Proc ASEAN Congr Trop Med Parasitol. 2008;3:78-83.

Available online at www.ptat.thaigov.net

Detection of Similar *Staphylococcus aureus* Strains on Hands and Nasal Tracts of Surgical-Ward Nurses and in Surgical-Wound Infections, Using *Coa* Gene PCR-RFLP

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ABSTRACT

Staphylococcus aureus is the most important cause of surgical wound infections. Nurses can become reservoirs and transmit *S. aureus* through contaminated hands, or colonized noses. *Alu1* restriction fragment length polymorphism (RFLP) analysis of the coagulase gene (*coa* gene) by polymerase chain reaction (PCR) method could detect similarities between 30 isolates from patients with surgical wound infections and 20 isolates from the hands and noses of the nurses in charge (15 isolates from hands, and 5 from noses). Seven distinct PCR products and 12 distinct RFLP patterns were observed, with most PCR products around 600 bp (15 samples), and most RFLP patterns nearing 300 bp. Five of 30 patients (17%) showed no PCR-RFLP similarity with any nurse. Ten of 15 nurses whose hands were positive for *S. aureus* had PCR-RFLP similarities with some patients. Only 1 of 5 nurses showed PCR-RFLP similarity with some patients. Statistically, the rate of similarity by PCR-RFLP was 0.12 (12%).

Nurses, as potential reservoirs, had 12% PCR-RFLP similarity for *S. aureus* with surgical wound infections. It may be necessary to examine other possible reservoirs of *S. aureus* surgical wound infection with PCR-RFLP method using the *coa* gene, to detect the source of nosocomial surgical wound infections.

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

Introduction

Surgical wound infections account for 8-17% of all nosocomial infection in developed countries [1,2]. Gram-positive organisms cause 56% of surgical wound infections, with *Staphylococcus aureus* causing 19% [2].

In surgical wound infections, people are important reservoirs of common hospital pathogens. Nurses, as healthcare workers in hospitals, can transmit pathogens through direct contact with patients, particularly during medical treatment. In surgical wound infections caused by *S. aureus*, transmission could occur through contaminated hands or noses colonized by this pathogen [1,4]. Transmission occurs through contact with infected patients, contaminated equipment, or carriers [2,4].

In epidemiologic studies of the spread of hospital infections, it is important to determine reservoirs and modes of transmission. It is necessary to identify isolates during the course of the investigation using

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typing techniques that can discriminate (*ie* are able to show differences or similarities between strains of the same species) [4]. This can only be done by genotyping at present [4,7].

S. aureus strains (polymorphism) could be discriminated by RFLP (restriction fragment length polymorphism) [8-10]. RFLP can be analyzed through amplification and subsequent restriction enzyme digestion of the polymerase chain reaction (PCR) product of the *coa* gene [5,7,10,12-16].

The *coa* gene, a genetic marker, has a slow evolutionary process, an important virulence factor, and has been utilized as an epidemiological marker in *S. aureus* [12,16,18]. The 3' end of the coagulase gene contains an 81-bp tandem Short Sequence Repeat (SSR) series, the number of which differs between strains. By determining the size and site of its restriction, this method can be utilized to analyze of *S. aureus* PCR-RFLP [11,14,15,17]. *Alu1* is the best digesting restriction endonuclease enzyme for depicting polymorphism [11,14,15].

By determining the similarities between *S. aureus* isolated from nurses and surgical wound infections using PCR-RFLP *coa* gene patterns, it was possible to identify the risk nurses pose as potential reservoirs of *S. aureus*.

Materials and methods

Bacterial strains

Thirty *S. aureus* isolates were collected from surgical wound infections and 20 obtained from the hands and noses of nurses caring for each patient with that infection, in the hospital surgical ward. Patients and nurses were required to meet the inclusion and exclusion criteria. Standard microbiological methods for identification of *S. aureus* included Gram staining, catalase test, and coagulation test.

DNA extraction for amplification

S. aureus bacteria, which had been cultured overnight, were treated with Wizard Genomic DNA Purification Kit (Promega), which consists of EDTA 50 mM pH 8.0, lysozyme 10 mg/ml, isopropanol, ethanol 70%, nuclei lysis solution, protein precipitation solution, and DNA rehydration solution.

