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EVALUATION OF THE SENSITIVITY RESPONSE OF Staphylococcus aureus ATCC 6538 TO ANTIBIOTICS INHIBITING THE SYNTHESIS OF BACTERIAL CELL WALLS

Putri Aisyah Qholbitsna Nurron[1*], Reza Anindita[1], Maulin Inggraini[2], Noor Andryan Ilsan[2]

Abstract

Introduction: The cause of nosocomial infections is bacteria. One of the bacteria that causes nosocomial infections is Staphylococcus aureus. The use of antibiotics with the right dose is the main choice in treating bacterial infections. But in practice antibiotics are often used inappropriately, causing bacterial resistance. Resistance is a condition where antibiotics fail to kill bacteria. To reduce this problem, it is necessary to evaluate the sensitivity to antibiotics. The purpose of this study was to determine the sensitivity response of Staphylococcus aureus ATCC 6538 to antibiotics in the class of inhibitors of bacterial cell wall synthesis with concentrations of 15, 20, 25, and 30 g.

Methods: This research is a laboratory experimental study using the Kirby Bauer method with Ampicillin as a sample of antibiotics. The data analysis used in this research is descriptive which shows the size of the inhibition zone diameter and turbidity in the MIC method.

Results: The results of this study showed that the antibiotic Ampicillin with a concentration of 30 g was able to inhibit the growth of S. aureus with an average diameter of the inhibition zone of 29 mm or included in the sensitive category.

Conclusion: The conclusion of this study is that the antibiotic Ampicillin is still effective in inhibiting the growth of S. aureus. With a sensitive category at a concentration of 30 g

Keywords: Antibiotics, Nosocomial infections, Kirby Bauer, Resistance, Staphylococcus aureus

INTRODUCTION

According to Sihombing (2020) Nosocomial infections are diseases that are generally acquired from hospitals. This disease can trigger an increase in morbidity and mortality worldwide.Khan *etal*. (2017) states that the percentage of nosocomial infections in developed countries is 7% while developing countries are 10%. According to research results Sihombing (2020) states that the percentage of hospitalized patients is at risk of nosocomial infection by 20%.

Research result Klevens *et al*. (2007) reported that every year as many as 1.7 million patients whoare undergoing treatment in hospitals are exposed to nosocomial infections. WHO (2005) in Sardi (2021) added that as many as 5% - 15% of patients in developed countries who were hospitalized had nosocomial infections. Abubakar (2017) stated that the most dominant nosocomial infection was bacteria. Erlin *et al*. (2020) stated that one of the bacteria that causes nosocomial infections is*Staphylococcus aureus*. According to Kuslovic (2020) in his research explained that the bacteria *S. aureus* is a type of bacteria with a pathogen.

Artati *et al*. (2016) stated that the use of antibiotics with the right dose is still the main choice in treating nosocomial infectious diseases caused by *S. aureus*. Roni *et al*. (2019) added, although theuse of antibiotics needs to pay attention to the right procedure, in practice antibiotics are oftenused inappropriately, causing bacterial resistance with varying impacts. Kuswandi (2019) explained that bacteria that are resistant to antibiotics can increase the impact of mortality on the risk of infectious diseases. Meanwhile, according to WHO in Tjay *et al* (2015) , Every year antibiotic resistance in European Union countries reaches 25,000 deaths. Given the problems and impacts caused by bacterial resistance to antibiotics, it is necessary to do research on *survailance*

returned to evaluate the resistance of nosocomial bacteria, such as *S. aureus*, to antibiotics. The importance of conducting research on surveillance of bacterial resistance to antibiotics is reported in the research results Iliya *et al* (2020) stated in his research that the isolates taken from patientsat the Klambu District health facility were tested for sensitivity using the disc diffusion method using several antibiotics and got the results that the highest sensitivity was obtained from chloramphenicol antibiotics and the lowest was penicillin.

METHOD

Research design

This study was conducted using an experimental design, with the aim of testing the sensitivity of the antibiotic cell wall synthesis inhibitor class to the growth of S. aureus bacteria.

