Detection of *Staphylococcus aureus*'s Strain Similarity on Surgical Ward Nurses's Hand and Nose and Post Operative Wound Infection Using *Coa* Gene Through PCR-RFLP Method

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ABSTRACT

Staphylococcus aureus (S.aureus) remains to be the most important cause of post operative wound infection. Nurses could become reservoirs to transmit *S.aureus* through contaminated hands transiently, or through colonized nose. Strain polymorphism could be determined by Restriction Fragment Length Polymorphism (RFLP), using *coa* gene and restriction endonuclease enzyme *Alu1*. There were 30 isolates of S.aureus's infection, and 20 isolates taken from hands and nose of the nurses in charge. From 50 isolate positive *S.aureus,* PCR results showed single and multiple bands within 300 to 900 base pairs (bp) in length, and multiple bands within 200 to 600 bp. Five out of 30 patients (17%) showed no PCR-RFLP similarity with any of the nurses. Ten out of 15 nurses which hands were positive for *S.aureus,* has PCR-RFLP similarity with some patients. There was only 1 out of 5 nurses which nose was positive for *S.aureus,* showed PCR-RFLP similarity with some patients. Statistically, the proportion of the similar PCR-RFLP between those samples in this study is 0.12 (12%). Conclusion: Nurses had 12 % PCR-RFLP similarity for *S.aureus* with post operative wound infection.

Keywords: post operative wound infection, similarity of strain, *S.aureus*, *coa* gene, PCR-RFLP

Among different types of nosocomial infection, post-operative wound infection occurs most frequently, with a rate of 8-17% of overall infection rate in hospitals in developed countries.[1,2] *National Nosocomial Infections Surveillance (NNIS), Central of Diasease Control (CDC),* reported that *S. aureus* with negative coagulation *Staphylococcus* and *Enterococcus* consist of 34% of overall nosocomial infection rate in developed countries. [1] In this group, *S. aureus* is the major cause of post-operation wound infection, with 19% out of 56% occurrence of post-operative wound infection. [2]

Post-operative wound infection reservoir could occur in human or contaminated objects.[1,2,4] This reservoir determines nosocomial infection transmission modes:

1. Through direct contact: i.e. from medical staff to the patients, from patients to patients, or from visitors to patients.
2. Through indirect contact: i.e. from contaminated objects or hospital ventilation system[1].

Nurses could transmit organism through direct contact to the patients, particularly during medical treatment. In *S. aureus* post-operative wound infection, transmission happened through contaminated hands transiently or through contaminated nose.[1,4] The transmission occurs after contact with infected patients, contact with contaminated equipments, or contact with carriers.[2,4]

In epidemiology, it is important to determine the reservoir or modes of transmission and this requires various laboratory examinations that determine similarities among different infection-causing strains.[4] In order to determine strain similarities, it is necessary to conduct an examination with highly accurate differentiation or strong polymorphism, which only can be performed through genotypic molecular examination. [4,7]

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Polymorphism may occurs from mutation and causes difference in single property. However, it does not affect genetic production [8]. RFLP (restriction fragment length polymorphism) [9,10] determines variation of a species and differentiated various strains, which is conducted through polymerase chain reaction (PCR) to determine the microorganism types at genetic level which is fast, easily available and affordable. [5,7,10,12,13,14,15,16]

The PCR-RFLP method utilizes chromosomal genes. In the case of *S. aureus*, this method utilizes the *coa* gene that code protein coagulation. The *coa* gene are the genetic marker with slow evolutionary process and possess repeating regio-polymorphic, which differentiate *S. aureus* strain. This gene is also an important virulence factor and has been utilised as epidemiology marker in *S. aureus*.[12,16,18] Genetic sequencing analysis of *coa* gene shows regio polymorphic that ends at 3' end coding region, and this region contains 81 bp tandem Short Sequence Repeat (SSR) with variable number and sequence. By determining the size and site of its restriction, this method can be utilised to analyse PCR-RFLP *S.aureus*.[11,14,15,17]A restriction endonuclease enzyme that works best in giving the depiction of polymorphism is *Alu1*.[11,14,15]

Nurses are an important component in transmission of S. aureus post-operative wound nosocomial infection. This can be further investigated through PCR-RFLP profile through *coa* gene examination, in order to detect the strain similarities. It is important that nurses are well informed about the potentials of bacterial reservoir in order to be more vigilant in handling the patients.