Coa gene amplification

Primers were selected using the gene sequence deposited in GenBank (accession number X16457) [14,24]. The forward primer was 5' ATA GAG ATG CTG GTA CAG G3' (1,513 to 1,531) and the reverse primer 5'GCT TCC GAT TGT TCG ATG C3' (2,188 to 2,168). Each amplification process was conducted in a sterile Eppendorf tube and comprised DNA template 3 μl and 0.5 μl for each primer, buffer 2.5 μl, Mg^{2+} 3 μl, dNTP 0.5 μl, Taq polymerase 0.125 μl, and ddH_2O 14.9 μl was added to a final volume of 25 μl. An initial denaturation at 94˚C for 2 minutes was followed by 30-cycle thermal cycling at 94˚C for 1 minute, 42˚C for 1 minute, and 72˚C for 1 minute, with a final step at 72˚C for 10 minutes. The size of the PCR product (5 μl aliquot) was determined by comparison with a 100 bp ladder marker by electrophoresis on 2% (w/v) agarose gels.

DNA restriction analysis of the PCR-amplified *coa* gene

About 800 ng (15-20 μl) of PCR product were digested with 0.1 U of restriction endonuclease *Alu1* at 37˚C overnight. Ten μl of digested PCR product were analyzed by electrophoresis on 2% (w/v) agarose gel.

Data analysis

For each patient with a similar PCR-RFLP pattern as a nurse, identification and statistical analysis by proportion testing were conducted.

Results

Patient and nurse characteristics

Of a total of 269 patients, 86 (32%) had surgical wound infections during the course of the study. Among the infected patients, 35% were due to *S. aureus*, 20% from general surgery, and 15% from orthopedic surgery. Of a total of 26 nurses, 20 were found positive for *S. aureus*. Among these, 19% were positive for nasal *S. aureus*, while 58% had *S. aureus* on their hands. No nurse was positive for *S. aureus* on both hands and nose.

PCR-RFLP patterns

In all, there were 50 *S. aureus* isolates; 30 patient isolates and 20 nurse isolates. With the exception of

coagulase-negative strain, *S. epidermidis* ATCC 12228, all strains examined produced a PCR amplicon which varied in size between 100-2,000 bp. *Alu1* restriction resulted in single or multiple DNA fragments (Figs 1 and 2).

Seven distinct PCR products and 12 distinct RFLP patterns were observed, with most PCR products near 600 bp (15 samples), and most RFLP patterns near 300 bp.

PCR-RFLP patterns in patients and nurses

Of 30 patients, 5 (15%) did not show similar PCR-RFLP patterns to any of the nurses. However, among the nurses positive for *S. aureus* on their hands, 10 had similar PCR-RFLP patterns to their patients. Of the 5 nurses positive for *S. aureus* in their noses, only one had a similar PCR-RFLP pattern to the patients.

Discussion

Surgical-wound infection analysis and *S. aureus* detection in patients

S. aureus caused surgical wound infections in 35% of patients from the total of surgical-wound infections (20% of general, and 15% orthopedic, surgery cases). Djojosugito reported that, among the gram-positive bacteria, *S. aureus* was the main cause of surgical wound infections in the same hospital (16.5% general, and 25.3% orthopedic, surgery) [3]. The current study showed an increase in the general surgery infection rate, but a decline in the orthopedic rate.

Analysis of *S. aureus* detection in nurses

Five nurses (19%) were positive for *S. aureus* in the nasal area, while 15 (58%) were positive on the hands. This result shows the ineffectiveness of the handwashing procedure prior to wound treatment in decreasing the *S. aureus* colonization rate. This research supported previous research by Roosyati [6], in which most *S. aureus* is found in the hand areas of nurses (9.3%) in the same hospital.

