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Research Article

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Detection of R plasmid-mediated antibiotic resistance in *Staphylococcus aureus* bacteria of MRSA from Jayapura, Papuan-Indonesia

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ABSTRACT

Bacteria originating from the hospital environment could lead to infection on humans and have a high risk of nosocomial infection. This study aims to identify and detection of DNA plasmid in Staphylococcus aureus (S. aureus), which comes from the hand swabs and swab clinical nurses in hospitals of Jayapura, Papuan Province of Indonesia. In this study were found 96 isolates of S. aureus. Identification of S. aureus conducted through fermentation test of mannitol salt agar (MSA), gram staining, coagulase test and catalase test. Here, we showed that all isolates of S. aureus were able to ferment MSA, coagulase and catalase positive test. Results of resistance on 96 isolates of Staphylococcus aureus on sensitivity test to antibiotics oxacilin 89.6%, vankomicin 47.9%, ampicillin 79.2%, tetraciklin 46.9% and gentamicin and norfloxacin are 0%. Results of plasmid isolation and electrophoresis test, in get 3 bands with the size of 9.5 kb DNA using Lambda Hind III DNA as marker. From the results of this study concluded that the high levels of Staphylococcus aureus bacteria resistant to antibiotics and allegedly mediated by plasmid DNA at Jayapura hospital, Papua-Indonesia.

Keywords: Plasmid R, Resistance, Antibiotics, *Staphylococcus aureus*, MRSA, and Hospital of Jayapura

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INTRODUCTION

The hospital is a place where illness peoples are treated. In this place patients receive therapy and treatment to be cured. However, hospitals in addition to getting a cure, is also a source of infection for various diseases, especially diseases caused by bacteria, which is a major cause of infectious diseases. Bacteria can lives and thrive in a hospital environment, such as water, air and floor [1-2].

Nosocomial infections are infections acquired during hospitalization. Nosocomial infection was found in patients after 48 hours of hospitalization. Nosocomial infections are mostly caused by microorganisms that are resistant to antibiotics, even by microorganisms that are resistant to many types of antibiotics (*multi-drug resistant infection*) or microorganisms that have been resistant to all existing antibiotics (*pan-resistant infection*). One cause of nosocomial infections, which has been widespread throughout the world is an infection caused by the bacteria *Staphylococcus aureus* [3-4].

Staphylococcus aureus is a bacterium shaped gram-positive cocci including family of *Micrococcaceae*, has a circular chromosome genome about 2,800 kb, containing plasmids and chromosomes. *Staphylococcus aureus* has been known since the 19th century as the cause of local and systemic infections. In the mid 20th century, the problem of infection caused by the bacterium *Staphylococcus aureus* has successfully treated with beta-lactam class of

antimicrobials that is penicillin. Penicillin beta-lactam antimicrobials bind binding protein (PBP) is a membrane peptidase enzyme which catalyzes the reaction transpeptidation the bacterial cell wall synthesis process [4]. Beta-lactam bond in the active site serine resulted in PBP is not active, cell wall synthesis failed and bacteria through lysis. Staphylococcus aureus has four kinds, namely PBP PBP 1 weighing 85 kDa, PBP2 weighing 81 kDa, PBP 3 weighing 75 kDa and PBP4 weighing 45 kDa. PBP1, 2 and 3 have transpeptidase activity and has a very high affinity for beta-lactam, thus giving beta-lactam antimicrobials will cause lethal for *Staphylococcus aureus* [5].

Four years since antibiotics began to be produced and marketed massive, emerging bacteria that are resistant to penicillin. The first bacteria resistant to penicillin was *Staphylococcus aureus*. This bacterium is actually a normal flora of the body, but can cause diseases such as pneumonia if growth is not controlled or due to toxins.

Therapeutic efficacy against *Staphylococcus aureus* infections that do not last long because then appeared strains resistant plasmid containing a gene *bla*Z. Blaz gene encodes an betalactamase enzyme that is an enzyme capable of degrading penicillins by breaking the beta-lactam ring. The end of the 1950s, the problem of resistance to beta-lactam can be overcome by administering antimicrobial resistant to methicillin betalactamase ie. *Staphylococcus aureus* isolates were sensitive to methicillin-called *Methicillin Sensitive Staphylococcus aureus* (MSSA). After the use of antibiotics methicillin during one year in the hospital, the problem of resistance resurfaced with the discovery of methicillin-resistant *Staphylococcus aureus* isolates called Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Staphylococcus aureus* MRSA strains were found to also have resistance to multiple antimicrobial properties or are multi-resistant. As a result of multi-resistant MRSA infection is the selection for antimicrobial therapy is becoming increasingly difficult [6].