METHODS

The subject of this research is the speciment from the hands and nose of the nurses from third class male surgery ward, as well as speciments from the post-operative wound infection from patients in the third class male surgery ward at Hasan Sadikin General Hospital in Bandung Indonesia. Both patients and nurses must fulfill the criteria for inclusion. According to the statistical requirements, the sample number must be at least thirty patients. The number of nurses is according to the ones who take care of the patients, and must fulfill the critera for nurse's inclusion, and hence twenty nurses are taken as samples. In this study, hand'swab before nurses did the handwashing procedure had not been taken.

The method of this research is cross-sectional with proportion testing analysis.

Identification of *S. aureus* Using Conventional Method

The speciment used is swab taken from the hands and nose of the nurses after hand-washing, and post-operative wound infection in the patients. Examination are done through microsopic Gram stain, culture, and identification of colony in the blood agar plate, catalase examination, and slide coagulation test. If the coagulation slide test result is negative, subsequent tube coagulation test is conducted.

Identification of *S. aureus* Using Molecular Method

A subsequent molecular examination is done in each speciment with positive *S. Aureus* result. For each isolated *S. aureus* samples the following method is conducted:

- DNA Isolation

After culturing in Luria Bertani broth, DNA isolation is conducted using Wizard Genomic DNA Purification Kit from Promega, which consists of EDTA 50 mM pH 8.0, Lysozyme 10 mg/ mL, Isopropanol, Ethanol 70%, nuclei lysis solution, protein precipitation solution, DNA rehidration solution

- PCR Amplification of *coa* gene

PCR Primer design for *coa* gene uses basic nucleotides with access No X16457[14,24]. Forward primer 5' ATA GAG ATG CTG GTA CAG G3' and reverse primer 5'GCT TCC GAT TGT TCG ATG C3' are used. Each amplification process is done in sterile eppendorf tube, in which each reaction consists of DNA template 3 ìL and 0,5 ìL for each

of the primer, buffer 2,5 ìL, Mg2+ 3 ìL, dNTP 0,5 ìL, Taq polymerase 0,125 ìL, and ddH2O 14,9 ìL to achieve the final volume of 25 ìL. Thermalcycler is used with PCR condition of 94 °C initial denaturation for two minutes. A 30-cycle amplification is conducted with 94°C denaturation for one minute, annealing 42°C for one minute, and elongation 72°C for one minute. Subsequently, a post-elongation procedure is done with 72 °C for 10 minutes and storage in 4°C. PCR product measurement (5 ì L aliquot) is determined through 100 bp ladder marker comparison in agarose gel electrophoresis of 2%.

- Endonuclease DNA restriction analysis in *coa* gene resulting from PCR amplification

The PCR products are purified through sedimentation in ethanol precipitation, and agarose gel electrophoreses is conducted to approximate DNA concentration by comparing it with *PUC19/ Hindf1* marker. Approximately 800 ng (15-20 ìL) of PCR product is cut with 0.1 U restriction endonuclease enzymes *Alu1* and placed in 1.5 mL sterile tubes and stored for one day in water bath of 37°C. Ten ìL restricted PCR products are analysed through agarose gel electrophoresis in order to obtain RFLP mapping.

- Data Analysis

For each patient with similar PCR-RFLP mapping with the nurses, identification and subsequent proportion test analysis are conducted

RESULT

The research is done from June to November 2005.

- Patient and Nurse Characteristics

There are 86 (32 %) patients with post-operative wound infection from a total of 269 patients during the course of the study. From all the infected patients, 35% is caused by *S. aureus*, with 20% from general surgery and 15% from orthopaedic surgery. Additionally, there are 20 nurses positively affected with *S. aureus* from a total of 26 nurses. Among the 20 nurses, 19 % has positive nasal *S. aureus*, while 58% has from hands. There is no nurse with positive *S. aureus* in both hands and nose.

