Basic Medical Research

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

Staphylococcus aureus is a bacterium that often causes infections in the skin and soft tissue (furuncles, carbuncles, and cellulitis), as well as in the bones (osteomyelitis), lungs (pneumonia and empyema), blood (bloodstream infection), heart (endocarditis infective), gastrointestinal tract (gastroenteritis), and lining of the brain (meningitis). Morphologically, it is a cocci-shaped gram-positive bacterium arranged in clusters like grapes.[1] In 2019, *S. aureus* infection was the most common infection that caused death related to antimicrobial resistance in high-income countries and the Southeast Asian region.[2] According to Kuntaman et al,[3] there is an 8.1% incidence of methicillin-resistant *S. aureus* (MRSA) based on nose and throat swab results.

Virulence factors play a role in various infections caused by *S. aureus* bacteria, including cell wall-anchored (CWA) protein, a surface protein bounded to peptidoglycan. Protein A, the major group of CWA protein in the staphylococcal protein A (*spa*) gene, can bind to various ligands that result in different

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 1×10^9 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 genomic binding buffer in the tube and vortexed, followed by another incubation at 70°C for 10 min with an additional 200 µl absolute ethanol and vortexed again. The genomic depletion (GD) column was stringed 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-AGACGATCCTTCGGTGAGC-3) and 1517R (5-GCTTTTGCAATGTCATTTACTG-3).[7] 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.[8]

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).

In the MRSA isolate group, several types of the *spa* genes were found, namely, two isolates for type t258 and one isolate each for types t1544, t148, t267, t050, t159, and t213. Three types of the *spa* genes

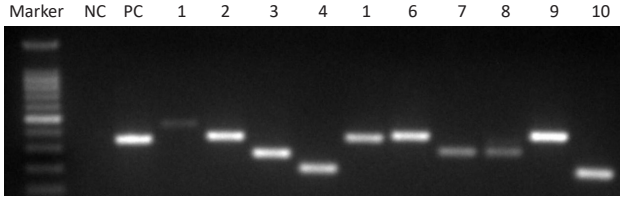

Figure 1. Gel electrophoresis results on MRSA isolates
MRSA=methicillin-resistant *Staphylococcus aureus*; NC=negative control; PC=positive control

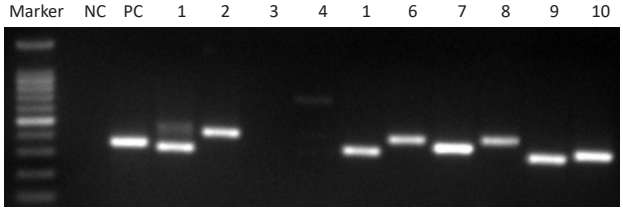

Figure 2. Gel electrophoresis results on MSSA isolates
MSSA=methicillin-susceptible *Staphylococcus aureus*; NC=negative control; PC=positive control

Table 1. *Spa* gene band length in isolates

Band (bp)	Frequency, n (%)	
	MRSA (N = 39)	MSSA (N = 40)
<300	3 (8)	0
300	8 (21)	3 (8)
350	14 (36)	7 (18)
>400	3 (8)	15 (38)
500	3 (8)	0

bp=base pairs; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*

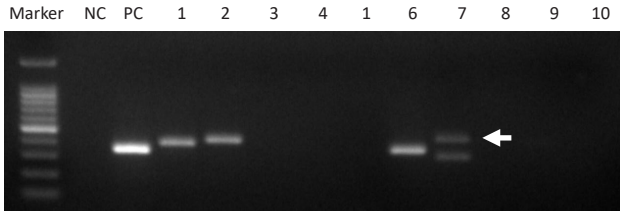

Figure 3. MSSA gel electrophoresis results, with the discovery of two bands on isolate number 17 (arrow)
MSSA=methicillin-resistant *Staphylococcus aureus*; NC=negative control; PC=positive control

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.[9–11] 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.[11,12] *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/,[13] 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.[13]

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.[14] Baum et al,[14] 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.[16]

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),[13] 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.[12] 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[16] 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;[17] 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.[18]

Since December 22, 2022, *Spa*Server has discovered 20,838 *spa* gene types, with *spa* t032 being the most prevalent gene type globally (9.79%).[9] The dominant *spa* gene types in the Asian region are t030, t037, t002, t437, t1081, t004, t001, and t2460.[19] 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.[20] 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.[14] Protein A can also act as a superantigen by binding to the V_H3 domain of the B cell receptor, triggering a disruption in the B cell response.[21] The expression of protein A can help colonize *S. aureus*

bacteria on the nose and skin surface.[22] 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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