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ORIGINAL ARTICLE

Staphylococcal aureus bacteraemia: Clinical characteristic & evaluation of Prolex Staph Xtra latex agglutination test in the rapid identification of *Staphylococcus aureus*

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Abstract

Background: Staphylococcus aureus is a leading cause of community as well as healthcare-associated bacteraemia. This study aimed to describe clinical characteristics of *S. aureus* bacteraemia (SAB) and to evaluate the performance of the Prolex Staph Xtra Latex agglutination test in the identification of *Staphylococcus aureus*. *Methods*: Cross-sectional study was conducted from Jun 2018 to May 2019. Isolates from first-positive peripheral blood cultures were tested with Prolex Staph Xtra Latex agglutination test, together with routine tube coagulase and DNase test. All isolates were further confirmed with Vitek2 GP. *Results:* Hundred isolates were tested with Prolex Staph Xtra Latex. Twelve isolates were excluded due to incomplete medical records. Eighty-eight isolates were analysed, yielded sensitivities, specificities, positive and negative predictive values of 100%, 91.7%, 98.7%, and 100%, respectively. Of these, 76 were identified as *S. aureus* and 12 CoNS. Seventy-six patients were included in the SAB analysis. Fifty-nine out of 76 (78.6%) had underlying comorbidities. Thirty-four percent of the episodes were considered as primary SAB. Skin and soft tissue infection were accounted for the highest source of bacteraemia, 24(31.6%). Both MRSA and MSSA bacteraemia were seen mostly among healthcare-associated bacteraemia (HCA) (7/16, 43.8% and 28/60, 46.7%). Liver cirrhosis was significantly associated with MRSA bacteraemia (P=0.048). Metastatic infection & complicated SAB were identified in 13(17.1%) and 30(39.5%) of cases, respectively. All-cause mortality was 22.4%. *Conclusion: S. aureus* bacteraemia is a serious infection associated with significant metastatic complications and mortality. Prolex Staph Xtra Latex agglutination test has excellent sensitivity and specificity with 100% and 91.7% respectively.

Keywords: *Staphylococcus aureus* bacteraemia, rapid identification, latex agglutination test

INTRODUCTION

Staphylococcus aureus is a leading cause of community as well as healthcare-associated bacteraemia, resulting in significant morbidity and mortality worldwide.[1] It is the second most common pathogen causing bacteraemia and a leading cause of osteoarticular, skin, soft tissue and device-related infections.[2,3] The annual incidence rate for *S. aureus* bacteraemia (SAB) ranges from 10 to 30 per 100,000 population-year.[1] Previous studies identified underlying comorbidities, presence of an indwelling intravenous catheter, prosthetic devices, haemodialysis-dependent patient and HIV-infected patients were the main risk factors associated with SAB.[1,4]

The detection of *S. aureus* in blood culture specimens should always be considered clinically significant as it can lead to serious complications. Delay in the initiation of appropriate empirical antibiotic treatment in SAB was associated with a higher mortality rate.[5] Metastatic infection occurred in 19% to 34% of patients with SAB and 30-day mortality is up to 20% to 30%.[6,7] Misidentification of *S. aureus* as coagulase-negative staphylococcus (CoNS), which is commonly associated with culture contamination can result in undertreated of significant SAB.

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Therefore, a rapid diagnostic test that accurately distinct *S. aureus* from CoNS is crucial for early diagnosis and initiation of appropriate empirical antibiotics, resulted in optimal management and improved outcomes for the patients.

Differentiating *S. aureus* from other CoNS has traditionally been done using a tube coagulase method. Although this test is reliable, it takes 4 to 24 hours to provide results.[8] The introduction of the latex agglutination test has allowed more rapid detection of *S. aureus*. However, some MRSA strains masking the cell wall protein detection, thus giving false negatives results in some agglutination assays with sensitivity as low as 49.2 % to 73.8 %.[9] Latest third-generation assays have been developed to detect capsular polysaccharides 5 and 8, which further improved the detection of MRSA strain.[10] Prolex Staph Xtra is a 4th generation agglutination assay that incorporated blue carboxylated microparticle, to further reduce nonspecific reactions, thus increasing its sensitivity and specificity. Evaluation of 4th generation assay has been proved to have comparable performance to 3rd generation latex assay.[11,12] However, there is a lack of studies evaluating the performance of Prolex Staph Xtra. The objective of this study was to determine the clinical characteristic of *S. aureus* bacteraemia and to evaluate the performance of the Prolex Staph Xtra Latex (Pro-Lab Diagnostics) agglutination test in the rapid identification of *S. aureus*.

MATERIAL AND METHODS

Study design and population

The retrospective cross-sectional study was conducted over a period of one year (2018 to 2019) at the Microbiology Unit, Pathology Department, Hospital Kuala Lumpur (HKL). First-positive peripheral blood cultures of hospitalised adult patients aged more than 18 years old, suspected to have *Staphylococcal aureus bacteraemia* were enrolled. Patients with polymicrobial bacteraemia were excluded to avoid the influence of multiple pathogens on the analysis of clinical characteristics and outcomes.