Population and Sample

The samples in this study were pure cultures of S. aureus ATCC 6538 collection of the Parasitology Laboratory of the University of Indonesia and cell wall inhibitor antibiotics, namely amoxicillin, co amoxiclav, penicillin, ampicillin, and cefadroxil.

Research Tools and Materials

The tools that will be used in this research are measuring cup (Pyrex, USA), erlenmeyer (Pyrex, USA), beaker glass (Pyrex, USA), test tube (Pyrex, USA), test tube rack, measuring pipette (Pyrex, USA). , 50-100μl micropipette (Socorex, Switzerland), yellow tip, analytical balance (Acuplus, China), laminar air flow (Esco, Singapore), autoclave (Hirayama, HG-80, 76L, Japan), incubator (Memmerth IN- 30, Germany), Bunsen, petri dish (Pyrex, USA), vortex mixer VM 300 (Gemmy, Germany), ose needle, paper disk, sterile cotton swab, tweezers, label paper, spray bottle, tube rack, cotton stopper, wrapping , aluminum. The ingredients to be used are pure isolate of S. aureus, antibiotics amoxicillin (Bernofarm), co amoxiclav (Indofarma), penicillin (Phapros), ampicillin (Errita Pharma), and cefadroxil (Dexamedica) withdoses of 45, 50, 55, 60μg , Mueller Hinton Agar (MHA), Nutrien Agar (NA), Nutrien Broth (NB), NaCl 0.9%, sterile Aquadest, and Mc Farland 0.5.

Tool Sterilization

All tools that will be used in research with microorganisms must be sterilized before use. The tools and
media will be sterilized by the wet heat method, namely autoclaving at 121 for 15 minutes. Before sterilization, tools and materials must be wrapped first. Tools made of iron that are not waterproof (wet) such as ose needles and tweezers are then sterilized using the fixation method or incandescent with a Bunsen flame. (Hadijah, 2021).

Production of Mueller Hinton Agar (MHA) Media

Weigh 38 grams of MHA media then put it into an Erlenmeyer and mix it with 1 liter of distilled water then heated on a hot plate until dissolved and sterilized using an autoclave for 15 minutes at a temperature of 121C. The MHA media was put into a petri dish using a pipette as much as 25 ml of a petri dish(Utomo *et al*., 2018).

Preparation of Nutrient Agar (NA) Media

Weigh 1.68 grams of NA medium then put it into the omogenyr and dissolve it in 60 ml of distilled water. Homogenize using a hotplate magnetic stirrer. The media was sterilized using an autoclave at 121C for 15 minutes, then poured 15 ml into each sterile petri dish and at room temperature for ± 30 minutes (Puspasari*et al*., 2020).

Preparation of 0.5 . Mac Farland Solution

Mac Farland's solution is a turbidity comparison solution which is equivalent to 1.5x108 CFU/ml bacteria (Amanda *et al*., 2019). Mac Farland 0.5 was made using 2 ingredients, namely 1% Barium Chloride and 1% Sulfuric Acid by inserting it into a test tube of 0.5 ml of 1% Barium Chloride then adding 9.95 ml of 1% Sulfuric Acid. Mix until homogeneous, then cover with cotton (Apriani dan Fathir, 2021).

Bacterial Rejuvenation

Bacterial rejuvenation was carried out using 5 ml of NA media which had been compacted in a test tube in the form of an inclined media with a slope of 30. S. aureus bacterial colonies were taken as much as one ose and then streaked on NA media and incubated in an incubator at 37C for 24 hours. (Lestari *et al*., 2020).

Bacterial Suspension Manufacturing

Bacteria that have been rejuvenated are taken with a sterile ose needle, suspended in 5 ml of 0.9% NaCl in a test tube until it reaches the same turbidity as the standard Mc Farland turbidity of 0.5 (Base *et al*., 2019).

Determination of Test Solution

1) Preparation of Antibiotic Solution Concentration 15 g / 50 l.
Antibiotics as much as 165 mg dissolved in 50 ml of distilled water (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.
2) Preparation of Antibiotic Solution Concentration 20 g / 50 l.
Antibiotics as much as 220 mg dissolved in 50 ml of distilled water (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.
3) Preparation of Antibiotic Solution Concentration 25 g / 50 l.
Antibiotics as much as 275 mg dissolved in 50 ml of aquadest (Solution A). Take 1 ml of solution A, add with aquadest to 10 ml. Drop on a paper disk as much as 50 l.
4) Preparation of Antibiotic Solution Concentration 30 g / 50 l.
330 mg of antibiotic dissolved in 50 ml of aquadest (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.