- PCR-RFLP Pattern

There are a total 50 *S. aureus* isolates, consisting 30 patients isolates and 20 nurses isolates. The isolates resulted in PCR product (with exception of strain *S.epidermidis* ATCC 12228 as negative control) with varied sizes between 100 bp to 2000 bp. The *Alu1* restriction resulted in single or multiple DNA fragments. (Figure 1 A dan B).

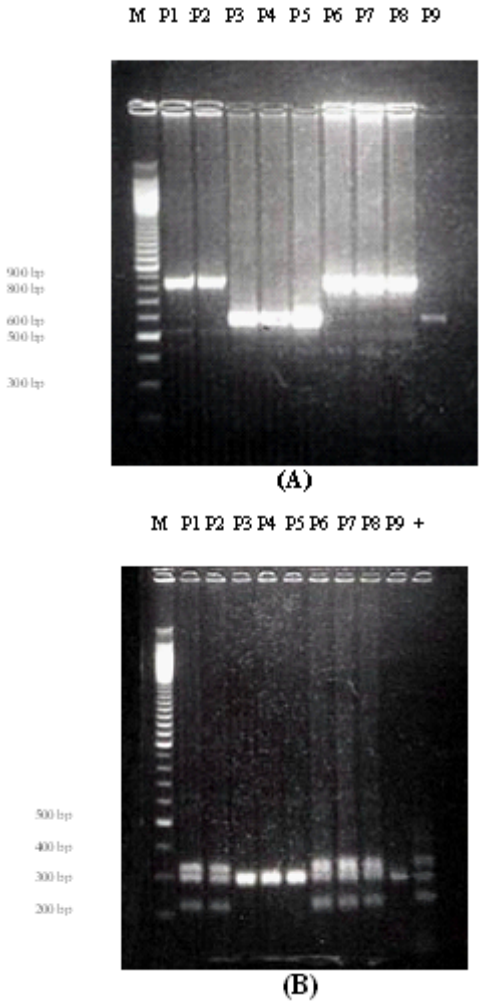
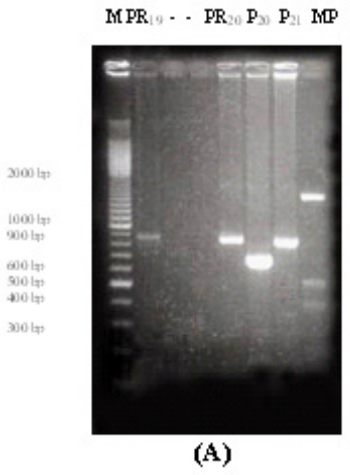


Figure 1 PCR-RFLP *Coa* Gene mapping with restriction endonuclease enzyme *Alu1* in 9 patients. (A) PCR results before restriction. (B) After restriction (RFLP). M = Marker 100 bp *ladder* ; P = patients; - = negative controls; + = positive controls

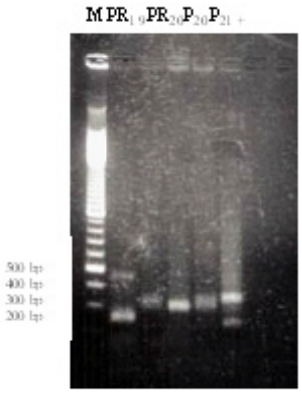
There are seven PCR product pattern, and there are 12 resulting PCR-RFLP mapping with most pattern is found in 15 samples with single-band PCR (nearing 600 bp) and single-band RFLP (nearing 300 bp)

PCR-RFLP Mapping in Patient and Nurse

From 30 Patients, five (15%) did not show similarity of PCR-RFLP mapping with any nurse. Moreover, among the nurses with positive *S. aureus* in their hands, ten nurses has similar PCR-RFLP mapping with their patients. Additionally, among the five nurses with positve *S. aureus* in their noses, only one nurse has similar PCR-RFLP mapping with the patients (Figure 2A and B)



(A)



(B)

Figure 2 PCR-RFLP *coa* gene mapping with restriction endonuclease enzyme *Alu1* in 2 patients (P) and 1 nurse (PR). (A) PCR results before restriction: Marker 100 bp ladder, PR19, - control, - control, PR20, P20, P21, Marker *PUC19/Hindf1*. (B) After restriction : Marker 100 bp ladder, PR19, PR20, P20, P21, + control.