Material and method

A positive blood culture demonstrated gram-positive cocci in cluster (GPCC) was plated onto an in-house blood agar plate as per standard protocol. Colony of *staphylococcus* spp. was identified by positive catalase (3% H^2O^2) test. A total of 100 pure and fresh isolates of suspected *S. aureus* were tested with Prolex Staph Xtra Latex agglutination test, according to the manufacturer's instructions, and together in conjunction with standard laboratory tube coagulase and DNase test. The tube coagulase test was performed by mixing a single colony into an in-house coagulase test broth, incubated at 37°C and examined for clot formation at 4 hours and 24 hours. DNase test was performed by using a DNase agar plate (Thermo Scientific™). A suspected *S. aureus* colony was streaked on the agar and incubated at 37°C for 24 hours. Toluidine Blue O (TBO) was then flooded on the plate and observed for bright pink zone of clearing around the colony, which is indicative of a positive test result. The antimicrobial susceptibility test was performed using the disc diffusion method following Clinical Laboratory Standards Institute guideline[12] (CLSI M100-S29) for *S. aureus*. Vitek2 GP (BioMérieux Inc. France), an automated bacterial identification was taken as the gold standard method in the identification of specific strains of isolates in this study.

Data collection

Patient's demographics and clinical data including age, gender, race, ward, comorbidities, setting of bacteraemia (hospital-acquired or community-acquired), sources of bacteraemia (primary or secondary), clinical findings and outcome were collected from the patient's hospital medical record and patient's clinical notes.

Study Definitions

Staphylococcus aureus bacteremia (SAB) was defined as the presence of one or more positive blood cultures for *S. aureus* in patients with signs and symptoms of infection. Primary bacteremia was defined as an infection that occurs with no source of infection was documented or identified after careful examination of clinical signs, microbiological findings or imaging results. The onset of SAB was defined as the date of collection of the first blood culture yielding positivity for *S. aureus*. Persistence SAB was defined as persistent blood culture positive for *S. aureus* bacteremia for 3 days or more from the onset of SAB.[13] Immunosuppressive therapy was defined as those with recent chemotherapy or on prolonged steroid therapy (received within one month before the onset of infection). Recent hospitalisation was defined as any admission to a hospital or health care facility for the past 90 days.[14] Diabetes mellitus was defined as the

patient having a clinical diagnosis of diabetes mellitus and taking at least an oral or non-insulin injectable hypoglycemic agent and/or insulin. Bacteraemia was considered community-acquired (CA) when the onset of SAB appeared within 48 h of admission while hospital-acquired (HA) was defined when the onset of SAB appeared after 48 h of admission. Healthcare-associated (HCA) infection was defined as a positive blood culture obtained from a patient at the time of hospital admission or within 48 hours of admission if the patient fulfilled any of the criteria by Friedman *et al*. (2002).[15] Complicated SAB was defined as a patient that fulfilled any of the following criteria; evidence of metastatic infection of *S. aureus*, infective endocarditis detected on echocardiography, those that did not achieve defervescence within 72hr or persistent positive culture of *S. aureus* at >4 days after intravenous therapy.[16] Metastatic infection was defined as a distant focus of infection that is anatomically unrelated to the implicated source. Mortality in this study was in-hospital death from all-cause mortality.

Statistical Analysis

Data were analysed using Statistical Package for the Social Science (SPPS) software (IBM, USA) version 23.0. Mean and median were used to describe the characteristics of the study population. Categorical variables were compared using the chi-square test. *P-value* less than 0.05 represents statistical significance.