Testing the Inhibitory Power of Antibiotics Using the Kirby Bauer Method

The bacterial suspension that has been made is scratched onto the solidified MHA media using the 4 quadrant scratch plate method. Blank disk measuring 6 mm was dripped with 50 l of antibiotic solution at each concentration of 15, 20, 25, and 30 g. Paper disks containing antibiotics were placed in a petri dish containing MHA media and bacterial culture using tweezers, then incubated at 37C for 24 hours. Then the diameter of the inhibition zone around the paper disk was measured using a caliper or ruler (Hadijah, 2021).

RESULTS

The sensitivity response test of Staphylococcus aureus bacteria to antibiotics amoxicillin, co amoxiclav, penicillin, ampicillin, and cefadroxil by disc diffusion method using concentrations of 15, 20, 25, 30 g,and negative control with three replications, shows the results as shown in table 5.1.

Table 1. Result of Measurement of Inhibitory Zone Diameter for Staphylococcus aureus growth after being given antibiotics

	Average Inhibition Zone Diameter (mm)
Treatment	Amp
15 µg	26,7
20 µg	27,2
25 µg	28,2
30 µg	29
Category	Sensitive

description : Ampisilin (Amp)

Based on the data above, it can be seen that the diameter of the inhibitory zone after being given antibioticswith various concentration levels showed a sensitive category to the antibiotic Ampicillin with a concentration of 30 g, the average diameter of the inhibition zone was 29 mm.

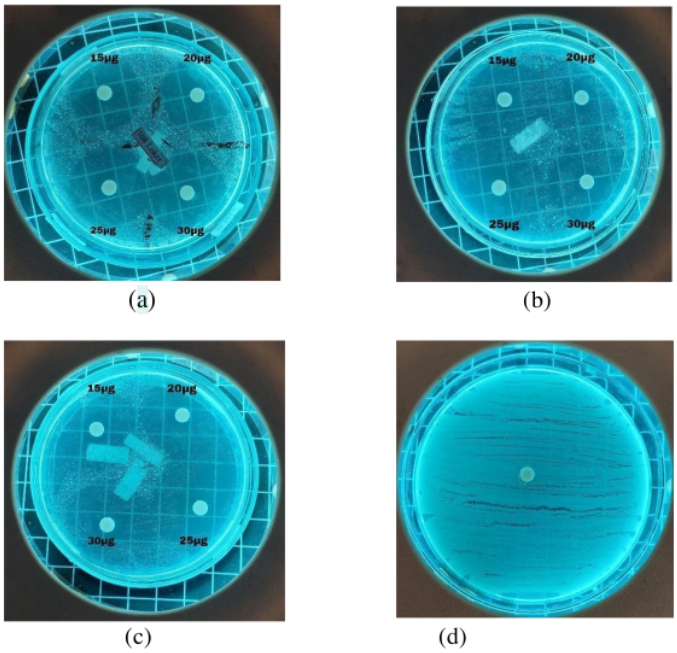

Figure 1. Replication 1 (a), Replication 2 (b), Replication 3 (c) and Negative control (d)

DISCUSSION

This study aims to determine the sensitivity response of S. aureus bacteria to antibiotics in the class of inhibitors of bacterial cell wall synthesis. The antibiotic used in this study was Ampicillin. According to Fatimah *et al*. (2022) Ampicillin is an antibiotic that is very effective in inhibiting the growth of Staphylococcus aureus and other gram-positive bacteria. This is reinforced by the statement Jamilatun (2019) in his research showed that the sensitivity test of several antibiotics against several isolates of S. aureus bacteria gave results, namely that among all isolates were sensitive to Ampicillin with a concentration of 10 g. This indicates that with a small concentration of Ampicillin still able to inhibit the growth of S. aureus. This statement is supported by Fatimah *et al*. (2022) that in his research, the results of the antibiotic Ampicillin had better sensitivity and were still more effective in inhibiting S. aureus bacteria.

