptidase works at the final stage of peptidoglycan formation. In contrast with the transpeptidase activation, the protein that has this transglycocylation activity is not sensitive to beta-lactam antibiotics. The double activities expressed by this PBP are probably due to the presence of 2 different polypeptides and are active at the same polypeptides. The amino end domain catalyzes transglycolation reaction and carboxyl end domain has transpeptidase activity that is sensitive towards beta-lactam antibiotics. The inhibition activity of betalactam antibiotic PBP leads to bacterial cell death. PBP 1 will cause rapid cell death, PBP 2 causes cell to change its shape into an ovoid one while PBP3 makes the cell change its shape into a filamentous cell. Eventually, the bacteria that bind with PBP 1-3 will experience lysis. Cephalosporin antibiotics have an affinity towards PBP 1 and PBP3 .[2,4,5]

Cephalosporin is a beta-lactam antibiotic agent that inhibits peptidoglycan synthesis. Initially, this Cephalosporin was isolated from *Cephalosporium acremomium* fungus that is frequently found in the coastal areas of Italy. According to Giuseppe Brotzu, the inventor of this antibiotic, the main structure of Cephalosporin at 7-aminocephalosporinic acid (7-ACA) is similar to the main structure of penicillin, i.e. 6-aminopenicillanic (6-APA). The R group in 7-ACA is then modified to have a better antimicrobial potential resulting in the first generation of Cephalosporin which is then followed by the next generations [6,7]

The work mechanism of Cephalosporin is triggered by the presence of a configuration that is similar to the terminal structure of acyl D-ala-D-ala, a part of pentapeptide chain in peptidoglycan. This Cephalosporin will acylate the Serine active side of transpeptidase so that this enzyme is unable to interrupt D-ala-D-ala in the peptapeptide chain that becomes tetrapeptide. As a result, a failure in inter-bridge development that is the final stage of peptidoglycan synthesis is found. It is in this phase that bacterial lysis occurs [5-7]

Currently, various types of Cephalosporin antibiotics are still used as the alternative in treating diseases caused by MRSA. The agents used include, among others, Cefoxitine which is the second generation Cephalosporin and Cefepime which is the fourth generation of Cephalosporin. Recently, it is suspected that some S. aureus are resistant towards Cefoxitine. According to several researchers, when S. aureus is resistant towards Cefoxitine, the bacterium is considered as MRSA .[8]

Based on those reasons, to deal with Cefoxitine-resistant S. aureus, a change in R group of 7-ACA is made to get the fourth generation of Cephalosporin, including Cefepime.

Method

Forty 40 MRSA isolates were collected from several hospitals and laboratories in Bandung. The isolates were then cultured and identified as methicillin resistant S. aureus at the Faculty of Medicine Laboratory, Unpad-Dr. Hasan Sadikin hospital, Bandung.

The isolation of S. aureus was conducted using blood agar media that was incubated for 24 hours under a temperature of 37^0C. Identification was then made using

Gram staining and coagulase testing. The sensitivity testing for the antibiotics was performed using Mueller Hinton's agar diffusion method. The inoculum turbidity is based on 0.5 Mc Farland. After that the sensitivity testing of S. aureus towards several antimicrobials was conducted using discs of Oxacillin that also shows the isolates are resistant towards Methicillin, Cefoxitine, and Cefepime.

The inhibition zone diameter formed was interpreted according to the NCCLS protocol. The inhibition zone diameter is the discriminatory antimicrobial concentrations used to determine whether the isolates tested is sensitive (S), intermediate (I) or resistant (R).

The inhibition zone diameter formed for several antimicrobials based on the NCCLS includes:

Breakpoint (diameter in mm)

	Sensitive	Intermediate	Resistant
Oxacillin	> 13	11-10	< 10
Cefoxitine	> 18	17-14	<14
Cefepime	> 18	17-14	< 14

Results

Table 1. Results of S. aureus inhibition zone towards Oxacillin

Tested Bacteria	Diameter	Notes	Tested Bacteria	Diameter	Notes
1	10.8	I	21	9.2	R
2	7.5	R	22	8.8	R
3	5.2	R	23	7.6	R
4	6.7	R	24	5.5	R
5	8.8	R	25	5.8	R
6	9.6	R	26	6.5	R
7	7.7	R	27	7.8	R
8	8.6	R	28	9.2	R

9	9.2	R	29	8.9	R
10	6.5	R	30	7.7	R
11	5.5	R	31	6.5	R
12	4.9	R	32	6.7	R
13	8.9	R	33	8.1	R
14	9.7	R	34	5.8	R
15	9.9	R	35	6.1	R
16	6.2	R	36	7.8	R
17	5.1	R	37	6.7	R
18	7.5	R	38	5.3	R
19	9.7	R	39	6.2	R
20	9.8	R	40	4.7	R