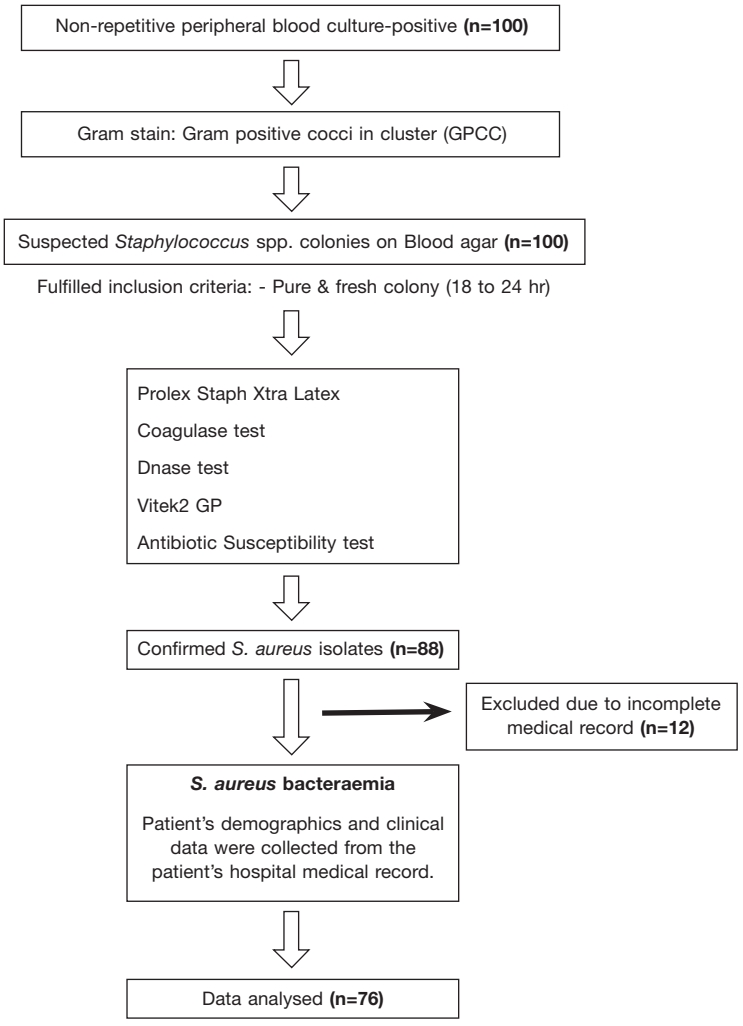

FIG. 1: Flowchart of the study

Table 1: Performance of Prolex Staph Xtra Latex Agglutination test

		Prolex Staph Xtra Latex Agglutination test		
		Positive	Negative	Total (n=100)
Vitek 2 GP	*S. aureus*	76	0	76
	CoNS *	1[#]	11	12
	Total	77	11	88
	Sensitivity (%)			100
	Specificity (%)			91.7
	PPV (%)			98.7
	NPV (%)			100

PPV, positive predictive value; NPV, negative predictive value.
* *coagulase-negative staphylococci*
[#] This false-positive reaction isolate corresponded to *S. pseudointermedius*

RESULTS

Evaluation of Prolex Staph Xtra Latex agglutination kit

A total of 100 isolates of suspected *staphylococcus* spp. from non-repetitive peripheral blood culture-positive were collected and were tested with Prolex Staph Xtra Latex agglutination kit. Twelve isolates were excluded due to incomplete medical records. Out of these, 76 isolates were identified as *S. aureus* and 12 isolates were identified as coagulase-negative staphylococci (CoNS). The overall sensitivity, specificity, positive and negative predictive values of the Prolex Staph Xtra latex test in *S. aureus* identification were 100%, 91.7%, 98.7%, and 100%, respectively (Table 1). All isolates demonstrated clear agglutination within 20 seconds of the recommended rocking period and no indeterminate result was obtained by the latex test. Among 12 CoNS isolates, 7 species were identified. There were 2 *S. epidermidis*, 2 *S. haemolyticus*, 2 *S. hominis*, 2 *S. capitis*, 1 *S. saprophyticus,* 1 *S. pseudointermedius* and 2 *S. warneri*. Eleven out of 12 CoNS isolates yielded negative results by the latex test, except one CoNS isolate gave false positive when tested with both latex and routine tests (tube coagulase and DNase test), which identified as *S. pseudointermedius* by Vitek2 compact system (BioMérieux Inc. USA).

Table 2 demonstrated comparable sensitivity and specificity of both tube coagulase and DNase tests (sensitivity 100%, specificity 91.7%) to the rapid Prolex Staph Xtra Latex test. Among 76 confirmed *S. aureus* isolates, 60 (78.9%) were methicillin-sensitive *S. aureus* (MSSA) strains and 16 (21.1%) were methicillin-resistant *S. aureus* (MRSA) strains. The latex test was able to accurately identify all of the MSSA and MRSA strains.

Fig. 2 shows the resistance rate of the *S. aureus* strains (MSSA and MRSA) to various antibiotics tested. Most of the strains (61/76) were resistant to penicillin (80.3%). All the strains were susceptible to vancomycin, co-trimoxazole, rifampicin & mupirocin. Resistance rates to other antibiotics were generally higher in MRSA as compared to MSSA; clindamycin (75% vs 3.3%), erythromycin (75% vs 5%), gentamicin (6.3% vs 0%) (Figure 3). The range of minimum inhibitory concentration (MIC) among MRSA isolates was 0.38 mcg/ml to 1.0 mcg/ml.