After experimenting with the antibiotic Ampicillin in table 1, it is known that the results obtained from the measurement of the diameter of the inhibitory zone of the antibiotic Ampicillin at a concentration of 15 g obtained an average inhibition zone of 26.7 mm, a concentration of 20 g was 27.2 mm, a concentration of25 g was equal to 28.2 mm, and the concentration of 30 g was 29 mm. From the measurement data that has been obtained, it is known that the antibiotic Ampicillin on S. aureus bacteria all showed sensitive results using graded concentrations.

The interpretation of the results of the category of bacterial growth response to the antibiotic Ampicillin in this study, especially for the disc diffusion method, was based on the formation of a clear zone or inhibition zone around the paper disc compared to *National Community for Clinical Laboratory Standard* (NCCLS) in Fatimah *et al*. (2022) which are grouped into three categories, namely Staphylococcus aureus bacteriaare said to be resistant if the clear zone formed is <15 mm, intermediate is 16-17 mm, and sensitive is 18 mm.

The results of the sensitivity test to the antibiotic Ampicillin showed that S. aureus was sensitive to the

antibiotic ampicillin. According to Sumampouw (2018) that ampicillin is very effective against S. aureus because both antibiotics belong to the semisynthetic beta-lactam chemical class which is effective in inhibiting gram-positive by inhibiting cell wall synthesis and murein production.

Several studies on antibiotic sensitivity tests gave different results. Based on this, it proves that the pattern of bacterial sensitivity differs from one area to another. Different habitats or sources of S. aureus bacteria allow different sensitivity characters to each antibiotic. This is in accordance with and reinforced by Bhavya's (2014) statement which says that bacterial resistance to antibiotics varies widely in an area because antibiotics have different sensitivities depending on the environment and the bacterial isolates themselves.

Staphylococcus aureus is one of the gram positive bacteria. The cell wall of gram-positive bacteria is composed of very thick peptidoglycan to maintain the integrity of the cell. If there is damage to the cell wall during its formation, bacterial cell lysis will occur so that the bacteria lose the ability to form colonies and will die. Inhibiting the formation of cell walls can occur due to the administration of antibiotics (Ajizah *et al*., 2007). In research Kurniawan *et al*. (2019) added that positive bacteria have cells that are pretty simple stuff so that's what makes it easier for antibiotics to get into cells and find targets to work on.

Antibiotics of the class of inhibitors of bacterial cell wall synthesis have a mechanism of action by preventing cross-linking of peptidoglycan during the final stage of cell wall synthesis, by inhibiting bound proteins. When the antibiotic experiences resistance, what happens is that the bacteria produce beta-lactamase enzymes in gram-negative or gram-positive bacteria which then secrete the beta-lactamaseenzyme to exit the cell in large enough quantities so that the drug that wants to penetrate the cell wall becomes inactive (Sagita *et al*., 2020).

In this study, the method used is disc diffusion. This method is done by applying a bacterial suspension thathas previously been made. The bacterial suspension was made by dissolving the bacteria into NaCl 0.9 and then equalizing the turbidity with Mac Farland 0.5. The process of balancing the bacterial suspension with Mac Farland 0.5 with the aim of estimating the number of bacteria in the liquid suspension by comparing the turbidity. Mac Farland standard 0.5 is the most commonly used standard in clinical microbiology laboratories with an estimated bacterial count of 1.5 x 108 CFU/ml (Aviany dan Pujiyanto, 2020).

CONCLUSION

Sensitivity testing using the Kirby Bauer method on the antibiotic Ampicillin, with concentrations of 15 g, 20 g, 25 g, and 30 g showed a sensitive category. Ampicillin antibiotics obtained the largest average diameter results, namely at concentrations of 15 g, 20 g, 25 g, and 30 g, the average inhibition zones were 26.7 mm, 27.2 mm, 28.2 mm, and 29 mm. Based on the results obtained, it can be concluded that the antibiotic Ampicillin is still effective in inhibiting the growth of Staphylococcus aureus and the use of higher concentrations will result in a larger diameter of the inhibition zone.

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