Article

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Characterization of Enterotoxin Genes and Antibiotic Resistance Patterns in *Staphylococcus aureus* Blood Culture Isolates

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Background: ***Staphylococcus aureus*** **is an opportunistic pathogen that can result in skin infections, respiratory infections, sinusitis, and food poisoning. Methicillin-resistant** ***Staphylococcus aureus*** **(MRSA), due to antibiotic resistance, is a major determinant of healthcare-associated infections. Although the resistance mechanisms of MRSA have been reported to be related to exotoxins, research on this subject remains limited. This study aimed to examine the relationship between MRSA isolated from blood cultures and Staphylococcal enterotoxin (SE) genes.**

Methods: A total of 135 ***S. aureus*** **clinical isolates were collected from blood cultures, including 99 MRSA and 36 methicillin-susceptible** ***Staphylococcus aureus*** **(MSSA). The eight SE genes, including** ***sea***, ***seb***, ***sec***, ***sed***, ***see***, ***seg***, ***seh***, **and** ***sei***, **were compared between MRSA and MSSA using quantitative PCR (qPCR) SYBR Green assay.**

Results: Detection rates of ***sec***, ***seg***, **and** ***sei*** **genes were significantly different ($p < 0.001$) between MRSA and MSSA. Among the 99 MRSA isolates, 88 (88.9%) harbored two or more SE genes, of which 43 (43.4%) isolates carried both** ***seg*** **and** ***sei*** **genes. The multi-positive SE gene profiles, which have more than three genes, accounted for 40 (40.4%) isolates, of which 38 (38.4%) harbored the** ***sec***, ***seg***, **, and** ***sei*** **genes. The MRSA group carried more diverse SE gene profiles than the MSSA group and demonstrated significant differences in positivity rates, such as the** ***sec***, ***seg***, **, and** ***sei*** **genes.**

Conclusions: In this study, the presence of the SE genes in clinical isolates was determined using a molecular diagnostic method. Among the SE genes, the ***sec***, ***seg***, **, and** ***sei*** **genes showed significant differences compared with the MSSA group due to the high positivity rate in the MRSA group. Moreover, most of the genes were found in combination with other SE genes. Our findings demonstrate distinct distribution profiles of SE genes between MRSA and MSSA isolates, highlighting the need for continuous molecular monitoring. These results will provide an important indicator for research on MRSA resistance and effective infection control.**

Keywords: staphylococcal enterotoxin genes; virulence factor; methicillin-resistant *Staphylococcus aureus*; methicillin-susceptible *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a bacterium that is broadly distributed in nature. It is an opportunistic pathogen that exists in and colonizes the skin, hair, nasal cavity, and larynx of more than 50% of healthy individuals [1]. *S. aureus* has been known to cause a broad spectrum of human infections, ranging from skin and soft tissue infections to invasive diseases such as sepsis and pneumonia [2]. Antibiotics have long been used to treat *S. aureus* infections, but since the emergence of methicillin-resistant strains, methicillin-resistant *S. aureus* (MRSA) has become widely recognized for its resistance to one or more conventional antimicrobial agents [3,4]. MRSA is recognized as the leading causative agent of healthcare-associated infections, posing significant challenges in patient care and becoming a major public health concern across regions, including Europe, the United States, North Africa, the Middle East, and East Asia [5–7].

According to the Centers for Disease Control and Prevention (CDC) report, the mortality rate of MRSA infection surpasses that of acquired immune deficiency syndrome (AIDS) and Parkinson's disease [8].

In addition, various diseases caused by MRSA infection occur in medical institutions in the Republic of Korea, posing challenges to clinical treatment [9]. Additionally, recent studies report that MRSA infections in the community are emerging as a new variant of community-associated (CA)-MRSA [10]. Although the prevalence of MRSA in Republic of Korea has shown a decreasing trend in recent years, annual reports of the Korean Antimicrobial Resistance Monitoring System (KARMS) continue to indicate a high prevalence rate of *S. aureus* and MRSA [11].