Demographic data

Table 3 displayed the demographic data and the clinical characteristic of the subjects studied (n=76). A total of 76 episodes of SAB were enrolled. The median age of the patients was 57 years with aged more than 60 years accounted for the highest percentage (43.4%). Forty-eight

Table 2: Sensitivity and specificity of Tube coagulase, DNase and Prolex Staph Xtra Latex test

	Tube coagulase test	DNase test	Prolex Staph Xtra Latex test
Sensitivity (%)	100	100	100
Specificity (%)	91.7	91.7	91.7

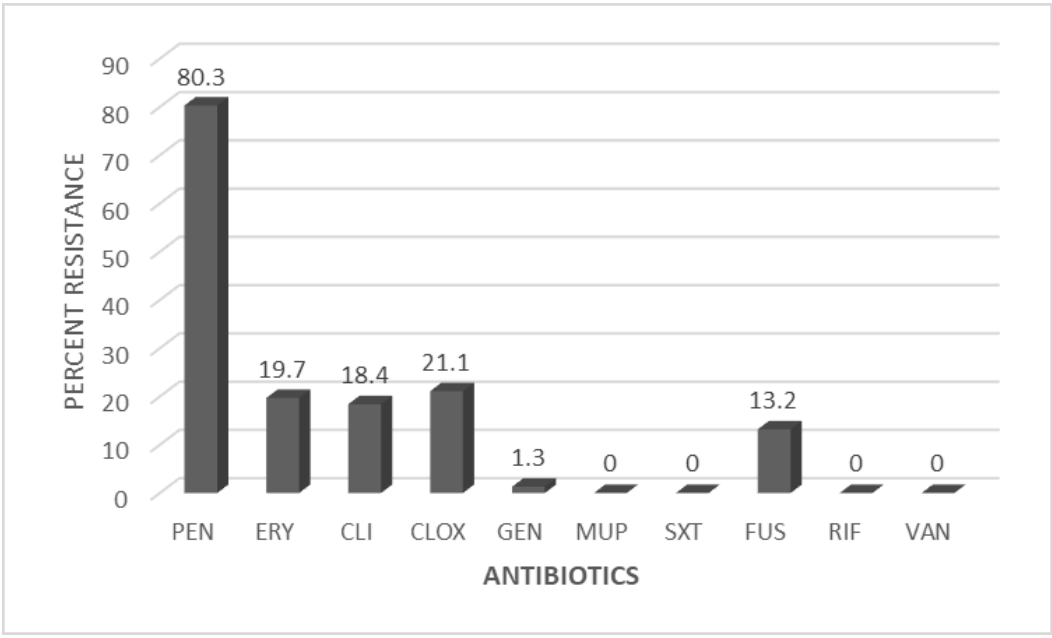

FIG. 2: Antibiotic-resistant rate of *S. aureus* (MSSA and MRSA).
Abbreviations: PEN, penicillin; ERY, erythromycin; CLI, clindamycin; CLOX, cloxacilin GEN, gentamicin; MUP, mupirocin; SXT, co-trimoxazole; FUS, fusidic acid; RIF, rifampicin; VAN, vancomycin.

(63.2%) were male, whereas 28 (36.8%) were female. Malay ethnic group recorded the highest number of cases 47 (61.8%), followed by the Chinese 12 (15.8%), India 12 (15.8%) and others (Myanmar, Bangladesh and Indonesia). The majority of the patients had underlying comorbidities, with 59 (78.6%) had a minimum of at least one comorbid. Diabetes mellitus was the most common comorbid 47 (61.8 %), followed by chronic kidney disease 35 (46.1%)

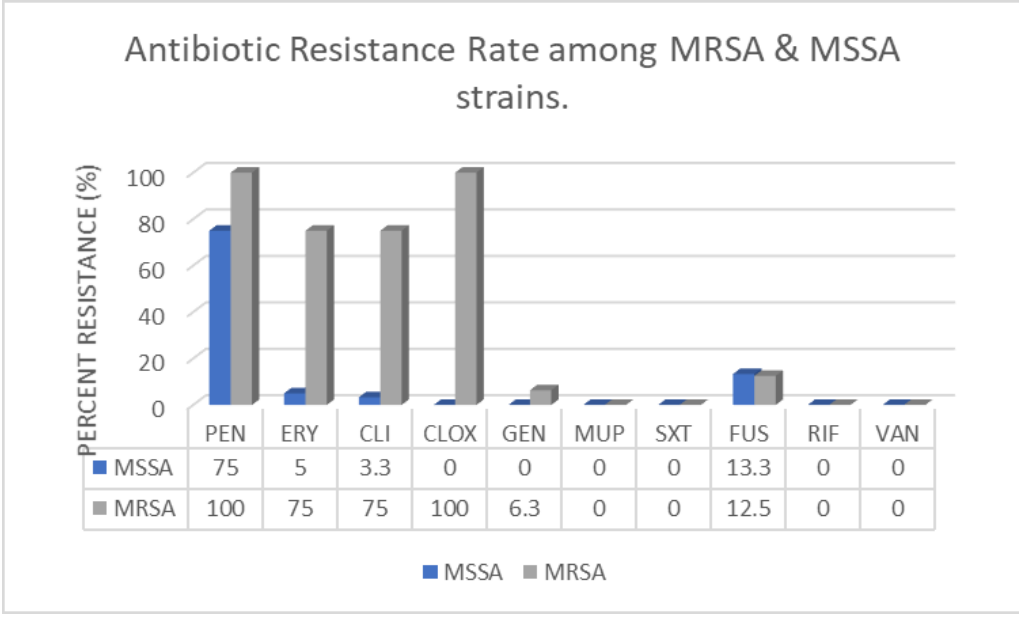

FIG. 3: Antibiotic-resistant rate of MRSA and MSSA strains.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*

