Diversity of Spa gene between methicillin-resistant and methicillin-sensitive Staphylococcus aureus bacteria in a tertiary referral hospital, Indonesia

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ABSTRACT

BACKGROUND Staphylococcal protein A (spa) typing is an effective and fast technique to identify the prevalence and spread of Staphylococcus aureus strains based on their spa gene profiles. The distribution of spa types will contribute to control the spread of S. aureus. Little is known regarding the spa types of S. aureus in Indonesia. This study aimed to investigate the diversity of spa gene among S. aureus carriage isolates in North Sumatra Province, Indonesia.

METHODS 79 S. aureus isolates consisting of 39 methicillin-resistant S. aureus (MRSA) and 40 methicillin-susceptible S. aureus (MSSA) carriage isolates were identified by VITEK2 Compact (BioMérieux, Indonesia) to detect mecA gene. All samples underwent spa typing and sequencing.

RESULTS Spa gene was detected among 31/39 (79%) of the MRSA isolates and 24/40 (60%) of the MSSA isolates. Most spa typing genes were identified between 350 and 400 base pair (bp). t258 and t852 were the most prevalence spa types among MRSA and MSSA isolates, respectively.

CONCLUSIONS Many MRSA and MSSA isolates encoded spa gene. The most genes detected were t258 and t852, identified in Germany and Portugal, respectively; while t18977 was initially identified in Malaysia. This result indicated a global spread of MRSA according to spa typing.

KEYWORDS bacterial typing techniques, Staphylococcus aureus, tertiary referral hospital
effects, such as IgG fragment, IgM fragment antigen-binding, and tumor necrosis factor receptor 1. These bindings allow protein A to inhibit opsonization and phagocytosis, act as superantigen, and trigger inflammation. Additionally, protein A can bind to the von Willebrand factor and has a role in endovascular infection and endocarditis.4

To understand the epidemiology of S. aureus, both the methicillin-sensitive S. aureus (MSSA) and MRSA, molecular investigations of S. aureus strains are required. Molecular typing can assist in monitoring and limiting the spread of S. aureus in healthcare facilities. In clinical applications, it can be used to determine whether an episode or event of S. aureus infection is a relapse of the initial infection or a second infection from a different S. aureus strain.5

Among various molecular typing methods, single-locus sequence typing is the most effective and fastest way to differentiate S. aureus isolates. This technique is based on various sequences and the number of tandem repeats in the X region of the spa gene. These spa typing results are in good agreement with the results of pulsed-field gel electrophoresis.6 Molecular spa typing studies in Indonesia are still limited and have never been performed in the North Sumatra Province. This study aimed to investigate the diversity of the spa gene in S. aureus isolates from patients with mucocutaneous infections in North Sumatra Province, provide information regarding epidemiological surveillance and public health tracing by spa typing, and identify MRSA and MSSA familial strains.

**METHODS**

**Sample collection**

Samples were collected from the isolates stored in our previous study.6 A total of 79 isolate samples, consisting of 40 MSSA isolates and 39 MRSA isolates, were included. These samples were collected in 2021 from Adam Malik General Hospital. We began this study by isolating bacterial DNA, followed by examination using conventional polymerase chain reaction (PCR), electrophoresis, visualization, and DNA sequencing.

**Bacterial DNA isolation**

DNA was extracted from the bacterial cells using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). A total of $1 \times 10^8$ bacterial cell colonies were placed in a sterile 1.5 ml tube, centrifuged at 13,000 rpm for 1 min, and the supernatant was discarded. A volume of 200 µl of buffer was added to the tube (0.8 mg/200 µl of lysozyme had previously been added) and was vortexed. This mixture was then incubated at 37°C for 30 min, added 20 µl of proteinase K, and vortexed again. Subsequently, it was incubated again at 60°C for 10 min with an additional 200 µl absolute ethanol and vortexed again. The genomic depletion (GD) column was strunged into a collection tube, and the sample was inserted into a GD column series. Next, 400 µl of W1 buffer was added and centrifuged at 13,000 rpm for 30 sec; then, the liquid was discarded in a collection tube. The GD column was reassembled using the same collection tube, and 600 µl of wash buffer was added. The column was centrifuged again at 13,000 rpm for 30 sec, and the liquid was discarded from the collection tube. The GD column was reassembled and centrifuged for 3 min. The collection tube was discarded, and the GD column was transferred to a 1.5 ml tube. Then, 100 µl of elution buffer (previously heated to 70°C) was added and left for 3 min at room temperature. After centrifugation for 30 sec, the GD column was discarded. Tubes containing DNA were stored at -20°C. This assay was performed according to the Geneaid protocol.