Antibiotic resistance in MRSA is primarily mediated by the penicillin-binding protein 2a (PBP2a), which is synthesized by the *mecA* gene located on the MRSA chromosomes. Therefore, widely used methods for identifying MRSA target either the *mecA* gene or PBP2a [12,13]. However, there is a limitation that a general *mecA* gene test may fail to detect the presence of a *mecC* gene with sequence homology [14]. Additional criteria for accurate identification of MRSA in clinical isolates are required to ensure appropriate treatment [15].

Research on antibiotic resistance mechanisms has reported that the resistance of MRSA is also related to exotoxins, which are important virulence factors; however, studies on this relationship remain limited [16,17]. Exotoxins are broadly classified into cytotoxins, superantigens, and cytotoxic enzymes. *S. aureus* produces more than 40 exotoxins, some of which have similar functions and structures [18]. Representative cytotoxins include staphylococcal enterotoxin (SE), exfoliative toxin (ET), and toxic shock syndrome toxin-1 (TSST-1), all of which target host cell membranes and induce cell lysis as well as inflammatory responses [19,20]. Among cytotoxins, SEs are gastrointestinal toxins associated with the induction of autoimmune diseases, such as allergic reactions and type I immediate hypersensitivity.

SEs have been classified into at least 26 types according to the characteristics of their antigens, biological activities, and toxicity, including sea-see, seg-seu, selw, selx, sey, selz, sel26, and sel27. Each SE has different antigenicity and includes the five classical SEs—SEA, SEB, SEC, SED, and SEE—and 21 additional serotypes [21]. Although various types of SEs and their characteristics have been identified, the factors that influence the expression of these virulence genes related to *S. aureus* antibiotic resistance have not been identified. As antibiotic resistance of *S. aureus* continues to be a concern, this study attempted to identify the relationship between SE genes and *S. aureus* resistance-related factors. Therefore, we analyzed the correlation among eight representative SE genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, and *sei*) to find the differences in enterotoxin factors that can distinguish MRSA from methicillin-susceptible *S. aureus* (MSSA). A total of 135 *S. aureus* clinical isolates, including 99 MRSA and 36 MSSA isolates, were isolated from blood cultures, and the molecular genetic characteristics of SE genes were analyzed.

Methods

Clinical Isolates

Positive blood culture samples were collected from the Department of Laboratory Medicine, Konyang University Hospital, a general hospital with a capacity of 850 beds, from August 2015 to June 2018.

Blood Cultures

Whole blood samples collected from the patients were cultured for five days using BACTEC FX (Cat. No. 441385; Becton Dickinson, Sparks, MD, USA). Among them, BACTEC PLUS Aerobic/F Medium (Cat. No. 442023; Becton Dickinson, Sparks, MD, USA) and BACTEC LYTIC Anaerobic/F BACTEC PLUS (Cat. No. 442021; Becton Dickinson, Sparks, MD, USA) were used for the adult culture samples, and BACTEC LYTIC Anaerobic/F BACTEC PLUS was used to culture the pediatric samples. The detected bacterial growth signals in the BACTEC FX system during sample incubation and the sample solutions were smeared and Gram-stained to distinguish Gram-positive and Gram-negative bacilli. Simultaneously, sample solutions were inoculated onto blood agar plates (BAP) and MacConkey plates and then incubated for 24 h at 35 °C in a 5% CO_2 incubator. After incubation, *S. aureus* isolates were identified based on colony morphology and Gram staining results.