Table 3: Descriptive characteristics of patients with *Staphylococcus aureus* bacteraemia

Demographic (n=76)	**n (%) [a]**
Age, years (median, IQR)	57 (29.75)
Age group (years)	
18-29	9 (11.8)
30-39	11 (14.5)
40-49	7 (9.2)
50-59	16 (21.1)
> 60	33 (43.4)
Gender	
male	48 (63.2)
female	28 (36.8)
Ethnicity n (%)	
Malay	47 (61.8)
Chinese	12 (15.8)
Indian	12 (15.8)
Others [b]	5 (6.6)
Discipline unit	
Medical	36 (47.4)
Nephrology/urology	22 (28.9)
Orthopaedic	5 (6.6)
Surgical	5 (6.6)
Others [c]	8 (10.5)
Setting of acquisition	
Community-acquired (CA)	18 (24)
Healthcare-associated (HCA)	33 (44)
Hospital-acquired (HA)	25 (33.3)
Charlson comorbid index (CCI) (median)	4.00
Low (0-1)	18 (23.7)
Medium (2-4)	21 (27.6)
High (≥5)	37 (48.7)
Presence of comorbid [d]	59 (78.6)
Comorbid diseases	
Diabetes mellitus	47 (61.8)
Chronic kidney disease (CKD)	35 (46.1)
Liver cirrhosis	3 (3.9)
Congestive heart failure (CHF)	12 (15.8)
HIV infection/AIDS	2 (2.6)
Active malignancy	5 (6.6)
Risk factor	
CVC (>72hr)	29 (38.2)
Recent hospitalisation	21 (27.6)
Intravenous drug user (IVDU)	1 (1.3)
Immunosuppressive therapy	2 (2.6)
Clinical finding	
Fever ≥ 38°C	60 (78.9)
Septic shock (systolic BP < 90 mm/Hg)	17 (22.6)
Pitt Bacteraemia score (mean/median)	(1.22/0.00)

Demographic (n=76)	n (%) [a]
Laboratory findings	
Leucocytosis (WBC count >10,000/mm3)	54 (72)
Thrombocytopenia (platelet <150,000/mm3)	22 (29.3)
Leukopenia (WBC count <4000/mm3)	2 (2.7)
Source of bacteraemia	
Unknown	26 (34.2)
CRBSI	23 (30.3)
Skin/soft tissue infection	24 (31.6)
Surgical site infection	3 (3.9)
Complicated SAB	30 (39.5)
Metastatic infection	13 (17.1)
Persistence bacteraemia [e]	19 (34.5)
Length of hospitalisation (days) (median, IQR)	13 (3)
Death after onset of SAB in days (median, IQR)	10 (24.5)
In-hospital death n (%)	17 (22.4)

Abbreviations: CVC, central venous catheter, CRBSI, Catheter-related blood stream infection

[a] Data represent No. (%) of case-patients or controls, unless otherwise specified.

[b] Myanmar, Bangladesh and Indonesia

[c] Obstetrics & gynecology, radiotherapy & ICU

[d] Presence of at least 1 comorbid

[e] Persistence (defined as bacteremia for ≥ 3 d). Denominator included patient with available follow-up blood culture (n=55)

and congestive heart failure 12 (15.8%). The median charlson comorbid index (CCI) was 4.0 with 37 (48.7%) of the patients had a high score (≥5). The majority of patients with SAB were from the medical and nephrology/urology unit, 47.4% and 28.9%, respectively. Of the 76 patients with SAB, 16 (21.1 %) had MRSA bacteraemia and 60 (78.9 %) had MSSA bacteraemia. MRSA bacteraemia was seen mostly among healthcare-associated (HCA) (7/16, 43.8%) and hospital-acquired (HA) infection, (6/16, 37.5%). A similar distribution was also observed in MSSA bacteraemia, mostly among HCA (28/60, 46.7%) and HA (20/60, 33.3%).

Clinical characteristics of patients with SAB

Thirty-four percent of the episodes were considered as primary SAB. Among patients with secondary SAB, the commonest source was from the skin and soft tissue infection (SSTI), 24 (31.6%), followed by CRBSI, 23 (30.3%) and the remaining were from surgical site infection (SSI) 3 (3.9%). Of these 24 SSTI cases, 12 (50%) were due to thrombophlebitis, 4 (16.7%) were due to cellulitis, and the remaining were due to limb abscess, carbuncle and infected diabetic foot ulcer. Sixty patients (78.9 %) documented a temperature of ≥38°C at the onset of SAB while hypotension (systolic pressure <90 mmHg) was observed in 17 (22.6 %) of the patients. Routine laboratory analyses at the onset of SAB revealed leucocytosis (>10,000/mm3) in 54 (72%) and thrombocytopenia (<150,000/mm3) in 22 (29.3 %) of the patients.

