Chan *et al.*
European Journal of Medical Research (2024) 29:246
https://doi.org/10.1186/s40001-024-01831-6

European Journal of Medical Research

RESEARCH

Open Access

Unveilling genetic profiles and correlations of biofilm-associated genes, quorum sensing, and antibiotic resistance in *Staphylococcus aureus* isolated from a Malaysian Teaching Hospital

Yun Li Chan[1], Chin Fei Chee[2], Soo Nee Tang[1*] and Sun Tee Tay[1*]

Abstract

Background *Staphylococcus aureus* is a notorious multidrug resistant pathogen prevalent in healthcare facilities worldwide. Unveiling the mechanisms underlying biofilm formation, quorum sensing and antibiotic resistance can help in developing more effective therapy for *S. aureus* infection. There is a scarcity of literature addressing the genetic profiles and correlations of biofilm-associated genes, quorum sensing, and antibiotic resistance among *S. aureus* isolates from Malaysia.

Methods Biofilm and slime production of 68 methicillin-susceptible *S. aureus* (MSSA) and 54 methicillin-resistant (MRSA) isolates were determined using a a plate-based crystal violet assay and Congo Red agar method, respectively. The minimum inhibitory concentration values against 14 antibiotics were determined using VITEK® AST-GP67 cards and interpreted according to CLSI-M100 guidelines. Genetic profiling of 11 *S. aureus* biofilm-associated genes and *agr/sar* quorum sensing genes was performed using single or multiplex polymerase chain reaction (PCR) assays.

Results In this study, 75.9% ($n=41$) of MRSA and 83.8% ($n=57$) of MSSA isolates showed strong biofilm-forming capabilities. Intermediate slime production was detected in approximately 70% of the isolates. Compared to MSSA, significantly higher resistance of clindamycin, erythromycin, and fluoroquinolones was noted among the MRSA isolates. The presence of intracellular adhesion A (*icaA*) gene was detected in all *S. aureus* isolates. All MSSA isolates harbored the laminin-binding protein (*eno*) gene, while all MRSA isolates harbored intracellular adhesion D (*icaD)*, clumping factors A and B (*clfA* and *clfB*) genes. The presence of *agrI* and elastin-binding protein (*ebpS*) genes was significantly associated with biofilm production in MSSA and MRSA isolates, respectively. In addition, *agrI* gene was also significantly correlated with oxacillin, cefoxitin, and fluoroquinolone resistance.

Conclusions The high prevalence of biofilm and slime production among MSSA and MRSA isolates correlates well with the detection of a high prevalence of biofilm-associated genes and *agr* quorum sensing system. A significant association of *agrI* gene was found with cefoxitin, oxacillin, and fluoroquinolone resistance. A more focused approach

*Correspondence:
Soo Nee Tang
soonee_t@um.edu.my
Sun Tee Tay
tayst@um.edu.my
Full list of author information is available at the end of the article

targeting biofilm-associated and quorum sensing genes is important in developing new surveillance and treatment strategies against *S. aureus* biofilm infection.

Keywords Biofilm production, MSSA, MRSA, Biofilm-associated gene, Antibiotic resistance

Introduction

Staphylococcus aureus is one of the leading causes of severe bacterial infections which may lead to life-threatening conditions, including sepsis, pneumonia, endocarditis, osteomyelitis, and implant-associated diseases [1–3]. The emergence of antibiotic resistance in *S. aureus* has posed a significant impact on the treatment and infection control practices in hospitals worldwide [4, 5]. Methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA) and vancomycin-intermediate *S. aureus* (VISA) are among the pathogens listed as "High" priority in the World Health Organization (WHO)'s priority pathogens list for research and development of new antibiotics [6].

The ability of *S. aureus* to resist antimicrobials is further enhanced by the strategy of biofilm formation. Currently available antibiotics cannot eradicate biofilms, especially of ESKAPE pathogens, which includes MRSA [7]. Several studies found no statistically significant difference in the biofilm formation among MRSA and MSSA strains [8, 9]. In contrary, a study reported that MRSA strains showed enhanced biofilm formation as compared to MSSA strains [10]. Staphylococcal polysaccharide intercellular adhesin (encoded by *icaABCD*) [11], collagen-binding protein (*cna*), fibrinogen binding protein (*fib*), elastin binding protein (*ebpS*), laminin binding protein (*eno*), fibronectin binding proteins A and B (*fnbA* and *fnbB*), and clumping factors A and B (*clfA* and *clfB*) [12] have been reported to play important roles in *S. aureus* adherence, which is the first step in biofilm production. The *icaABCD* operon is also known for its function in slime production [13].