**Spa gene detection by conventional PCR**

A PCR master mix was prepared by diluting GoTaq Green Master Mix 2X (Geneaid) with forward/reverse primers, nuclease-free water, and DNA templates. Initially, the samples were vortexed using a spindle for ± 10 sec. Then, the PCR mix was prepared with a mixture of GoTaq Green Master Mix 2X (Geneaid) (12.5 µl), 10 µM forward primer (1 µl), 10 µM reverse primer (1 µl), nuclease-free water (8.5 µl), and DNA template (2 µl), with a total mix volume of 25 µl for one sample. The PCR mix was transferred to a 1.5 ml tube, vortexed for homogeneity, and arranged on a 0.2 ml PCR cooling block tube. The 23 µl PCR mix was distributed into the PCR tubes, and 2 µl of DNA template was added to the PCR tube and spun down to reduce all reagents in the tube. PCR conditions in the thermal cycler were initially denatured at 94°C for 5 min, followed by denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec. This process was repeated 35 times. The final extension step was performed at 72°C for 5 min. PCR products were analyzed by...
electrophoresis and visualization. This assay was performed according to the Geneaid protocol.

**Electrophoresis and visualization**

Initially, agarose gel electrophoresis was performed using 1 liter of Tris-acetate-EDTA (TAE) 1x buffer (100 ml TAE 10X + 900 ml distilled water). Next, 2 g of agarose was weighed to prepare a 2% agarose gel placed in an Erlenmeyer flask. Next, 100 ml of 1X TAE buffer solution was added. The solution was then heated until it boiled and became transparent. After cooling it down until warm, 1 µl of ethidium bromide was added and mixed thoroughly. The gel was poured into a caster and allowed to solidify for ± 30 min. TAE 1X buffer was then added, allowing the gel to submerge in the electrophoresis chamber. The PCR ladder for the marker was 100 base pairs (bp).

Next, 8 µl of PCR product was added to the agarose gel wells, and 5 µl of DNA ladder was added to the far left or right well. Results were visualized using the GelDoc tool (Bio-Rad, USA) and subsequently analyzed. Based on the PCR results, variations in the spa gene bands were categorized into five groups: <300, 300, 350, >400, and 500 bp. The target spa gene for both MRSA and MSSA was 350 bp long. Examples of the gel electrophoresis results are shown in Figures 1 and 2.

**Spa typing sequencing**

The samples used for sequencing exhibited thick bands. Selection was carried out through discussions with three authors (SA, RLK, and RB). Fifteen samples of MRSA isolates and 15 samples of MSSA isolates were selected, whereas one ATCC sample of MSSA and MRSA (a total of 32 samples) was selected for sequencing. Region X of the spa gene was amplified by PCR using the primers 1095F (5-AGACGATCCTTCGGTAGC-3) and 1517R (5-GCTTTTGCAATGTCATTTACTG-3). Each 50 µl used 50 µl primer. Gene spa products were sent to the Apical Scientific Laboratory (Selangor, Malaysia). The spa gene results were entered into SeqSphere+ version 8.4 (http://spaserver.ridom.de/ [Ridom GmBH, Germany]) to analyze the spa type.

**RESULTS**

The spa gene was detected in 31 MRSA (79%) and 24 MSSA isolates (60%). Based on the division of the bands, most MRSA bacteria (36%) had a spa gene length of 350 bp, whereas the majority of MSSA bacteria (38%) had a spa gene band length >400 bp (Table 1). One MSSA isolate (sample 17) showed two spa gene bands (Figure 3).

Results of Spa typing sequencing show that variations in the spa gene bands were categorized into five groups: <300, 300, 350, >400, and 500 bp. The target spa gene for both MRSA and MSSA was 350 bp long. Examples of the gel electrophoresis results are shown in Figures 1 and 2.

**Table 1. Spa gene band length in isolates**

<table>
<thead>
<tr>
<th>Band (bp)</th>
<th>MRSA (N = 39)</th>
<th>MSSA (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;300</td>
<td>3 (8)</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>8 (21)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>350</td>
<td>14 (36)</td>
<td>7 (18)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>3 (8)</td>
<td>15 (38)</td>
</tr>
<tr>
<td>500</td>
<td>3 (8)</td>
<td>0</td>
</tr>
</tbody>
</table>

bp=base pairs; MRSA=methicillin-resistant Staphylococcus aureus; MSSA=methicillin-susceptible Staphylococcus aureus
were found in the MSSA isolates: t852 (five isolates), t701 (one isolate), and t18977 (one isolate). Owing to poor reliability, the remaining seven MRSA isolates and eight MSSA isolates did not show spa gene sequencing results.