The median length of hospitalisation was 13 days and metastatic infection was observed in 13 (17.1%) of patients. During the study period, death in the hospital occurred in 17 (22.4 %) out of 76 cases of SAB. Among the 17 patients who died, the median days of death from the onset of SAB was 10 days (interquartile range of 24.5).

Characteristics of patients with MRSA in comparison to MSSA staphylococcal bacteraemia were demonstrated in Table 4. In both groups, SAB is predominantly seen in males and patients aged 60 years or more. Diabetes mellitus was the most common premorbid illness seen in both groups and no significant association in premorbid conditions except for liver cirrhosis which was significantly associated with MRSA bacteraemia (P= 0.048). Healthcare-associated infection was predominantly observed in both MRSA and MSSA bacteraemia, 7/16 (43.8%) and 28/60 (46.7%) respectively. Primary bacteraemia

Table 4: Characteristics of patients with staphylococcal bacteraemia caused by MRSA compared with patients with staphylococcal bacteraemia caused by MSSA

Demographic data	MRSA (n= 16) n (%)	MSSA (n= 60) n (%)	χ^2	P value
Age (years)			7.02	0.135
18-29	1 (6.3)	8 (13.3)		
30-39	1 (6.3)	10 (16.7)		
40-49	4 (25)	3 (5)		
50-59	3 (18.8)	13 (21.7)		
>60	7 (43.8)	26 (43.3)		
Gender				
Male	9 (56.3)	39 (65)	0.42	0.519
Female	7 (43.8)	21 (35)		
Baseline diseases (n, %)				
CCI score				
Low	3 (18.8)	12 (20)	0.49	0.780
Medium	8 (50)	29 (48.3)		
High	5 (31.3)	19 (31.7)		
Diabetes mellitus	11 (68.8)	36 (60)	0.41	0.522
Chronic kidney disease	6 (37.5)	29 (48.3)	0.59	0.44
Liver cirrhosis	2 (12.5)	1 (1.7)	3.91	0.048*
Active malignancy	0 (0)	5 (8.3)	1.43	0.232
HIV infection/AIDS	1 (6.3)	1 (1.7)	1.04	0.309
Risk factors				
CVC (>72h)	7 (43.8)	22 (36.7)	0.27	0.604
Recent hospitalisation	7 (43.8)	14 (23.3)	2.63	0.105
Immunosuppression	1 (6.3)	2 (3.3)	0.28	0.594
Setting of acquisition				
Community-acquired	3 (18.8)	12 (20)	0.012	0.911
Healthcare-associated	7 (43.8)	28 (46.7)	0.008	0.929
Hospital-acquired	6 (37.5)	20 (33.3)	0.034	0.853
Source of bacteraemia				
Unknown	4 (25)	22 (36.7)	0.764	0.382
CRBSI	4 (25)	19 (31.7)	0.266	0.606
Skin/soft tissue infection (SSTI)	8 (50)	16 (26.7)	3.183	0.074
Surgical site infection	0 (0)	3 (5)	0.833	0.361
Clinical presentation				
Fever ≥ 38 ◦C	10 (62.5)	50 (83.3)	3.299	0.069
Systolic BP < 90 mm/Hg	2 (12.5)	15 (25)	1.137	0.286
Complicated SAB				
Yes	6 (37.5)	24 (40)	3.80	0.150
No	10 (62.5)	36 (60)		
Outcome				
Survived	14 (87.5)	45 (75)	1.14	0.286
Persistence bacteraemia	5 (31.3)	14 (23.3)	2.14	0.343
Metastasis infection	1 (6.3)	12 (20)	1.68	0.194
In-hospital death	2 (12.5)	15 (25)	1.14	0.286

Abbreviations: CVC, central venous catheter, CCI, Charlson comorbid index, CRBSI, Catheter-related blood stream infection, MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*

*Significant P <0.05

was the most common clinical manifestation observed in 22/60 patients with the MSSA group (36.7%), whereas soft tissue/skin infection was the predominant source of MRSA bacteraemia, 8/16 (50%). There was no significant association between the severity of clinical presentation and the outcome of SAB in both groups.

Table 5 demonstrated a comparison of variables between survivors and patients with fatal outcomes in SAB. Persistent bacteraemia, shock at presentation and complicated bacteraemia are significantly associated with mortality ($P < 0.001$). There was no significant correlation found between mortality with age, premorbid condition and MRSA bacteraemia.