MALDI-TOF Mass Spectrometer

All clinical isolates cultured and isolated as single colonies from blood cultures were tested for microbial species identification using a MALDI Biotyper (Bruker Daltonics, Bremen, Germany). Subsequent test results were analyzed using the MALDI Biotyper RTC software version 3.1 (Bruker Daltonics, Bremen, Germany). Briefly, fresh single colonies cultured for 16–18 h were plated on an MSP 96 Target Polished Steel BC Microscout Target Plate (Cat. No. 8280800; Bruker Daltonics, Bremen, Germany) using a sterile wooden applicator. After the bacteria were dried, 1 µL of a reagent solution containing α-cyano-4-hydroxycinnamic acid saturated with matrix solution (2.5% trifluoroacetic acid and 50% acetonitrile) was added. After the reagent solution was completely dried at room temperature, the test plate was mounted on a Microflex MALDI Biotyper for analysis. The judging criteria were as follows: cut-off scores $\geq$2.0 were considered sufficient for species identification, cut-off scores $\geq$1.7 and $<$2.0 were considered sufficient for genus identification, and cut-off scores $<$1.7 were considered unreliable.

Antimicrobial Susceptibility Test

To test susceptibility, the MicroScan® WalkAway 96 Plus System (Beckman Coulter, West Sacramento, CA) was used to determine the minimum inhibitory concentration (MIC) of amoxicillin-clavulanic acid, ampicillin, azithromycin, cefepime, cefuroxime, ciprofloxacin, clindamycin, daptomycin, ertapenem, erythromycin, fosfomycin, fusidic acid, gentamicin, imipenem, levofloxacin, linezolid, meropenem, moxifloxacin, mupirocin, penicillin, rifampin, synercid, teicoplanin, tetracycline, tobramycin, trimethoprim-sulfamethoxazole, and vancomycin. The MIC results were analyzed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100 S30).

Genomic DNA Extraction

Genomic DNA (gDNA) was extracted from a single colony isolated from a blood agar plate for genetic analysis of the *S. aureus* clinical isolates. During gDNA extraction, a single bacterial colony was collected using a disposable loop, resuspended in 500 uL of 5% Chelex® Resin (Cat. No. 1421253; Bio-Rad Laboratories, Hercules, CA, USA), boiled for 10 min, and then centrifuged at 3000 g for 10 min. After centrifugation, the extracted gDNA in the supernatant was transferred to a sterile tube, and the concentration and purity were measured using a NanoDropTM 2000 Spectrophotometer (Cat. No. ND-2000; Thermo Fisher Scientific, Waltham, MA, USA). The extracted gDNA was stored at –20 °C before analysis.

SYBR Green qPCR Assay

To identify *S. aureus* and detect the *mecA* gene and SE genes, including *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, and *sei*, a SYBR green real-time quantitative PCR (qPCR) assay was performed. A 20 µL mixture consisting of 10 µL THUNDERBIRD® SYBR qPCR Mix (Cat. No. QPS-201; Toyobo Co., Ltd., Osaka, Japan), 10 pmol/µL of forward and reverse primers, 3 µL of gDNA template, and 5 µL of distilled water was prepared. The qPCR assay was performed using the Applied Biosystems™ 7500 FAST Real-Time PCR System (Cat. No. 4351106; Thermo Fisher Scientific, Waltham, MA, USA). In this study, the oligonucleotide sequences of each forward and reverse primer were modified based on existing research data (Table 1). The PCR conditions for detecting each type of *S. aureus* gene were as follows: pre-denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 50–61 °C (with each gene amplified separately using its appropriate annealing temperatures) for 30 s, and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The annealing temperature for each primer is listed in Table 1. After the PCR procedure was completed, a melting curve analysis was conducted by increasing the amplification product temperature by 0.5 °C from 55 °C to 95 °C in the melting curve analysis step.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). To compare the categorical data of SE gene distribution patterns between the MRSA and MSSA groups, Fisher's exact test was used. A p-value < 0.05 was considered statistically significant.