**DISCUSSION**

In the present study, the spa gene was detected in 56 isolates (71%) of *S. aureus* (31 [79%] MRSA and 24 [60%] MSSA). In general, recent studies have revealed that approximately 63.5–97.5% of *S. aureus* bacteria have the spa gene. It was detected in 67.2–96.6% of MRSA isolates and 46.15–95% of MSSA isolates. This study also revealed that one isolate had two bands of the spa gene in MSSA bacteria. This finding is in line with previous research reporting two spa gene bands in both MRSA and MSSA isolates. Spa typing was used to study *S. aureus* familial strains. Although this method is not clinically significant, it is valuable for epidemiological tracing.

Based on data from https://spa.ridom.de/¹, the seven types of the spa genes found in MRSA isolates were the gene types that have been detected in Indonesia for the first time. Previously, the gene types spa t1544 (two strains), t148 (one strain), t050 (one strain), and t159 (one strain) were detected in Indonesia (all in 2007), but in MSSA bacteria, not in MRSA bacteria as in this study. Only the t701 spa gene type was discovered in Indonesia out of the three types of the spa genes detected in MSSA isolates (2007). In 2019, Malaysian researchers identified a new type of the spa gene, t18977, in one strain.²

The lack of the spa gene in the *S. aureus* isolate might be explained by a mutation that prevented the spa primer from annealing to the target DNA, or by the fact that the isolate did not have the spa gene.³ Baum et al.⁴ conducted a study to analyze the non-spa-typeable in patients with invasive infections due to *S. aureus*. Sequencing of the spa locus revealed deletion mutations in the IgG-binding domain C, with only two strains showing unfavorable results. Despite lacking the spa gene, the bacteria remained virulent and caused invasive infections. In addition, the deficiency of forward spa primers in the IgG-binding region can lead to undetectable spa genes, making 1–2% of strains non-typeable.⁵

The majority of the spa gene band lengths in this study were 350 bp in MRSA isolates and >400 bp in MSSA isolates. In recent studies, MRSA bacteria have been shown to have a spa gene length of approximately 150–400 bp (the majority of the spa gene length is 300 bp),⁶ but another study discovered that the length of the spa gene in *S. aureus* (MSSA and MRSA) ranged from 1,150 to 1,500 bp.⁷ The sequencing results in this study showed that the dominant spa gene type was t258 and t852 in MRSA and MSSA bacteria, respectively. This study also identified the spa gene type t18977, which is the first to be identified in North Sumatra. Previously, Deurenberg et al.⁸ identified 62 MSSA isolates from 440 individuals in Yogyakarta and 37 different spa genes. Several types of spa genes were also found in this study, including the t701 type (in MSSA). Other spa gene types, such as t1544, t148, t050, and t159, were found in MSSA by Zukancic et al.;⁹ however, the present study found different spa gene types in MRSA. This difference might be due to the horizontal transfer of the genes between *S. aureus* bacteria (both MRSA and MSSA) or *S. aureus* bacteria and other *Staphylococcus* bacteria.¹⁰

Since December 22, 2022, SpaServer has discovered 20,838 spa gene types, with spa t032 being the most prevalent gene type globally (9.79%). The dominant spa gene types in the Asian region are t030, t037, t022, t437, t1081, t004, t001, and t2460. This type of spa gene represents a variety of tandem repeat sequences that often undergo polymerase staggering during DNA replication that are faster than most protein-encoding regions of the *S. aureus* genome. In addition, it is suspected that the variation in the spa type is a result of selection from the host immune system because the product of the spa gene is released as a virulence factor.¹¹ Although spa typing has no clinical impact, it can have an epidemiological impact. Two of the spa gene types identified were initially found in Germany and Portugal and then spread to China and Indonesia, while the newest one came from Malaysia before reaching Medan, a city destination for international travelers predominantly coming from Penang and Kuala Lumpur.

The spa gene encodes for protein A, a surface protein found in *S. aureus*. Protein A plays a role in the pathogenesis by binding to IgG. This will result in the bacteria being inaccessible to opsonins and could avoid phagocytosis.¹² Protein A can also act as a superantigen by binding to the V\(_i\)-3 domain of the B cell receptor, triggering a disruption in the B cell response.¹³ The expression of protein A can help colonize *S. aureus*...
bacteria on the nose and skin surface.\textsuperscript{21} The spa typing technique could allow sequencing of the X polymorphic region or short sequence repeats.

The limitations of this study were that not all spa genes were examined; the examined spa genes were selected based on the thickness of the spa band. Additionally, this study did not observe a clinical association in patients infected with S. aureus with the spa gene; therefore, we could not determine whether the spa gene’s presence or absence affected the patient.

In conclusion, the number of detected spa genes in MRSA and MSSA isolates was 79% and 60%, respectively. Seven types of spa genes were identified in MRSA: t258, t1544, t148, t267, t050, t159, and t213, whereas three spa genes MSSA were identified: t852, t701, and t18977. The first spa gene detected in Indonesia was t18977.

Conflict of Interest
The authors affirm no conflict of interest in this study.

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