Humoral immune consequences of *Staphylococcus aureus* ST239-associated bacteremia

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Abstract The humoral immune responses against 46 different staphylococcal antigens in 27 bacteremia patients infected by clonally related methicillin-resistant *Staphylococcus aureus* (MRSA) strains of a single sequence type (ST) 239 were investigated. A group of non-infected patients ($n = 31$) hospitalized for different reasons served as controls. All strains were confirmed as ST 239 by *S. aureus* and *mecA*-specific PCR, *spa*, and multi-locus sequence typing (MLST). In each bacteremia patient, a unique pattern of *S. aureus* antigen-specific immune responses after infection was observed. Antibody levels among bacteremia patients were significantly higher than controls for HlgB ($P = 0.001$), LukD ($P = 0.009$), LukF ($P = 0.0001$), SEA ($P = 0.0001$), SEB ($P = 0.011$), SEC ($P = 0.010$), SEQ ($P = 0.049$), IsaA ($P = 0.043$), IsdA ($P = 0.038$), IsdH ($P = 0.01$), SdrD ($P = 0.001$), SdrE ($P = 0.046$), EsxA ($P = 0.0001$), and SA0104 ($P = 0.0001$). On the other hand, the antibody levels were significantly higher among controls for SSL3 ($P = 0.009$), SSL9 ($P = 0.002$), and SSL10 ($P = 0.007$) when the IgG level on the day of infection was compared with that

measured on the day of admission. Diversity was observed in the immune response against the antigens. However, a set of antigens (IsaA, IsdA, IsdH, SdrD, and HlgB) triggered a similar type of immune response in different individuals. We suggest that these antigens could be considered when developing a multi-component (passive) vaccine. SEA and/or its specific antibodies seem to play a critical role during ST239 MRSA bacteremia and SEA-targeted therapy may be a strategy to be considered.

Introduction

Staphylococcus aureus is a well-recognized nosocomial pathogen. Nosocomial methicillin-resistant *S. aureus* (MRSA) infections increase hospital stay, antibiotic use, overall hospitalization costs, morbidity, and mortality [1–3]. Among all bacterial infections, mortality due to MRSA remains the most frequent. The two main factors that render *S. aureus* a successful nosocomial pathogen are its frequent and multiple drug-resistant properties and its multi-virulence. The declining trend observed in the development of new antibiotics is likely due to huge costs and the lack of significant returns on investment owing to rapidly emerging resistance. Producing protective immunity against *S. aureus* was and still is important, but using live bacterial cells or killed bacteria has not yet been successful [4]. This requires alternative approaches, including the prophylactic or therapeutic use of antibodies.

Many geographical regions harbor selective and local epidemic MRSA strains. Several large-scale epidemiological investigations demonstrated that most hospital-acquired MRSA infections are caused by a relatively small number of MRSA clones spread worldwide. The most frequently encountered clones are the Iberian (ST247-IA), Brazilian/Hungarian (ST239-III), Berlin (ST45-IV), New York/Japan (ST5-II),

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pediatric (ST5-IV), EMRSA-15 (ST22-IV), and EMRSA-16 (ST36-II) clones [5]. For example, all strains from Korea and Japan belong to clonal complex 5 (CC5) whereas strains from the rest of the Asian countries are primarily CC239 [6]. ST239 is the most successful clone isolated from hospitalized patients in Malaysia [7, 8].

Different staphylococcal determinants, such as purified capsular polysaccharide (types 5 and 8) or individual surface proteins, e.g., clumping factor A (ClfA) [9], clumping factor B (ClfB) [10], iron-regulated surface determinant B (IsdB) [11], or fibronectin-binding protein (FnBP) [12] only generated immune responses that gave partial protection against *S. aureus* in experimental animals [13], whereas vaccination in humans failed [14–16]. A successful vaccine candidate should be immunogenic and should play an essential role in staphylococcal survival. Failure of human experiments indicates that animal models are not always good mimics for the human body. In addition, there is a gap in the knowledge of in vivo expression of virulence factors of *S. aureus*, emphasizing the need to better understand the role and expression status of *S. aureus* virulence factors in humans [17]. Transcriptomics of MRSA USA300 cultured in human blood or serum revealed up-regulation of only a limited number of genes encoding putative *S. aureus* virulence factors [18]. Genes encoding the cytolytic toxins HlgA, HlgB, and HlgC were among the most frequently up-regulated genes at various time points [18].