Results

Isolation Frequency of S. aureus From Blood Cultures

From a total of 85,225 blood cultures collected during the study period, MRSA was isolated from 99 (1.3%) of 7670 (8.9%) positive blood samples. A total of 135 *S. aureus* clinical isolates were obtained from blood culture-positive samples and identified as *S. aureus* using a MALDI Biotyper with a cut-off score >2.0. *S. aureus*-specific qPCR was additionally performed to confirm *S. aureus*, and the detection of MRSA was verified through mecA PCR. As a result, out of a total of 135 *S. aureus* cases, 99 strains that tested positive on mecA PCR were classified as MRSA, and 36 strains that tested negative were classified as MSSA. The results of the two test methods showed 100% concordance (Table 2).

Antimicrobial Resistance Patterns of S. aureus Clinical Isolates

In the MRSA group, all isolates were resistant to penicillin, ampicillin, amoxicillin-clavulanic acid, and imipenem. In addition, resistance to amoxicillin-clavulanic acid, fosfomycin, imipenem, and rifampin antibiotics was revealed only in the MRSA group. Although the overall resistance to tobramycin was low, it was more frequently observed in the MSSA group (11.1%) than in the MRSA group (1.0%). Only one MRSA isolate was resistant to cefepime, cefuroxime, ertapenem, and meropenem. None of the isolates were resistant to daptomycin, linezolid, synercid, teicoplanin, trimethoprim-sulfamethoxazole, or vancomycin. The antimicrobial susceptibility test results are shown in Table 2. MRSA clinical isolates were identified by detecting the mecA gene in all *S. aureus* clinical isolates using *mecA* gene qPCR. The results revealed that 100% (99/99) of the MRSA isolates carried the *mecA* gene (Table 2).

Existence Pattern of Staphylococcal Enterotoxin (SE) Genes of S. aureus Isolated From Blood Cultures

A qPCR SYBR Green assay was performed to analyze the SE gene existence patterns between the MRSA and MSSA groups. As a result, the seg gene was identified in 93 (93.9%) of 99 MRSA clinical isolates, showing the highest detection rate, followed by 83 (83.8%), 42 (42.4%), and 4 (4.0%) *sei*, *sec*, and *sed* genes, respectively. The rest of the SE genes—the *sea*, *seb*, *see*, and *seh*—were all negative in the MRSA group (Fig. 1, Table 3). In the results for the

Table 1. Oligonucleotide primers used in this study.

Target	Primer	Sequence (5′ to 3′)	Tm (°C)	Product size (bp)	Gene accession no.
S. aureus	*SA*-F	GCGATTGATGGTGATACGGTT	55	267	CP068143.1
	SA-R	AGCCAAGCCTTGACGAACTAAAGC			
mecA	*mecA*-F	GTGAAGATATACCAAGTGATT	50	147	X52593.1
	mecA-R	ATGCGCTATAGATTGAAAGGAT			
sea	*sea*-F	GGTTATCAATGTGCGGGTGG	60	102	M18970
	sea-R	CGGCACTTTTTTCTCTTCGG			
seb	*seb*-F	GTATGGTGGTGTAACTGAGC	58	164	M11118
	seb-R	CCAAATAGTGACGAGTTAGG			
sec	*sec*-F	CTTGTATGTATGGAGGAATAACAA	58	284	X05815
	sec-R	TGCAGGCATCATATCATACCA			
sed	*sed*-F	GTGGTGAAATAGATAGGACTGC	60	385	M28521
	sed-R	TCATATGAAGGTGCTCTGTGG			
see	*see*-F	GTACCAATTAACTTGTGGATAGAC	60	173	AY518387
	see-R	CCTCTTTGCACCTTACCGC			
seg	*seg*-F	CAGGTAACAATCGACAATAGACA	59	139	AB060535
	seg-R	AGAACCATCAAACTCGTATAGC			
seh	*seh*-F	AGGTGATAGTGGCAATGATTTG	58	200	AB060536
	seh-R	TAGCACCAATCACCCTTTCC			
sei	*sei*-F	CAACTCGAATTTTCAACAGGTACC	61	469	AB060537
	sei-R	TTACAGGCAGTCCATCTCCTG			

MSSA group, the *seg* gene was identified in 17 (47.2%) of 36 isolates, revealing the highest detection rate, followed by 8 (22.2%) *sea* genes, 4 (11.1%) *seb* genes, and 1 (2.8%) *sec* gene. The rest of the SE genes—*sed*, *see*, *seh*, and *sei*—were all negative in the MSSA group (Fig. 1, Table 3).