The causes of resistance in *S. aureus* due to by genes that encode antibiotic resistance trait found in chromosomal DNA and DNA outside the chromosome (plasmid). Plasmids are circular DNA molecules are relatively small on the outside of the chromosomes present in prokaryotic cells, especially bacteria. Genes contained in plasmid in general are not essential for the growth and survival of individual bacteria, but often encode protein synthesis for antibiotic resistance [7].

Plasmids present in the cytoplasm of prokaryotic organisms and simple unicellular eukaryote. Plasmids can be grouped based on the nature of that encoded by the genes they contain, namely: *first*, Plasmid F (fertility) carrying the gene *tra*, which is responsible for the process of conjugation; *second*, Plasmid R (resistance) contain resistance genes against antibiotics or heavy metals; and the third plasmid containing genes encoding toxins and bacteriocins [8].

The results of previous studies, identification of bacterial isolates from the air and the hands of nurses showed that the dominance of species of bacteria are *Staphylococcus epidermidis* respectively 51.79% (29 isolates), *Staphylococcus aureus* 23.21% (13 isolates), *Lactobacillus sp* 17.86% (10 isolates), *Pseudomonas aeruginosa* 5.36% (3 Isolate), *M. luteus* 1.79% (1 Isolates), and 100% of *Staphylococcus aureus* has obtained are resistant to methicillin [9-10].

Based on the above, it has carried out a study to analyze the causes of resistance in *Staphylococcus aureus* bacteria found in the environment General Hospital Dok II Jayapura. This study focused on the detection of R-plasmids that are expected to get an overview electrophoresis on plasmid DNA that mediates resistance to antibiotics on *Staphylococcus aureus* MRSA strains in the environment of the Regional General Hospital (Hospital Dok II), Jayapura, Papua Province, Indonesia.

EXPERIMENTAL SECTION

RESEARCH METHODOLOGY

Places of sampling done in Hospital of Dok II, Jayapura, Papua, Indonesia. The population in this study is the identification of *S. aureus* bacterial culture obtained from Hospital Environment (Swab hands health officer and clinical isolates) Jayapura. The sample in this study was the identification of *S. aureus* bacterial culture obtained from Hospital Environment Jayapura (96 samples). The sampling method is performed as follows:

$$\mathbf{n} = \frac{z\alpha^2 \mathbf{PQ}}{d^2}$$

where:
P = The proportion of isolates or circumstances to be searched (0.50)
d = The absolute level of accuracy preferred (10%)
α = level of significance (0.025), zα = 1.960 (viewed from distribution table/z)
Q = 1-P

$$n = \frac{(1.960)^2 x\ 0.5\ (1 - 0.5)}{0.1^2}$$

$$n = \frac{0.9604}{0.01}$$

$$n = 96$$

Samples were grown on media fertilizer (*NaCl broth*) and purified on Media Blood To Plate (BAP), the incubation temperature of 37 °C for 24 h. Having seen the growth of the colony and haemolysis on BAP, then carried gram stain, catalase test, test and test fermentation mannitolt coagulase in colonies that grow [11].

Catalase test

Catalase testing is a way of identifying bacteria by dripping liquid H_2O_2 3% in the bacterial suspension on the slide. Catalase test is positive if there is an air bubble in the suspension of bacteria which drops of H_2O_2 become H_2O and O_2 by the catalase enzyme.

Mannitol fermentation test

Mannitol fermentation test conducted by growing colonies of *Staphylococcus sp* on MSA media, then incubated 37 °C for 24 h, where bacteria can grow and positive results occur mannitol fermentation media color change from red to yellow. Mannitol fermentation is characteristic of *S.aureus*.

Coagulase test

Coagulase test conducted by making a suspension of bacteria using 0.9% NaCl on a glass slide. Bacterial suspension was mixed with plasma citrate (9 parts of plasma: 1 Sodium citrate 3.8%), then shaken and after 19 sec, check whether there is coagulase to see whether there is a white blob on the slide. If the negative can be confirmed by coagulase test tube method. How to tubes made by inoculating colonies growing on MSA media to media BHI (*Brain Heart Infution*), after incubation at 37 °C for 24 h. Then coupled plasma citrate, then incubated back at 37 °C for 24 h. Positive results in case of agglutination or clots.

Sensitivity test

Examination of bacteria to antibiotics sensitivity test is performed with sensitivity test. This study uses the disk diffusion method, the bacteria used in this method is to have turbidity in accordance with the standard Mac Farland No. 0.5-1. Once accordance with the standard turbidity, inoculated 0.2 mL suspension in the MHA sample, then place the paper disk antibiotics, Tetracycline 30 µg, Kanamycin µg and Erythromycin µg. Incubate 37 °C for 24 h, the test results of this method, shown by the clear zone/clear around the paper discs, as the obstacle area (zone of inhibition) the growth of germs. Zone of inhibition results were analyzed using a standard interpretation of inhibition zone Beuer Kirby. Results are interpreted according to the diameter contained in Table LCSI. At 5 µg methicillin discs, resistant *S.aureus* methisilin have growth inhibitory zone diameter ≤9 mm, while methicillin-sensitive S. aureus have a growth inhibitory zone ≥14 mm.