DISCUSSION

The findings in this study supported those of previous studies which consistently identified males and advanced age are at increased risk for SAB acquisition.[7,18] Highest episodes of SAB were observed in patients aged over 60 years old, likely attributed to their underlying comorbidities. SAB is often associated with hospital setting acquisition.[19] As seen in our cohort, a high percentage of cases were related to healthcare-associated (HCA) and hospital-acquired (HA) infection, 33 (44%) and 25 (33.3%) respectively, highlighting the importance of the clinical setting in identifying patient populations at risk, particularly in MRSA bacteraemia.[20] This is likely attributed to their underlying comorbidities and history of recent hospitalisation, particularly those with underlying diabetes mellitus and ESRF. Furthermore, significant numbers of them, 4 (25%) in MRSA and 19 (31.7%) in the MSSA group were dialysis-dependent patients with indwelling catheterisation. Higher rate of *S. aureus* colonisation in dialysis patient makes them at greater risk for SAB and often, catheter-related infection in these group resulted from autoinfection.[21,22]

In MSSA bacteraemia, 20/60 (33.3%) were hospital-acquired infections, with more than half (12, 60%) were those who were initially admitted for dengue fever, subsequently developed thrombophlebitis from the peripheral cannula insertion site. A study by Blauw *et al*. (2019) identified peripheral venous catheter (PVC), was the most common cause of hospital-onset SAB, observed in 36% and likely to occur in PVC duration ≥ 4 days.[23] This reflected the importance of proper care and monitoring of peripheral cannula site, in order to minimise the risk of SAB and its complications.

Table 5: Comparison of variables between survivors and patients with fatal outcome in Staphylococcus aureus bacteraemia

Variable	Fatal (n = 17)	Survivor (n= 59)	*P* value
Median (IQR, age) (years)			
Age < 60 years	11 (64.7)	35 (59.3)	0.689
Age ≥ 60 years	6 (35.3)	24 (40.7)	
Comorbid condition, n (%)	15 (88.2)	45 (76.3)	0.286
Charlson comorbidity index score, n (%)			
Low (0-1)	6 (35.3)	12 (20.3)	0.190
Medium (2-4)	2 (11.8)	19 (32.2)	
High (≥5)	9 (52.9)	28 (47.5)	
Methicillin-resistant S. aureus, n (%)	2 (11.8)	14 (23.7)	0.286
Hypotension (arterial tension <90mmHg)	12 (70.6)	5 (8.5)	**<0.001***
Persistent bacteraemia, n (%)			
Yes	7 (41.2)	12 (20.3)	**<0.001***
No	1 (5.9)	35 (59.3)	
NA	9 (52.9)	12 (20.3)	
Complicated SAB, n (%)			
Yes	16 (94.1)	14 (23.7)	**<0.001***
No	1 (5.9)	45 (76.3)	

*Significant P <0.05

Almost 40% of the patients in this study developed complicated SAB, similar as reported in the previous study.[6] Only 55 patients have completed follow-up blood culture (55/76). Persistence bacteraemia was observed in 19 out of 55 patients (34.5%). A prospective study by Khatib *et al*. (2006) also reported a high rate of persistence bacteraemia of 38.4% and complication rate increased steadily with bacteremia duration. In contrast, other studies reported a much lower rate (15.7%) of persistence.[17] These differences are likely due to differences in the definition used in each study, (≥7 days) versus (≥3 days) as being used in our study. Chong *et al*. (2013) reported MRSA bacteraemia was significantly associated with a greater risk of persistence.[17] Independent risk factors in this group were vancomycin trough level < 15 mg/L and CVC-related infection, particularly those with longer delays in the removal of the catheter. Similarly, in the present study, persistent bacteraemia was seen higher in MRSA (31.3%) compared to MSSA bacteraemia, (23.3%), however, it was not statistically significant.

Metastatic infections developed in 13 out of 76 patients (17.1%) as follows; vertebral osteomyelitis (5), suppurative pneumonia (2), infective endocarditis (2), septic arthritis (2), endophthalmitis (1), and psoas abscess (1). Similar rates had been reported in other studies.[24] The percentage was considered substantial, highlighted the importance of early recognition of possible metastatic seeding, particularly in those with persistent bacteraemia, as it was significantly associated with longer hospitalisations, higher relapse rates, and higher mortality in compared to patients with resolving bacteremia.[17] In this study, it was noted that liver cirrhosis was significantly associated with MRSA bacteraemia as compared to MSSA bacteraemia. Previous studies had reported a significant association of liver cirrhotic patients with multidrug-resistant bacteria, due to frequent exposure to the healthcare environment.[25] Furthermore, the use of invasive instrumentation like CVC, makes them more predisposed to gram-positive infection, particularly *S. aureus*.[26]