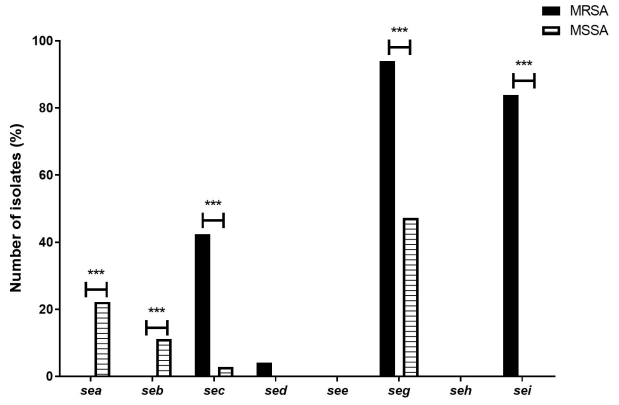

Fig. 1. Distribution of staphylococcal enterotoxin genes between MRSA and MSSA groups. ***: $p < 0.001$. MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-susceptible *Staphylococcus aureus*.

Profiles of Eight Staphylococcal Enterotoxin (SE) Genes

The co-existence patterns of eight types of SE genes were analyzed in 135 *S. aureus* clinical isolates from blood cultures. The results showed that 88 (88.9%) out of 99 isolates in the MRSA group harbored two or more SE genes, and 48 (48.5%) carried two SE genes. Of these, the MRSA isolates possessing both *seg* and *sei* genes accounted for the majority of SEs, with a total of 43 (43.4%) isolates, followed by 3 (3%) isolates with *sec* and *seg* genes and 2 (2%) isolates with *sed* and *seg* genes. A total of 40 (40.4%) isolates possessed 3 multiple SE genes, including 38 (38.4%) isolates with *sec*, *seg*, and *sei* genes and 2 (2.0%) isolates with *sed*, *seg*, and *sei* genes. In contrast, 7 (7.1%) isolates carried only 1 gene, including 1 clinical isolate with the *sec* gene and 6 isolates with the *seg* gene. None of the *sea*, *seb*, or *see* genes were detected in the MRSA isolates. Out of 36 MSSA clinical isolates, 5 (13.9%) harbored 2 or more SE genes. Moreover, 4 (11.1%) isolates possessed both *sea* and *seg* genes, and 1 (2.8%) isolate possessed 3 genes, including *sea*, *sec*, and *seg*; 8 (22.2%) isolates possessed the *sea* genes, and 4 (11.1%) isolates possessed the *seb* gene. No *sed*, *see*, *seh*, or *sei* genes were detected in the MSSA isolates. The proportion of the clinical isolates with 2 or more SE genes was 68.9%, with MRSA and MSSA isolates accounting for 94.6% and 5.4% of the isolates, respectively (Table 4).

Discussion

Infections caused by *S. aureus* range from skin and soft tissue infections to food poisoning, pneumonia, toxic shock syndrome (TSS), and other invasive infections. In particular, bloodstream infection by MRSA is a major challenge in clinical treatment. In 2019, the Antimicrobial Resistance Threat Report published by the U.S. CDC clas-

Table 2. *S. aureus* results, *mecA* gene PCR, and resistance rates of *S. aureus* isolates.