Quorum sensing is a mechanism, whereby bacterial cells communicate and coordinate their behaviours based on population density [14]. The accessory gene regulator (*agr*) quorum-sensing system plays a key role in *S. aureus* pathogenesis, while the staphylococcal accessory regulator (*sarA*) gene is essential in controlling staphylococcal virulence factors [15]. Both *agr* and *sarA* quorum sensing genes have been reported to regulate *S. aureus* biofilm formation [15–18]. To date, four polymorphic *agr* types (*agrI*, *agrII*, *agrIII*, and *agrIV*) have been reported [19].

Previously, a high prevalence of *icaADBC* genes and varied occurrence of biofilm associated genes, i.e., *cna* (42.7–93%), *fib* (24.7–90%), *ebps* (11.1–100%), *fnbA* (0–100%) and *fnbB* (1.1–53.33%) have been reported in Malaysian *S. aureus* clinical isolates [20–22]. The *agr1* was the most prevalent type reported in Malaysian isolates of *S.aureus,* followed by *agrII* and *agrIII*; however, no *agrIV* was detected [21, 23]. Understanding differences in biofilm and slime production between MRSA and MSSA and the associated genetic elements contributes to a better understanding of the epidemiology and spread of *S. aureus* infections, further aiding in developing more targeted surveillance and treatment strategies. Hence, this study was performed to analyze biofilm and slime production of a collection of MSSA and MRSA clinical isolates and to investigate possible correlations between biofilm-associated genes and the *agr*/*sar* quorum sensing systems in relation to antibiotic resistance.

Methods

Collection of clinical isolates

A total of 68 MSSA and 54 MRSA isolates collected from patients attending Universiti Malaya Medical Centre (UMMC) from August 2020 to June 2022 were investigated in this study. The isolates were primarily collected from the blood ($n=38$, 31.1%), and tissue ($n=36$, 29.5%), followed by pus ($n=15$, 12.3%), wound swab ($n=11$, 9%), and lower respiratory tract ($n=18$, 14.8%) (Additional file 1: Table S1). The identity of the isolates was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (VITEK MS system, bioMérieux Clinical Diagnostics, France).

Antibiotic susceptibility testing

The minimum inhibitory concentration values (MIC) of *S. aureus* against 14 antibiotics, i.e., clindamycin, penicillin, erythromycin, gentamicin, linezolid, oxacillin, rifampicin, cotrimoxazole, tetracycline, vancomycin, ciprofloxacin, levofloxacin, moxifloxacin, and cefoxitin were determined using VITEK® AST-GP67 card (bioMérieux Clinical Diagnostics, France), and interpreted according to the Clinical Laboratory Standards Institute (CLSI-M100) guidelines [24]. Methicillin susceptibility of the clinical isolates was determined using the CLSI disk–diffusion method with cefoxitin 30-µg disk and VITEK® AST-GP67 card. For 10 isolates with missing MIC data, vancomycin susceptibility testing was carried out using microbroth dilution method, as recommended by CLSI-M100 guidelines.

Biofilm quantitation assay

Quantitation of *S. aureus* biofilm production was performed as described by Atshan et al. [22] and Stepanović et al. [25], with slight modifications. Briefly, 100 µl of bacterial suspension (adjusted to 1×10^6 CFU/ml in Mueller Hinton broth containing 1% glucose) were seeded into each well of a sterile 96-well flat bottom microtitre plate (BIOFIL®, Guangzhou, China) and incubated at 37°C for 24 h. After incubation, the wells were washed thrice, fixed with methanol, and stained using 0.1% (v/v) crystal violet (Cat. No: C6158, Sigma, USA). *S. aureus* ATCC® 29213™ (MSSA) and ATCC® 33591™ (MRSA) were used as biofilm-producing controls, while microtiter wells with no inoculum served as negative controls. The amount of biofilm was quantitated by measuring the absorbance of each well at 570 nm using a microplate reader (Tecan, Sunrise™, Swiss). Biofilm was graded into four categories as described by Moghadam et al. [26]: no biofilm ($ODs \leq ODc$), weak ($ODc \leq ODs \leq 2 \times ODc$), moderate ($2 \times ODc \leq ODs \leq 4 \times ODc$), and strong ($4 \times ODc < ODs$). ODc and ODs represent the OD of the negative and the test isolates, respectively.