Isolation of plasmid DNA

The first step is to get a plasmid DNA by growing bacterial cells containing the recombinant plasmid. After the cells were harvested, walls and cell membranes are broken down so that the contents of the cell (cell extracts) out. Cell extract is then purified by a series of treatments in order to obtain pure plasmid DNA. Isolation of DNA were performed in this study according to standard procedures that are commonly performed. Homogenization of cells with this procedure will result in DNA intact as this process causes cell distruption and cleaned the cell components other than DNA. Addition of chloroform after centrifugation to separate the solution into liquid and solid phase wherein the liquid phase is DNA and solid phase is a mixture of proteins and DNA [11-13].

Electrophoresis of plasmid DNA

The isolated plasmid then electrophoresed in 1% agarose dissolved in 1x TAE buffer, with the following procedures: Agarose 1% (0.3 g and 30 ml of buffer TAE) where TAE buffer consist of Tris-HCl pH 8.3 40 mM, acetic acid concentrated 1.98 mM, 1 mM EDTA).

RESULTS AND DISCUSSION

The identification results are derived from the hands of nurses swab samples, and swabs were derived from clinical samples, obtained Staphylococcus aureus were 96 isolates with characteristics of the gram staining spherical shape (cocci), flocking, gram-positive (purple) and positive on the test catalase (air bubbles) and coagulase (clots

agglutination). In the MSA can memfrementasi mannitol media were the mark with the the growth of bacterial colonies are yellow.

Based on result of research, sensitivity pattern of *Staphylococcus aureus* to antibiotics oxacilin, vankomicin, ampicillin, tetraciklin, gentamicin and norfloxacin. In Table 1, we can see the bacteria *Staphylococcus aureus* is still sensitive to the antibiotic gentamicin and norfloxacin. Previous research, in hospitals Abepura, Papua Province, most bacteria found are of species *Stapylococcus aureus* (37%) with a high level of resistance and the identification of bacterial isolates from the air and get the hands of nurses in *Staphylococcus aureus* (13 isolates) and 100% were resistant to methicillin.

Table 1. Results of antibiotic sensitivity test

Antibiotics	Resistance levels (%)	Sensitivity level (%)
Oxacilin	89.6	10.4
Vancomicin	47.9	52.1
Ampicilin	79.2	20.8
Tetracicline	46.9	53.1
Gentamicin	0	100
Norfloxacin	0	100

Fig 1. Colonies of bacteria *Staphylococcus aureus* on MSA media

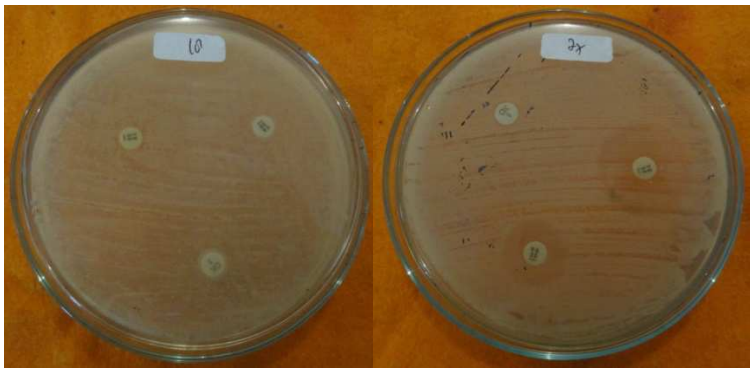

Fig 2. The results of antibiotic sensitivity test on the bacteria *Staphylococcus aureus*

From the results obtained three DNA electrophoresis with size of each approximately 9.5 kb by using Lambda Hind III DNA as a marker on electrophoresis test (Fig. 3). In the previous study, obtained 64 isolates of bacteria, which is found 33.9% were resistant to antibiotics and 7 isolates of which were *Staphylococcus aureus* colonies with characteristic are yellow and coagulase test positive. And the electrophoresis test obtained two DNA plasmids with a size of 1.5 kb [11].

DNA plasmid of the bacterium *Staphylococcus aureus* has categorized into three classes. Class I plasmids with a size of 1-5 kb and occur in large amounts (*high copy number*). Class II is a plasmid with a medium size (*intermediate copy number*) and usually encodes for beta-lactam class of antibiotics, and Class III plasmid with 40-50kb size, this plasmid carries multi-resistance trait (*multiple resistance determinants*) [4].