	MRSA group	MSSA group	Total
	n = 99, (%)	n = 36, (%)	n = 135, (%)
S. aureus PCR	99 (100.0)	36 (100.0)	135 (100.0)
mecA PCR	99 (100.0)	0 (0.00)	99 (73.3)
Antibiotics			
Amox/KClav	99 (100.0)	0 (0.0)	99 (73.3)
Ampicillin	99 (100.0)	32 (88.8)	131 (97.0)
Azithromycin	60 (60.6)	1 (2.7)	61 (45.2)
Cefepime	1 (1.0)	0 (0.0)	1 (0.7)
Cefuroxime	1 (1.0)	0 (0.0)	1 (0.7)
Ciprofloxacin	61 (61.6)	3 (8.3)	64 (47.4)
Clindamycin	61 (61.6)	1 (2.7)	62 (45.9)
Daptomycin	0 (0.0)	0 (0.0)	0 (0.0)
Ertapenem	1 (1.0)	0 (0.0)	1 (0.7)
Erythromycin	62 (62.6)	1 (2.7)	63 (46.6)
Fosfomycin	24 (24.2)	0 (0.0)	24 (17.7)
FusidicAcid	43 (43.4)	9 (25.0)	52 (38.5)
Gentamicin	51 (51.5)	6 (16.6)	57 (42.2)
Imipenem	99 (100.0)	0 (0.0)	99 (73.3)
Levofloxacin	60 (60.6)	1 (2.7)	61 (45.1)
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem	1 (1.0)	0 (0.0)	1 (0.7)
Moxifloxacin	61 (61.6)	1 (2.7)	62 (45.9)
Mupirocin	23 (23.2)	2 (5.5)	25 (18.5)
Penicillin	99 (100.0)	33 (91.6)	132 (97.7)
Rifampin	14 (14.1)	0 (0.0)	14 (10.4)
Synercid	0 (0.0)	0 (0.0)	0 (0.0)
Teicoplanin	0 (0.0)	0 (0.0)	0 (0.0)
Tetracycline	50 (50.5)	3 (8.3)	53 (39.2)
Tobramycin	1 (1.0)	4 (11.1)	5 (3.7)
Trimetho-sulfa	0 (0.0)	0 (0.0)	0 (0.0)
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)

Amox/kClav, Amoxicillin-clavulanic acid; Trimetho-sulfa, Trimethoprim-sulfamethoxazole; MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-susceptible *Staphylococcus aureus*; PCR, Polymerase chain reaction.

sified MRSA as a serious threat [22]. Accumulating research has been conducted on representative resistance-related genes and proteins, such as the *mecA* gene and PBP2a, to explore the antibiotic resistance mechanisms of MRSA [23]. Since antibiotic resistance is one of the virulence factors, along with adhesion, hemolysin, and superantigens, analyzing their correlation is important for understanding resistance mechanisms. Among them, the enterotoxin included in superantigens is widely used to study the biological properties of MRSA [24].

The antibiotic resistance rate of the MRSA clinical isolates from blood cultures was 100% in amoxicillin-clavulanic acid, ampicillin, imipenem, and penicillin. Ampicillin and penicillin showed high resistance rates in both the MRSA and MSSA groups, whereas resistance to the other antibiotics was found only in the MRSA group. This suggests that the rate of MRSA with multidrug resistance is increasing. Conversely, resistance to cefepime, cefuroxime, ertapenem, meropenem, and tobramycin was detected in only one isolate each, and all MRSA isolates were susceptible to daptomycin, linezolid, synercid, teicoplanin, trimethoprim-sulfamethoxazole, and vancomycin. The results indicate that these antibiotics could be therapeutic agents for controlling MRSA infections.

The present study analyzed the relationships between the molecular genetic characteristics of eight types of SE genes in 135 *S. aureus* clinical isolates from blood cultures. Our study attempted to determine the differences in SE gene distribution between the MRSA and MSSA groups using the molecular and biological properties of blood cultures. The results showed that the MRSA group carried a higher prevalence and diversity of SE genes than the MSSA group. Although the positive rate of the MRSA group was not high in the classical SE types, a difference in the distribution of SE genes between the MRSA and MSSA groups was confirmed in the *sec* gene (MRSA: 42.4%, MSSA: 2.8%). According to a recent study, a specific subtype of sec co-produced with tsst-1 caused a human vaginal infection [25]. Additionally, other studies have reported that four human-related *sec* subtypes are involved in immune system stimulation and are linked to human infections such as TSS, purpura fulminans, and necrotizing pneumonia [25]. Epidemiological studies have demonstrated a strong association between *S. aureus* infective endocarditis and a select group of superantigen (SAg) genes [26].