Studies on antibody repertoires against *S. aureus* antigens among healthy blood donors and infected patients revealed marked differences, whereas a noticeable heterogeneity was observed between given individuals [19]. More recent studies revealed that patients infected with *S. aureus* had clearly different, individualized humoral responses [20, 21], but no clear association among specific infection and humoral response against one or more virulence factors was documented. Sera from patients with *S. aureus* blood infection were examined; antibody levels increased against immune modulatory proteins and MSCRAMMs such as iron-regulated surface determinant A (IsdA) in most cases [20]. However, the situation was different in other sets of bacteremia patients, where antibodies against a putative iron-regulated ABC transporter, SA0688, showed the highest serum levels after challenge [17].

S. aureus strains responsible for human infections have clearly different genetic backgrounds with variable core genome sequences [22]. Most of these differences cover genes related to virulence located on pathogenicity island or other mobile genetic elements [17]. Hence, most pathogenic *S. aureus* strains share a vast arsenal of virulence factors. It could very well be that the diversity found in anti-staphylococcal humoral immune response is the result of staphylococcal genetic diversity. This indicates that different *S. aureus* strains express different spectra of virulence factors during infection. On the other hand, the ability of different

individuals to mount a humoral response against specific antigens could be different as well.

We tried to come to a better understanding of the human immune responses against *S. aureus* virulence factors encoded by a single dominant sequence type (ST) in different infected and non-infected individuals. In the present study, IgG antibody levels against an array of staphylococcal antigens was compared in bacteremia patients infected by ST239 *S. aureus* strains.

Materials and methods

Study cases

The study involved patients admitted to Hospital Kuala Lumpur (HKL) from the 19 September 2011 to the 14 February 2012, who developed confirmed MRSA bacteremia during their hospital stay. Two serum samples were collected from each patient. The first blood sample was taken on the day of onset of infection whereas a second sampling was performed 7 days later. Thirty-six adult patients were included in the study; at the end, 27 patients remained. Nine patients were excluded as 8 (21%) passed away during the first week of infection before a second blood sample could be collected and 1 patient was infected with a genetically different MRSA strain. The mean age of the patients was 52.8 years (range 16–77 years) and 70.3% were male. According to the three major ethnic groups in Malaysia, the study population comprised Malay ($n = 13$; 48.1%), Chinese ($n = 7$; 25.9%), and Indians ($n = 7$; 25.9%). All patients were treated with antibiotics according to hospital guidelines.

A group of hospitalized patients ($n = 31$) in HKL without staphylococcal infection during hospitalization served as controls, where serum samples on admission day and day 7 after admission were collected. The mean age for the control group was 46.2 (range 27–70 years), 70.9% of whom were male.

All patients and controls were subjected to nasal swab culture to determine their staphylococcal carrier status. A sterile transwab (Medical Wire, UK) was rotated four times in both anterior nasal vestibules and cultured on mannitol salt agar and chrome agar medium (BD Diagnostics, Heidelberg, Germany).

The study was performed with the approval of the Universiti Putra Malaysia ethics committee according to the rules of good clinical practice and the Malaysian statutory definition of clinical trials. Patients received verbal and written information from nursing personnel. Patients or relatives provided verbal consent, which was noted in the nursery record. Patients had to be mentally and physically capable of taking the decision to participate in the study. For patients unable to make such a decision, guardians were informed and their initial agreement was obtained. Before official

participation, either the patients or their guardians had to provide official written consent.