PCR-RFLP pattern analysis in patients and nurses

Most nurses with similar PCR-RFLP patterns as their patients were positive on the hands. Although the nurses had washed their hands before treating the next patient, it appears this procedure was ineffective

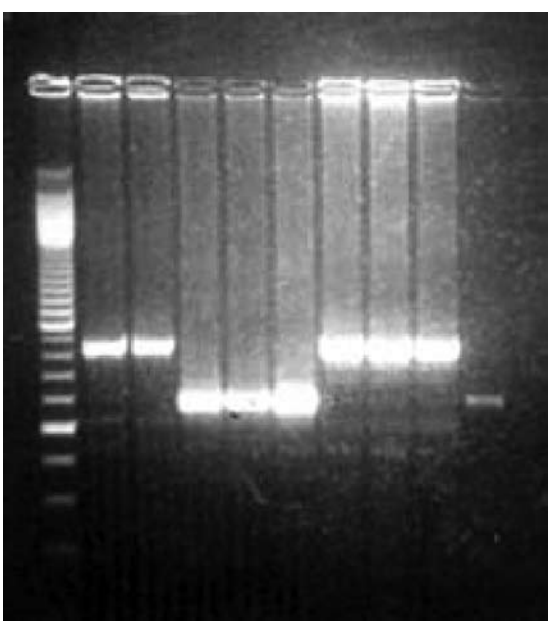

Fig 1 Example of PCR *coa* gene product from *S. aureus* isolates of 9 patients. Lane 1 contains 100 bp ladder markers, lanes 2 to 10, *S. aureus* isolates from 9 different patients.

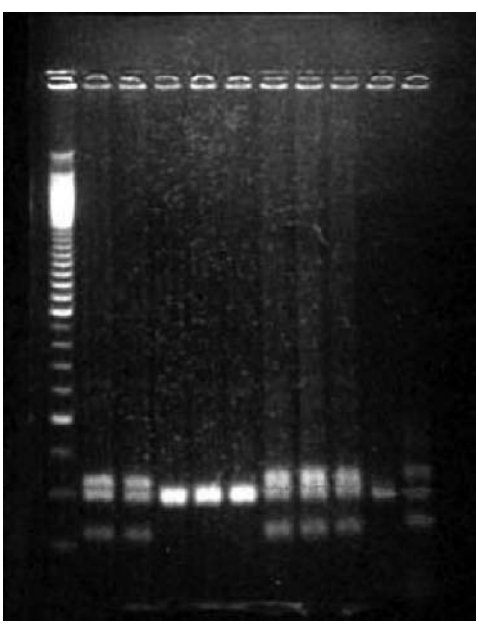

Fig 2 Example of *Alu1* restriction enzyme digest of the PCR *coa* gene product of 9 patients. Lane 1 contains 100 bp ladder marker, Lane 2 to 10 are isolates from 9 patients, lane 11 contains positive control.

in preventing *S. aureus* transmission.

Proportion statistical analysis showed that 12% of nurses had similar PCR-RFLP patterns to their patients. Therefore, of 20 nurses positive for *S. aureus* (58% in the hand area and 19% in the nasal area), only 12% had similar PCR-RFLP patterns as the *S. aureus* surgical wound infections.

S. aureus from 7 patients had similar PCR-RFLP patterns to the strain from one nurse-in-charge, so that this nurse was a nasal carrier and could be a reservoir of *S. aureus* from patients. Interestingly, 3 patients in the same ward had adjacent beds, and all had surgical-wound infections on day 3 post-surgery. None of these patients had a similar PCR-RFLP pattern to a nurse in charge. Therefore, the infections in these patients may have derived from other sources, such as contaminated operating instruments.

A sample from one nurse showed a similar PCR-RFLP pattern to 5 patients. Three of these 5 patients were in adjacent beds in the same ward, and had surgical-wound infections on day 4 post-surgery. This means that the hand-washing procedure used by that nurse was ineffective and *S. aureus* was transmitted among these patients by this nurse's hands.

In conclusion, 12% of the nurses had similar PCR-RFLP patterns to the patients. Thus, nurses were a 12% risk as potential reservoirs of *S. aureus* for surgical-wound infections during this investigation. Further research should examine all possible reservoirs of *S. aureus* surgical-wound infections with the PCR-RFLP method using the *coa* gene, to determine the causes of nosocomial surgical-wound infections.

Acknowledgements

We would like to express appreciation to Ridad Agoes from the Medical Faculty, Padjajaran University, Bandung, Indonesia for critical reading of the manuscript.

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