Despite the *sea* and *seb* genes being positive only in the MSSA group (*sea*: 22.2%, *seb*: 11.1%), it is difficult to determine whether this result was significant due to the low positive rate. Furthermore, in other studies, the positive rate for the *seb* gene was high or was being intensively studied, highlighting the need for additional genetic analysis [27]. The remaining classic enterotoxin genes, *sed* and *see*, showed negative or very low positive rates in both groups. Although the seg gene showed the highest positive rate in the MRSA group (93.9%), a significant number of positive rates were also detected in the MSSA group (47.2%). One study reported that seg induces T cell proliferation in mice through simultaneous cytokine production of interleukin-2 and gamma interferon, and is consequently associated with scarlet fever and toxic shock [28]. Compared to other SE genes, the seg gene was present in the entire SE gene profile; therefore, it has a high potential as the main target of virulence research. [29]. In addition, the *sei* gene showed a more significant difference between the MRSA (83.8%) and MSSA groups (0.0%). As a result of single gene analysis, significant differences were confirmed in the *seg* and *sei* genes, which are non-classical SE, and the *sei* gene result was found to be the most significant. Enteropathy associated with seg and sei is a life-threatening disease that causes reversible destruction of enterocyte ultrastruc-

Table 3. Frequencies of staphylococcal enterotoxin genes between MRSA and MSSA.

Type of SEs	Existence of SEs, n (%)			
	MRSA group (n = 99)	MSSA group (n = 36)	Total (n = 135)	*p*-value
sea	0 (0.0)	8 (22.2)	8 (5.9)	<0.001***
seb	0 (0.0)	4 (11.1)	4 (2.9)	0.0007***
sec	42 (42.4)	1 (2.8)	43 (31.8)	<0.001***
sed	4 (4.0)	0 (0.0)	4 (2.9)	0.2239
see	0 (0.0)	0 (0.0)	0 (0.0)	-
seg	93 (93.9)	17 (47.2)	110 (81.5)	<0.001***
seh	0 (0.0)	0 (0.0)	0 (0.0)	-
sei	83 (83.8)	0 (0.0)	83 (61.5)	<0.001***

SEs, Staphylococcal enterotoxin genes. ***: $p < 0.001$.

Table 4. Staphylococcal enterotoxin gene patterns of *S. aureus* isolates.

Gene profile	Total	MRSA group	MSSA group
	n = 135, (%)	n = 99, (%)	n = 36, (%)
sea	8 (5.9)	0 (0.0)	8 (22.2)
seb	4 (3.0)	0 (0.0)	4 (11.1)
sec	1 (0.7)	1 (1.0)	0 (0.0)
seg	18 (13.3)	6 (6.1)	12 (33.3)
sea, seg	4 (3.0)	0 (0.0)	4 (11.1)
sec, seg	3 (2.2)	3 (3.0)	0 (0.0)
sed, seg	2 (1.5)	2 (2.0)	0 (0.0)
seg, sei	43 (31.9)	43 (43.4)	0 (0.0)
sea, sec, seg	1 (0.7)	0 (0.0)	1 (2.8)
sec, seg, sei	38 (28.1)	38 (38.4)	0 (0.0)
sed, seg, sei	2 (1.5)	2 (2.0)	0 (0.0)
ND	16 (11.9)	4 (4.0)	12 (33.3)

Data are presented as the number of isolates and percentages (%). Percentages were calculated based on the total number of isolates in each group (Total: n = 135; MRSA: n = 99; MSSA: n = 36).