Bacterial isolation and growth conditions

Blood samples were cultured using the Becton Dickinson blood culture system (BD BACTEC™). All Gram-positive cocci resembling staphylococci were subjected to catalase testing, coagulase tube testing, and *Sa442* PCR for definitive species confirmation [23]. MRSA screening was performed by a cefoxitin disk diffusion test on a Mueller Hinton agar plate for all isolates. Whenever resistance to cefoxitin was observed, MRSA status was confirmed by *mecA* gene PCR [5].

Genotyping and virulence gene profiles of *S. aureus* isolates

For genotyping, chromosomal DNA was extracted using the DNeasy Kit (Qiagen). Staphylococcal protein A (*spa*) typing and multi-locus sequence typing (MLST) was performed as described earlier [24, 25]. Sequence types (STs) and clonal clusters (CC) were assigned using the *S. aureus* MLST database (www.mlst.net) hosted by Imperial College in London, UK. SCCmec typing of MRSA strains was performed as described before [7, 26].

For all 27 patients with staphylococcal bacteremia, *S. aureus* strains isolated from blood were screened for the presence of 46 genes encoding different antigens by PCR [17].

Antigens

All 6X His-tagged recombinant protein antigens were coupled with xMAP® beads (Luminex Corporation, Austin, TX, USA) for fluorescence-activated cell sorting (FACS) using Luminex experiments. The antigens investigated in the present study include: protein secretion system ESX-1-associated factors EsxA and EsxB; nuclease (Nuc); peptidoglycan hydrolase LytM; immune-dominant antigen A (IsaA); glucosaminidase; lipase; foldase-protein PrsA; clumping factor A and B (ClfA and ClfB); SD-repeat containing proteins D and E (SdrD and SdrE); iron-responsive surface determinants A and H (IsdA and IsdH); fibronectin-binding proteins A and B (FnbpA and FnbpB); extracellular fibrinogen-binding protein (Efb); *S. aureus* surface protein G (SasG); staphylococcal complement inhibitor (SCIN); chemotaxis inhibitory protein of *S. aureus* (CHIPS); formyl peptide receptor-like inhibitory protein (FLIPr); formyl peptide receptor-like inhibitory like protein (FLIPr-L); alpha toxin; gamma-hemolysin B (HlgB); leukocidins D and F (LukD, LukF); staphylococcal enterotoxins A–E, G–J, M–O, Q, R (SEA–SEE, SEG–SEJ, SEM–SEO, SEQ, SER); exfoliative toxins A (ETA); toxic shock syndrome toxin 1 (TSST-1); staphylococcal superantigen-

like proteins 1, 3, 5, 9, 10, and 11 (SSL1, SSL3, SSL5, SSL9, SSL10, and SSL11); and hypothetical proteins (SA0104 and SA2097).

SasG, SdrD, SdrE, ClfB, IsdA, IsdH, FnbpA, and FnbpB were purified as described previously [21, 27]. The genetic constructs were kindly provided by T. Foster (Trinity College, Dublin, Ireland). Alpha toxin, HlgB, LukD, LukE, LukF, LukS, SEA, and SEC were prepared as described previously [17]. All other proteins were kindly provided by other research groups, as indicated in the acknowledgments. Coupling protein antigens with xMAP® beads was performed using a protocol described earlier [28, 29].

Measurement of antibodies against *S. aureus* antigens

Fluorescence-activated cell sorting (xMAP®; Luminex Corporation) was used for measuring antibodies against staphylococcal antigens in serum samples of bacteremia patients, as described before [20, 28, 30]. Duplicate measurements were performed for all serum samples diluted 100 times in PBS. A 200 times-diluted secondary phycoerythrin (PE)-labeled goat anti-human antibody against total IgG was used for the detection of antibody binding. Median fluorescence intensities (MFIs), a semi-quantitative measure of antibody levels, were averaged. All duplicated measurements that had a coefficient of variation (CV) of more than 25% were excluded from statistical analysis.