Fig 3. Results of electrophoresis of three DNA plasmids found with a size of 9.5 kb. By using the lambda *Hind* III DNA as marker

MRSA is a strain of *Staphylococcus aureus* that are resistant to beta-lactam antibiotics, including penicillin and its derivatives (Methicillin, Oxacilin, dicloxacilin, Nafcilin and Sephalosporin). Methicillin is an antibiotic with a narrow spectrum beta-lactam group. Methicillin was introduced in 1959 to combat *S. aureus* resistant gram-positive bacteria producing betalactamase. How it works closely with beta-lactam antibiotics generally only methicillin-resistant betalactamase enzyme and inhibit the formation of bacterial cell wall synthesis end peptidoglycan transpeptidase facilitated known as Penicillin Binding Proteins (PBP). This antibiotic binds to PBP2 thus inhibiting peptidoglycan and eventual lysis. MRSA occurs because of changes PBP2 be PBP2a that in the coded by the mecA gene so that this Methicillin low affinity that causes the bacteria can not bind to PBP2a until the final stage of peptidoglycan formation is not disturbed and the bacteria become resistant [14].

Most of the genetic information encoded by the bacterial chromosome, but not all. Some bacteria have genes either extrachromosomal plasmid (DNA double strand twisting each pair measuring 2-200 kb) or bacteriophages (bacterial viruses that can be integrated into the bacterial chromosome). Extrachromosomal genetic elements can be transmitted vertically (from bacteria to their offspring through binary fission) and more importantly the horizontal transmission that can traverse the species and genus. Transferred the resistance factor can last from chromosome to a plasmid or vice versa at transposons and integron. Not every plasmid can be transferred. Which can be moved is the R factor plasmid, called plasmids transmitters (infectious plasmids). R factor itself consists of two units: the segment transfer resistance factor (RTF) and determinant-r (unit-r).

RTF segment allows the transfer factor R. Each unit-r carrying properties of the antimicrobial resistance. Thus the various units of the 1-r on R factor plasmids carrying the resistance to various antimicrobial properties as well. R factor is transmitted primarily among enterobacteria. Transposon an individual gene or a small group of genes resistant bound directly reverse or repeat DNA sequences (*like bookends*). Integron consists of two segments of DNA where on one side there is a gene that is resistant to antibiotics. Bacteria that are resistant to many antibiotics is caused by plasmid who develop resistance or the presence of multiple genes within the chromosome that carries the nature of resistance [15].

MRSA resistance to beta-lactam antimicrobial class caused by these bacteria have a protein mutant penicillin-binding protein 2a (PBP2a or PBP2') which is encoded by the mecA gene. PBP is a group of enzymes in the cell membrane of *S. aureus* transpeptidation to catalyze the formation of webbing (*cross-linkage*) peptidoglycan chains. PBP2a affinity for beta-lactam class of antimicrobials is so low that MRSA will remain alive despite the antimicrobial exposure in high concentrations.

MRSA resistance detection can be conducted by using oxacillin disk method (not methicillin). Oxacillin is used as a chemical class with methicillin, more stable, test results between methicillin and oxacillin together and at the moment methicillin is no longer produced commercially in the market so that there is oxacillin [5].

Resistance is the ability of bacteria to neutralize and weaken the antibiotics. This can happen in several ways, namely: damage to the enzyme produced antibiotics, antibiotics alter receptor capture point, change the physico-chemical antibiotics target bacterial cells, antibiotics can not penetrate the cell wall, due to the changing nature of the bacterial cell wall and antibiotics entry into bacterial cells, but was immediately removed from the cell through an active transport mechanism to the outside of the cell. To be able to show activities as a bactericidal or bacteriostatic, antibiotic should have some properties as follows: Activities microbiology antibiotics should be tied to specific binding sites (eg, ribosomes or penicillin binding proteins). Levels of antibiotic at the site of infection should be high enough. The higher levels of antibiotics, the more places bondage in bacteriology cells. Antibiotics should remain in place for a considerable time bondage is sufficient to effect adequate obtained. Minimal inhibitory concentration. These levels describe a minimal amount of drug needed to inhibit the growth of bacteria [18-22].

CONCLUSION

The causes of resistance in *Staphylococcus aureus* bacteria in hospital of Dok II Jayapura, Papua is the role of DNA plasmid R. It can be proved by the discovery of three DNA bands in the electrophoresis test with a size of approximately 9.5 kb. Bands obtained plasmid DNA into class II, in which Class II is a plasmid with medium size and usually contain a gene which codes for antibiotic inhibits the action of the beta-lactam group. Advanced research paths proposed is to sort and find mutations in the DNA of the bacteria *Staphylococcus aureus* isolates in Papua and compare it with the data on the sequence of nucleotides that have been published in GenBank.

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