Table 2. Results of S. aureus inhibition zone towards Ceptoxilin

Tested Bacteria	Diameter	Notes	Tested Bacteria	Diameter	Notes
1	20.8	S	21	14.8	S
2	18.6	S	22	17.2	S
3	17.5	I	23	18.1	S
4	19.2	S	24	20.2	S
5	22.1	S	25	16.5	S
6	13.8	I	26	22.6	S
7	16.5	I	27	15.7	S
8	17.8	I	28	22.3	S
9	19.1	S	29	24.2	S
10	18.0	S	30	18.1	S
11	14.1	I	31	16.8	S
12	15.3	I	32	20.1	S
13	20.9	S	33	21.2	S
14	16.8	I	34	17.8	S
15	13.4	I	35	18.6	S
16	16.7	S	36	18.5	S
17	22.3	S	37	19.8	S
18	23.1	S	38	22.5	S
19	13.8	R	39	19.1	S
20	14.5	S	40	21.4	S

Table 3 Results of MRSA inhibition zone towards Cefepime

Tested Bacteria	Diameter	Notes	Tested Bacteria	Diameter	Notes
1	26.3	S	21	18.5	S
2	24.8	S	22	20.7	S
3	20.1	S	23	19.8	S
4	19.3	S	24	22.1	S
5	25.7	S	25	26.3	S
6	27.5	S	26	21.8	S
7	22.8	S	27	27.3	S
8	20.0	S	28	19.3	S
9	18.9	S	29	24.5	S
10	25.8	S	30	26.8	S
11	22.7	S	31	24.3	S
12	21.5	S	32	25.6	S
13	18.7	S	33	18.6	S
14	22.5	S	34	19.7	S
15	23.8	S	35	23.1	S
16	20.3	S	36	22.8	S
17	23.0	S	37	24.5	S
18	19.3	S	38	19.3	S
19	21.4	S	39	20.9	S
20	22.7	S	40	22.6	S

Discussions

In this study, 40 MRSA isolates that have already been tested using Oxacillin disc were tested for their sensitivity towards Cephalosporin antibiotics, i.e. Cefoxitine and Cefepime, which are the beta-lactamase enzyme resistant antibiotics.

The MRSA resistance towards various antimicrobials is carried out by 2 groups, i.e. toward beta-lactam antibiotics and non beta-lactam antibiotics. The MRSA resistance towards beta-lactam antibiotics is caused by the mutation of PBP2 into PBP2a. The function of PBP2 that is inhibited by beta-lactam will be compensated by PBP2a making the synthesis of cell wall in MRSA continue to happen.[9] This mutation is

caused by an insertion of some of the nucleotide base from beta-lactamase operon gene that is substituted in PBP2 forming gene called gen mec.[10 ,11] The MRSA resistance which is not caused by beta-lactam antibiotics is mostly caused by the changes in antimicrobial receptor pumped actively from the cells or better known as the efflux mechanism. [10]

PBP2a is coded by mec A gene which is a conserved part of the genetic elements called mecDNA or Staphylococcal cassette chromosome (SCC mec). These genetic elements can be transferred to other bacteria through plasmid. The gene cannot be found in Staphylococcus that is sensitive towards Methicillin. [10,12]

In the MRSA sensitivity testing towards Cefoxitine, there are 7 isolates that are intermediate. It is assumed that several MRSA, which is then referred as pre-MRSA, still have mecA gene. However, these bacteria are still sensitive towards methicillin. The bacteria will receive weak induction stimulation from methicillin that triggers a weak stimulation for PBP2a production. Molecularly, a weak induction towards mecR1, which is a signal transmitting gene, will lead to a repression by MecI on mecR1 promoter gene so that the PBP2a produced will be less. The beta-lactam antibiotic induction on beta-lactamase enzyme formation regulation gene, blaI, is ten times stronger than beta-lactam induction on mec RI.[9].[13]

In MRSA sensitivity testing towards Cefepime, it is demonstrated that all isolates are sensitive. Based on the results of several studies, it stated that the Cefepime has a good affinity with PBP2a. This is due to the availability of structures in the R group of Cefepime that has the ability to stabilize the main structure of 7 -ACA so that it cannot be hydrolyzed by beta-lactamase (Guntur). This configuration accelerates the acylation process of Cefepime Serine towards the PBP2a Serine active side at Ser 403.

In this process, the Cefepime Serine active side becomes the proton donor that plays a role in the acylation process towards the PBP2a Serine active side leading to hydrogen binding with the amino acid at the residual end of Cefepime. [14]

Cefepime has a good affinity for binding PBP2a despite the mutation in gen mec operon that regulates PBP2a formation. However, Cefepime still has a good affinity to bind PBP2a because the Cefepime Serine active side has a structure that is similar to the PBP2a Serine active side.[14]

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