Statistics

The (fold) increase or decrease in the MFI values for each antigen on day 1 and day 7 among patients and controls were calculated. The nonparametric Mann–Whitney *U* test was used to compare the antibody levels of bacteremia patients and controls, and the nonparametric Wilcoxon signed ranks test was used to compare the antibody levels of bacteremia patients and controls on days 1 and 7. All statistical analyses were performed using SPSS version 15.0 and *P* values less than 0.05 were considered significant.

Results

Thirty-seven *S. aureus* strains were isolated from 27 bacteremia patients (10 from anterior nares and 27 from the blood stream). Among the nasal isolates, 8 were typed as t037/ST239 whereas the other two were typed as t421/ST239, and t6811/non-typeable by MLST. One blood isolate was typed as t421/ST239 whereas all others were t037/ST239. All carrier patients showed nondiscriminate types for the isolates from blood and nares (Table 1). Among controls, 8 were MSSA nasal carrier with bacteria showing genetically different backgrounds (t8021/NT,

Table 1 Patients' demographic data and strain typing results. First blood was taken after confirmation of bacteremia in the patient

Patient ID	Carrier status	Race	Age	Date blood culture taken	Bacteremia confirmation date	First blood taken date	Second blood taken date	Blood-isolated strain <i>spa</i> type (MLST)	Nasally isolated strain <i>spa</i> type (MLST)	Ward	Reason for hospitalization
P1	Noncarrier patients	Chinese	65	18.9.11	21.9.11	19.9.11	22.9.11	t037 (ST239)	–	U2B	ESRF on dialysis
P2		Malay	74	20.9.11	22.9.11	21.9.11	26.9.11	t037 (ST239)	–	SW14	Lung infection, sepsis
P4		Malay	57.8	1.10.11	4.10.11	4.10.11	7.10.11	t037 (ST239)	–	SW27	Line-related sepsis
P5		Malay	55.8	11.10.11	13.10.11	14.10.11	19.10.11	t037 (ST239)	–	SW13	Wound
P6		Malay	23.2	14.10.11	16.10.11	17.10.11	20.10.11	t037 (ST239)	–	NW3	Burn
P9		Indian	22	21.10.11	23.10.11	22.10.11	27.10.11	t037 (ST239)	–	NW3	Burn
P10		Chinese	71	25.10.11	26.10.11	27.10.11	1.11.11	t037 (ST239)	–	SW6	Wound
P15		Malay	55	27.10.11	29.10.11	30.10.11	2.12.11	t037 (ST239)	–	SW13	Wound
P17		Chinese	81	17.11.11	18.11.11	18.11.11	22.11.11	t037 (ST239)	–	SW13	Wound
P19		Indian	60.6	6.12.11	7.12.11	7.12.11	10.12.11	t037 (ST239)	–	SW26	Wound
P22	Carrier patients	Indian	46.2	4.1.12	5.1.12	5.1.12	10.1.12	t037 (ST239)	–	U2B	Dialysis, renal failure
P23		Malay	45	4.1.12	5.1.12	5.1.12	9.1.12	t037 (ST239)	–	SW26	Unknown
P25		Malay	77	11.1.12	13.1.12	11.1.12	16.1.12	t037 (ST239)	–	SW27	Wound, ESRF
P26		Malay	55	17.1.12	18.1.12	17.1.12	27.1.12	t037 (ST239)	–	GICU.NW10	Sacral sore grade 2
P27		Malay	20.2	20.1.12	21.1.12	20.1.12	26.1.12	t037 (ST239)	–	NW11	Unknown
P28		Malay	16.3	27.1.12	28.1.12	27.1.12	1.2.12	t037 (ST239)	–	Burn	Burn
P31		Malay	62.5	12.2.12	13.2.12	14.2.12	17.2.12	t037 (ST239)	–	U2B	Dialysis, renal failure
P3		Indian	69.2	24.9.11	26.9.11	25.9.11	2.10.11	t037 (ST239)	t037 (ST239)	NW23	Sepsis
P8		Chinese	45	18.10.11	20.10.11	21.10.11	24.10.11	t037 (ST239)	t037 (ST239)	UN2B	ESRF
P11		Indian	57.9	29.10.11	30.10.11	31.10.11	3.11.11	t037 (ST239)	t037 (ST239)	U1B	Dialysis, renal failure
P12		Chinese	25.7	30.10.11	1.11.11	3.11.11	8.11.11	t421(ST239)	t421(ST239)	UN1B	Dialysis, renal failure
P13		Chinese	81	3.11.11	5.11.11	4.11.11	8.11.11	t037 (ST239)	t6811(NT)	NW2	Unknown
P14		Chinese	48	29.10.11	31.11.11	29.10.11	9.11.11	t037 (ST239)	t037 (ST239)	UN2B	Dialysis, renal failure
P16		Indian	67	22.11.11	24.11.11	23.11.11	27.11.11	t037 (ST239)	t037 (ST239)	GICU	Unknown
P18		Malay	65	4.12.11	6.12.11	5.12.11	9.12.11	t037 (ST239)	t037 (ST239)	UN2A	Dialysis, renal failure
P21		Malay	65.1	3.1.12	4.1.12	4.1.12	11.1.12	t037 (ST239)	t037 (ST239)	U2A	Dialysis, renal failure
P24		Indian	61	9.1.12	10.1.12	10.1.12	14.1.12	t037 (ST239)	t037 (ST239)	GICU.NW10	Unknown