The mortality rate of SAB in this cohort was 22.4%, comparable to other studies.[27,28] Several studies have highlighted significant risk factors associated with mortality among patients with SAB which includes older age[28,29], presence of shock[5,19] and acute severity of illness at onset of SAB.[24,27] Aged over 60 years at onset of SAB and underlying premorbid were identified as the most important predictor of mortality for SAB.[27,28] However, in the present study, we did not find a significant association between mortality with age and premorbid, likely due to our small sample size compared to other studies. Contrary to other reports of higher mortality rates among patients with MRSA bacteraemia compared to MSSA bacteraemia[18,28], in this study, there was no significant difference observed in the mortality rate for both groups. The higher mortality seen in previous studies were associated with delay or inappropriate empirical antibiotic coverage, particularly in settings where MRSA is least suspected by clinicians.[30-32]

Rapid and accurate identification of *S. aureus* is crucial for early initiation of appropriate antimicrobial therapy in patients with SAB. Previous studies have reported lower sensitivity (81% to 91%) for tube coagulase tests, particularly in certain *S. aureus* isolates which have a weak coagulase despite being positive for the coagulase gene.[33-36] However, in our study, both tube coagulase and DNase gave comparable results to rapid Prolex Staph Xtra Latex test latex (sensitivity 100%, specificity 91.7%). The usage of latex test leads to rapid and timely identification of STAU and clinical diagnosis of SAB.

Personne *et al*. (1997) had reported improved sensitivity of the 4th generation latex agglutination assay in comparison to the previous 3rd generation latex assay.[37] With regards to utilising the Prolex Staph Xtra Latex agglutination test in rapid identification of *S. aureus*, we found that it has excellent sensitivity (100%) and high specificity (91.7%). Thus, a negative result is valuable in excluding bacteraemia caused by *S. aureus* (negative predictive value, 100%). The specificity (91.7%) achieved in this study was attributed to small numbers of CoNS being tested as compared to other studies.[11] This is due to most of the CoNS isolates included in this study being from significant bacteraemia instead of contamination or colonisation.

False-positive agglutination with several latex test kits has been reported for strains of *S. haemolyticus* and *S. hominis*, due to their possession of type 8 capsular polysaccharides.[9,10] In this study, Prolex Staph Xtra Latex test was able to give true-negative results for all two *S. haemolyticus* and two *S. hominis* isolates. Davies *et al*. (2008) also reported one false positive CoNS to isolate with Prolex Staph Xtra Latex test, indicating an issue with antibody

specificity of the test.[12] Similarly in this study, 1 CoNS isolate gave false-positive result for latex, which was identified as *Staphylococcus pseudintermedius*. This bacterium belongs to *S. intermedius* group, a coloniser of the dog's skin and mucous membrane, also a major cause of canine pyoderma.[38] Although true infections involving *S. pseudintermedius* in humans are rare, sporadic cases have been reported.[39, 40] It shares several virulent factors and similar laboratory characteristics as *S. aureus* include beta haemolysis colony, positive for both tube coagulase and DNase test making it a possible significant pathogen in humans.[41] This organism is likely underreported due to all coagulase-positive staphylococci are routinely grouped together as *S. aureus*. MRSA strains have been documented to give false-negative results in some agglutination assay (sensitivity of 49.2% to 73.8%), due to capsular polysaccharides produced by these strains masking the cell wall protein detection.[9] However, in this study, Prolex Staph Xtra was able to detect all MRSA isolates accurately, similarly as reported in a previous study.[12] In view of its rapid, excellent sensitivity, it could serve as a stand-alone test for reliable *S. aureus* identification.

LIMITATION OF THE STUDY

There are several potential limitations of the present study. First, the study was a retrospective design which make identifying the actual source of SAB was difficult. Furthermore, infectious disease (ID) referral was not a routine practice for all SAB cases, thus no standardization in terms of surveillance or follow-up blood culture. Some of the patient's clinical data were not retrievable due to technical issues or missing files. A prospective data from larger cohorts and proper standardisation are required to resolve this issue.

Second, our study was limited by the small numbers of CoNS isolates being tested which had affected the specificity of this latex assay. A wider range of staphylococcal species might be useful to support the accuracy of this test. Furthermore, the tests used were limited to latex assay and automated identification tests. A further molecular test that detects the presence of a specific DNA sequence belonged to *S. aureus* is beneficial.

CONCLUSION

S. aureus bacteremia is a serious infection, associated with significant metastatic complications and mortality, particularly in those with persistent bacteraemia. The majority of MRSA and MSSA bacteraemia were healthcare-associated infections. The peripheral venous catheter (PVC) represents a significant source of hospital-onset SAB and involved staff should be emphasised on proper monitoring and care of PVC. For identification of *S. aureus* isolates in microbiology laboratory, the Prolex Staph Xtra agglutination test has excellent sensitivity, is relatively specific, and proved to be comparable to conventional tests, thus it can be used as a stand-alone test for rapid and reliable identification of *S. aureus*. This is a crucial step for the early initiation of empirical antibiotics, which further improves the outcome of patients with SAB.

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