Post-Operative Wound Infection Analysis and *S.aureus* Detection in Patients

S.Aureus causes post-operative wound infection in 35% of patients from the total of post-operative wound infection cases (20% general surgery cases and 15% orthopaedic surgery cases). Djojosugito 1989 reported that *S.aureus* is the main cause of post-operative wound infection, among the positive gram bacteria group (16.5% general surgery and 25.3 % orthopaedic surgery) [3] , and this research shows an increase in general surgery infection rate, but a declining in orthopaedic surgery infection rate.

Analysis of *S.aureus* Detection in Nurses

Five nurses (19% of total nurse) are positive *S.aureus* in the nasal area while fifteen nurses (58%) are positive in the hands. This result shows ineffectiveness in hand washing procedure prior to wound treatment in decreasing *S.aureus* colonization rate. This research supported previous research by Roosyati 1998[6], in which most *S.aureus* is found in the hand area of the nurses (9.3%).

Analysis of PCR-RFLP Pattern

Several PCR result shows multiple PCR product, and this shows different result from the research of Hookey et. al [14], in which all isolates reveals single PCR product. However, this research shows similarity to Goh et.al[15], in which many isolates resulted in multiple PCR product.

Twelve PCR-RFLP patterns from restriction result of 7 PCR patterns shows that the *Alu1* enzyme can differentiate *S.aureus* strains, and this supports both Hookey et.al[14] and Goh et.al[15].

Statistical Analysis

Based on the PCR-RFLP mapping of both the patients and the nurses, we can obtain the proportion result and its average. The average proportion is 12%

for 30 patients with similar PCR-RFLP mapping with their nurses.

The test for significance shows that H_0 is not rejected since t count < t table. This means that the similarity of PCR-RFLP mapping in *S.aureus* isolates obtained from the hands and noses of the nurses and the ones obtained from the patients is less than 50%.

PCR-RFLP Analysis in Patient and Nurses

Most nurses who have similar PCR-RFLP mapping with the patients positively have *S.aureus* in their hands. Although the nurses has performed hand-washing each time they begin to treat different patients, it seems that this procedure has not been effective in preventing *S.aureus* transmission.

Proportion test analysis and its significance show that nurses with similar PCR-RFLP mapping with the patients is 12 % (with average proportion less than 50%). Therefore, from 20 nurses with positive *S.aureus* (58% in the hand area and 19% in nasal area), only 12 % has similar PCR-RFLP mapping with post-operative wound infection which was caused by *S.aureus*.

Seven patients has similar PCR-RFLP mapping with nurse 3. This means that the nasal area of nurse 3 tend to become carrier that contracts *S.aureus* infection, and this conforms with the theory stating that the nasal area of the hospital worker potentially act as transmission base of *S.aureus* to the patient.

Besides, based on the available data, there are three patients in the same ward with adjacent beds, and all had post-operative wound infection on the third day after opening the wound covering. While the PCR-RFLP mapping of all the three patients are not the same, no similar PCR-RFLP mapping with the nurse is found. Therefore, the infection in the three patients may have occurred from other carrier such as contaminated operating instrument, etc.

Sample from one nurse shows similar PCR-RFLP mapping with five patients. Based on the available data, three out of five of the patients are in the same ward with adjacent beds, and the three patients had post-operative wound infection on the fourth day after opening the wound covering. This means that the hand-washing procedure conducted by the nurse has not been effective and *S.aureus* was transmitted to the patient through the nurse's hand.

This research does not prove the actual transmission direction, whether it occurred from the patient to the nurse or vice versa. The available data only shows similarity of PCR-RFLP between the *S.aureus* strain found in the nurse and in the patient.

CONCLUSION

The nurse has 12% similarity in PCR-RFLP mapping with the patient. For further research, it may be necessary to examine other possible *S.aureus* post-operative wound infection transmission sources with PCR-RFLP method using *coa* gene in order to find similarities between the different sources.

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