ESRF end-stage renal failure, *GICU* general intensive care unit, *U* and *UN* urology wards, *SW* surgery ward, *NW* general medicine, *NT* nontypeable

t189/ST188 [$n = 2$], t735/ST97, t03/ST1153, t10560/ST96, t1445/ST1209, 127/ST10).

Polymer chain reaction

Genes for staphylococcal super antigen-like proteins (SSL1, SSL3, SSL5, SSL9, SSL10, SSL11), alpha toxin, clumping

factor A and B, fnbpA, fnbpB, Efb, immune-dominant staphylococcal antigen (IsaA), Iron-regulated surface determinant proteins (IsdA, IsdH), PrsA, sasG, serine-aspartate (SD) repeat-containing proteins (sdrD, sdrE), SEA, SEJ, SEQ, SEM, leukocidin D (LUKD), chemotaxis inhibitory protein of staphylococci (CHIPS), EsxA, HlgB, FlipR, FlipR-L, and lipase were observed in all ST239 strains tested, whereas other

genes were not detected (Table 2, Supplementary Tables 1, 2). The ST239 strains could not be discriminated by this type of PCR testing, as the results for all genes were equal for all isolates.

MFI values on day 1 and day 7 among patients

Mean MFI values reflecting IgG antibody levels against the corresponding antigens are illustrated in Table 2 and Supplementary Tables 1 and 2. MFIs measured on day 1 and day 7, when compared with the individual patients and controls, showed no significant differences for most antigens (Supplementary Tables 1, 2).

However, the mean MFI was significantly higher on day 7 for ClfA ($P = 0.001$), FnbpA ($P = 0.020$), sasG ($P = 0.015$), SEH ($P = 0.037$), SA0104 ($P = 0.045$), and SA2097 ($P = 0.011$) in the control group. For the patient group a higher MFI was observed for ClfA ($P = 0.02$), FnbpA ($P = 0.008$), IsaA ($P = 0.004$), and SER ($P = 0.024$; Table 2, Supplementary Tables 1, 2).