The see and seh genes were also analyzed but were not detected in any isolates, and thus were not included in the table values.

ture during antibiotic treatment, and differential diagnosis is required, particularly in neonates with early-onset disease [30].

In other experimental results [31,32], the positive rates of *seg* and *sei* genes in the MRSA groups were high. Specifically, the positivity rate of the *seg* gene was predominantly higher than that of the other genes. In this study, MRSA isolates obtained from bloodstream infections were analyzed to provide clinically relevant insights into SE gene distribution.

The SE gene pattern analysis results showed that 88 (88.9%) MRSA clinical isolates and five (13.8%) MSSA clinical isolates harbored two or more SE genes. These results indicate that the MRSA clinical isolate shows a high tendency to harbor multiple SE genes. A total of 12 SE gene profiles were detected in the MRSA and MSSA groups, except for the negative results. In the MRSA group, the SE gene profiles, the *sec*, *seg*, and *sei* genes, which showed high positive rates, were detected in 42.4%, 93.9%, and 83.8% of isolates, respectively. Meanwhile, combinations containing all three SE genes, including *sec*, *seg*, and *sei*, accounted for 38.4%. Consistent with several other studies, the isolation rates of clinical isolates with two or more SE genes were also high in the present study. However, it seems that there may be differences due to the different types of SE genes selected in the experiments and the different origins of the clinical isolates [33–37].

Despite the above important findings, this study has several limitations. To date, no definitive evidence has demonstrated that specific SE genes are definitively associated with antibiotic resistance beyond differences in prevalence of SE genes [38]. Additionally, the prevalence of SE genes between the two groups may be due to natural variation in the genomes of different isolates. No clinical isolates harboring four or more enterotoxin genes simultaneously were identified in this study. Additional SE genes will need to be selected and screened to obtain more accurate diagnostic criteria. While this study focused on the relationship between specific SE genes and antibiotic resistance, future research is needed to expand the scope by integrating analysis of genetic factors associated with SEs and phylogenetic backgrounds, potentially through whole-genome sequencing of isolated strains.

Conclusions

In conclusion, the MRSA group carried more diverse SE genes than the MSSA group. Among the SE genes, the *sec*, *seg*, and *sei* genes showed significant differences from the MSSA group due to the high positivity rate in the MRSA group. Moreover, most of the genes were found to be in combination with other SE genes. These findings suggest that specific SE genes may be associated with distinct virulence-related characteristics in MRSA bloodstream isolates. To address the limitations mentioned in the discussion, future studies should include diverse clinical samples and larger sample sizes to improve validation, along with additional experiments to enhance the reliability

of the results of this study. Based on these results, accurately identifying the differences in SE genes between the MRSA and MSSA groups will be an important indicator for understanding MRSA resistance mechanisms and improving infection control strategies.

Abbreviations

gDNA, genomic DNA; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; PBP2a, penicillin-binding protein 2a; SEs, staphylococcal enterotoxins.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Author Contributions

SHK and JL wrote the main manuscript text and participated the conception while HP was responsible for critically reviewing and editing it. JB dedicated to conducting formal analysis and acquisition of data. SBP and HJ conducted an investigation for this study and the interpretation of data. MSL and JB performed validation and data analysis. YKK and SK made contributions to the acquisition of data and revising it critically for intellectual content. SHK and JL were developed the theory by visualization. HP and SK supervised the study, critically revised the manuscript, and contributed to the study design. All authors contributed to significant editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study protocol was reviewed and determined to be exempt from IRB approval by the Institutional Review Board of Catholic University of Pusan (IRB No. CUPIRB-24-01-008), as this was a retrospective study utilizing de-identified whole blood samples previously collected from patients, and did not involve direct human participants or identifiable private information. All procedures were conducted in accordance with the Declaration of Helsinki.

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Conflict of Interest

Jiyoung Lee is an employee of Dream Dx Inc., and Jinyoung Bae is an employee of Q-Solutions. The remaining authors declare that they have no related conflicts of interest.

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