MFI values among patients and controls

In each bacteremia patient a unique pattern of *S. aureus*-specific antibody levels after infection were observed (Supplementary Figs. 1–16). The median MFI among patients increased compared with controls for most of the antigens. Antibody levels among bacteremia patients were significantly higher for the following antigens: HlgB ($P = 0.001$), LukD ($P = 0.009$), LukF ($P = 0.0001$), SEA ($P = 0.0001$), SEB ($P = 0.011$), SEC ($P = 0.010$), SEQ ($P = 0.049$), IsaA ($P = 0.043$), IsdA ($P = 0.038$), IsdH ($P = 0.01$), SdrD ($P = 0.001$), SdrE ($P = 0.046$), EsxA ($P = 0.0001$), SA2097 ($P = 0.001$), and SA0104 ($P = 0.0001$) when MFIs on the day of infection (day 1) were compared with day 1 in the control group (Table 2, Supplementary Tables 1, 2; Supplementary Figs. 1–16).

When MFI on day 7 after infection was compared with those of controls, antibody levels were still significantly higher among bacteremia patients for most of the above-mentioned antigens except for SEB ($P = 0.057$), SEQ ($P = 0.061$), and SdrE ($P = 0.387$; Table 2, Supplementary Tables 1, 2). However, for certain antigens, such as SCIN ($P = 0.046$), SEI ($P = 0.038$), and EAP ($P = 0.047$; Table 2, Supplementary Tables 1, 2), the MFI value was significantly higher only on day 7 after infection.

On the other hand, for SSL3 ($P = 0.009$, $P = 0.002$), SSL9 ($P = 0.002$, $P = 0.0001$), and SSL10 ($P = 0.007$, $P = 0.001$), significantly lower MFIs were detected on day 1 and day 7 of infection. For lipase ($P = 0.001$), the MFIs measured for patients were lower after 7 days (Table 2, Supplementary Tables 1, 2).

Discussion

In the present study, we investigated the humoral immune response against 46 staphylococcal antigens in bacteremia patients, all infected by a similar MRSA type (ST239), versus controls. The presence of the genes corresponding to the antigens was also investigated. The observation of unique patterns of *S. aureus*-specific immune responses in each patient is in concordance with data reported by Verkaik et al. (2010) [20]. The genetic diversity among the strains and expression of different staphylococcal proteins during infection or host factors were suggested as possible causes for this variability [20]. In the current study, all bacteremia patients were infected by highly homologous MRSA ST239 strains. Therefore, differences in the level of specific IgG antibodies between some patients here could be due to the difference in the level of immunity among patients as a result of various past exposures.

We observed significant increases in the antibody levels against leukocidin F, whereas the PCR result for the corresponding genes was negative in ST239 MRSA strains. Such an increased response against certain antigens in the absence of the corresponding genes has been reported before [20] and could be due to cross-reactivity with different antigenic components with structurally similar domains [31–33]. Den Reijer and colleagues reported the immunological cross-reactivity between leukocidins F and D and HlgB [17]. Interestingly, genes for leukocidin D and HlgB were present in the genomes of the ST239 MRSA strains involved and IgG antibody levels against these toxins were significantly higher among bacteremia patients than among controls. Cross-reactivity may also explain the higher MFI levels against SEB and SEC among patients, again in the absence of the corresponding genes, whereas clearly higher levels of antibodies against the structurally similar enterotoxins (SEA and SEQ) were detected.

We observed significantly higher levels of IgG antibody against HlgB in bacteremia patients. Recently, Malachowa et al. showed the up-regulation of *hlgABC* in USA300 during culture in human blood in combination with the induction of in vitro lysis of human PMNs [18]. Similar findings were also presented by den Reijer et al. in two distinct *S. aureus* strains (t209, t122), where the IgG-binding protein *sbi* and the gamma-hemolysin A and B precursors were up-regulated when strains were cultured in blood [17]. Our data, together with those of the above studies, suggest that HlgB could be a potential vaccine candidate, as it seems to trigger an immune response irrespective of the strain type.

The multi-virulent nature of *S. aureus* and the fact that monoclonal vaccines failed emphasize the importance of multi-component vaccines [34]. A combined vaccine of four cell-wall-anchored proteins (IsdA, IsdH, SdrD, and SdrE) showed successful protection in an animal model [13]. On the other hand, a recent study on IsaA, which is a secreted protein with an ill-defined housekeeping function, put it

Table 2 Luminex assay and statistical analysis

Antigen	PCR	Study group	Mean MFI	Mean MFI	MFI on day 1 compared with day 7 group (<i>P</i> *)	Ratio		MFI among patients in comparison to controls (<i>P</i> **)	
			Day 1	Day7		Patient: control	Patient: control	Day 1	Day7
HIgb	Positive	Patients	15,379	15,622	0.400	1.23:1	1.25:1	0.001	0.000
		Controls	12,552	12,481	0.800				
LUKD	Positive	Patients	15,413	15,630	0.486	1.18:1	1.20:1	0.009	0.005
		Controls	13,084	13,074	0.936				
LUKF	Negative	Patients	4,748	5,168	0.137	1.63:1	1.71:1	0.000	0.000
		Controls	2,905	3,016	0.189				
IsdA	Positive	Patients	6,169	6,461	0.130	1.40:1	1.39:1	0.038	0.046
		Controls	4,403	4,663	0.658				
IsdH	Positive	Patients	4,118	4,185	0.683	3.97:1	3.57:1	0.010	0.016
		Controls	1,038	1,172	0.060				
IsaA	Positive	Patients	9,912	11,010	0.004	1.51:1	1.58:1	0.043	0.010
		Controls	6,544	6,979	0.204				
SdrD	Positive	Patients	486	765	0.209	3.54:1	4.97:1	0.001	0.005
		Controls	137	154	0.043				
SEA	Positive	Patients	14,726	15,257	0.101	2.71:1	2.75:1	0.000	0.000
		Controls	5,443	5,540	0.491				
SEC	Negative	Patients	13,307	13,470	0.683	1.24:1	1.17:1	0.010	0.040
		Controls	10,690	11,501	0.280				
SSL3	Positive	Patients	6,079	5,939	0.773	1:1.42	1:1.51	0.009	0.002
		Controls	8,613	8,950	0.261				
SSL9	Positive	Patients	3,583	3,460	0.564	1:1.63	1:1.77	0.002	0.000
		Controls	5,838	6,124	0.237				
SSL10	Positive	Patients	5,454	5,319	0.269	1:1.47	1:1.57	0.007	0.001
		Controls	8,014	8,346	0.106				
EsxA	Positive	Patients	29	26	0.927	2.22:1	2.21:1	0.000	0.000
		Controls	13	12	0.367				
SA0104	Negative	Patients	296	326	0.304	5.93:1	5.72:1	0.000	0.000
		Controls	50	57	0.045				
IsdA	Positive	Patients	6,169	6,461	0.130	1.40:1	1.39:1	0.038	0.046
		Controls	4,403	4,663	0.658				
IsdH	Positive	Patients	4,118	4,185	0.683	3.97:1	3.57:1	0.010	0.016
		Controls	1,038	1,172	0.060				
SA2097	Negative	Patients	49	80	0.134	3.08:1	3.81:1	0.001	0.001
		control	16	21	0.011				
IsaA	Positive	Patients	9,912	11,010	0.004	1.51:1	1.58:1	0.043	0.010
		Controls	6,544	6,979	0.204				

Patients (day 1: day of infection, day7: 7th day after infection); controls (day 1: first day of hospital admission, day 7: 7th day after admission)

Italic font indicates statistical significance

*Wilcoxon signed rank test

**Mann–Whitney *U* test

forward as a candidate for antibody-based therapy with a potentially promising ability to improve the outcome of *S. aureus* infection [35]. In a recent study, high levels of specific IgG against IsaA and IsdA among patients with the blistering disease epidermolysis bullosa were reported [36]. Our finding of elevated levels of antibodies against IsaA, IsdA,

IsdH, and SdrD among bacteremia patients is in agreement with data reported from different geographic regions [17, 20]. This suggests that these antigens could be good targets to be included in a multi-component vaccine.

Many toxins are expressed in vivo during staphylococcal infection, which supports their role in *S. aureus* pathogenesis

[27]. Among our bacteremia patients, no significant increase in antibodies level against a group of *S. aureus* super antigens in the *egc* enterotoxin gene cluster (*egc*) was observed. Corresponding genes for these SAGs were absent among ST239 strains, except for *sem*. This is in line with the data reported among bacteremia patients previously [17, 20, 37]. Furunculosis patients also developed a low level of antibodies against *egc* SAGs [38]. In contrast, patients suffering from epidermolysis bullosa showed a higher level of antibodies for this *egc* SAGs compared with healthy individual controls [36]. On the other hand, genes in the *egc* cluster are more frequently observed among nasal strains than in strains from infections [39–41]. All these studies indicate that SAGs (*egc*) may not play critical roles in pathogenesis during staphylococcal bacteremia and furunculosis, whereas they may represent significant factors in epidermolysis bullosa. Although *egc* and non-*egc* SAGs are not greatly different with regard to in vitro properties such as T cell activation, gene regulation, cytokine secretion, or induction of T cell proliferation [42], diversity in immune response against different types of *S. aureus* infection emphasizes the fact that they may not be good candidates for vaccine development.

The staphylococcal enterotoxin mainly associated with staphylococcal food poisoning [43–46] is SEA and this is also reported to play an inflammatory role in skin and lung infections [47, 48]. Antibodies against SEA are commonly observed in healthy individuals [49, 50]. An association between bacteremia and the presence of genes for *sea* and *tst* in the corresponding strains has been reported before [21]. In previous studies among bacteremia patients, no significant differences in IgG levels against SEA were reported [17, 20], whereas the prevalence of the corresponding gene among causative strains was quite low (7.6% and 14.6%). Here, we observed significantly higher levels of anti-SEA IgG among all bacteremia patients infected with *sea* gene-positive ST239 MRSA. Our results suggest that SEA could represent a potential active vaccination candidate and that anti-SEA IgG could be used as a passive vaccine.

Staphylococcal superantigen-like proteins (SSLs) are structurally similar to superantigens, but do not share superantigenic activity [51–53]. These proteins interrupt the action of host immune factors: SSL3, for instance, binds to toll-like receptor 2 (TLR2) and interrupts the cytokine production by macrophages [54, 55]. SSL10 inhibits the classical complement pathway by binding to C1q and IgG in general [56]. FC receptor-mediated phagocytosis of neutrophils is interrupted by SSL10 binding to IgG [57]. Gene expression studies of *S. aureus* strains grown in blood revealed no significant up-regulation of SSL genes [17, 18]. In our study, no statistically significant difference in the level of IgG antibodies against SSL1 and SSL10 among bacteremia patients and controls was observed, whereas antibody levels against SSL3, 9, and 10 were significantly reduced among bacteremia patients.

Conclusion

Clonal isolates of MRSA ST237 induce different immune responses in different patients suffering from bacteremia. However, some antigens induce comparable responses in all patients. We recommend including these antigens (IsaA, IsdA, IsdH, SdrD, and HlgB) in future multi-component vaccines. SEA plays an important role during ST239 MRSA bacteremia and antibody-based therapy using anti-SEA IgG could be a good passive vaccination strategy to improve the outcome of infection. To test the efficacy, additional investigations on the details of the immune response against SEA in other infection models and during in vivo expression are still needed. Our data indicate that as yet ill-defined human factors play an important role in anti-staphylococcal immune regulation.

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Compliance with ethical standards The study is in compliance with ethical standards.

Conflicts of interest None to declare except that Alex van Belkum is an employee of bioMérieux, a company developing and selling infectious disease diagnostics.

Ethics approval This study was performed upon approval from the National Institutes of Health for conducting research in the Ministry of Health Malaysia (NMRR-10-488-5953).

Informed consent All subjects were included in the study only if they or their close relatives provided oral consent.

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