Congo red agar assay for determination of slime production

Bacterial slime production was determined qualitatively as described by Freeman et al. [27] and Thilakavathy et al. [28]. Congo red agar was prepared using brain heart infusion (BHI) broth (37 g/L), sucrose (50 g/L), agar no.1 (10 g/L), and Congo red stain (0.8 g/L). Slime producers are expected to form black colonies with a dry, crystalline consistency, while non-slime producers form pink coloured colonies. Intermediate slime production is indicated by the growth of smooth blackish-red colonies. The positive and negative control strains included in the test were *Staphylococcus epidermidis* ATCC® 35984™ and *Staphylococcus hominis* ATCC® 35982™, respectively.

Bacterial genomic DNA extraction

Genomic DNA was extracted from overnight cultures of *S. aureus* in Luria–Bertani broth, using either MasterPure™ Complete DNA and RNA Purification Kit (Lucigen, Middleton, WI, USA) or QIAamp DNA Mini Kit (Qiagen, Germany) following manufacturers' instructions. Amplification of the 16S rRNA gene from the bacterial DNA extract was performed to rule out the possibility of having PCR inhibitors, using universal oligonucleotide primers (27F and 1492R) as described by Gumaa et al. [29].

PCR detection of biofilm-associated genes

PCR profiling of *bap, cna, icaA,* and *icaD* genes was performed using singleplex PCR assays, while *ebpS*, *eno*, *fnbA*, *clfA*, *clfB*, *fib*, and *fnbB* genes were amplified using multiplex PCR assays as described by Tristan et al. and Vancraeynest et al. [30, 31]. The primers and PCR thermal cycling conditions are shown in Additional file 1: Table S2. s*arA* gene was amplified using *sar*AF and *sar*AR primers as described by Gowrishankar et al. [32]. Meanwhile, *agr* typing (types I–IV) was performed using primers and amplification conditions as described by Shopsin et al. [19]. The amplified products were then subjected to electrophoresis using 1% (w/v) agarose gel, pre-stained with nucleic acid staining dye (Bioteke Corporation, China). Sequence analyses were performed to confirm that correct genes were amplified.

Statistical analysis

Paired sample *t* tests were used to compare biofilm and slime production between MSSA and MRSA isolates. Pearson's Chi-square test was used to determine the correlation of antibiotic resistance with other parameters. Statistical analysis was performed using SPSS software version 20.0 (IBM, Armonk, USA). A *p* value of less than 0.05 was considered statistically significant.

Results

Antibiotic susceptibility profiling of *S. aureus* clinical isolates

MRSA isolates exhibited higher rates of resistance to erythromycin (53.7% vs 17.6%), ciprofloxacin (83.3% vs 2.9%), levofloxacin (83.3% vs 1.5%) and moxifloxacin (75.9% vs 0%), compared to MSSA isolates (Table 1). Clindamycin resistance was observed in 16.2% and 7.6% of MSSA and MRSA isolates, respectively, while inducible clindamycin resistance was detected in 23 (42.6%) MRSA isolates and 1 (1.5%) MSSA isolate. The MRSA MIC_{90}s against clindamycin (0.5 vs 8 µg/ml), erythromycin (0.5 vs 8 µg/ml), gentamicin (0.5 vs 8 µg/ml), ciprofloxacin (0.5 vs 8 µg/ml), and levofloxacin (0.25 vs 8 µg/ml) were 16–32 folds higher than those of MSSA isolates (Additional file 1: Table S3). Meanwhile, all isolates exhibited high susceptibility towards linezolid (100%), vancomycin (100%), rifampicin (99.2%), cotrimoxazole (86%), tetracycline (84.4%) and gentamicin (83.6%). In this study, no isolate showed resistance to vancomycin and linezolid. The MRSA vancomycin and linezolid MICs ranged from 0.5 to 2 µg/ml and 1 to 2 µg/ml, respectively.

Table 1 Antibiotic susceptibility profiles of *S. aureus* isolates investigated in this study

Antibiotics	No. (%) MSSA ($n=68$)			No. (%) MRSA ($n=54$)			Overall resistance No. (%)	*p* value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant		
Clindamycin	56 (82.4)	0 (0)	11 (16.2)[a]	27 (50)	0 (0)	4 (7.4)[b]	15(12.4%)	0.000[d]
Erythromycin	56 (82.4)	0 (0)	12 (17.6)	25 (46.3)	0 (0)	29 (53.7)	41(33.6)	0.000[d]
Gentamicin	57 (83.8)	1 (1.5)	10 (14.7)	45 (83.3)	3 (50)	6 (11.1)	16 (13.1)	0.401
Linezolid	68 (100)	0 (0)	0 (0)	54 (100)	0 (0)	0 (0)	0 (0)	–
Oxacillin	68 (100)	0 (0)	0 (0)	0 (0)	0 (0)	54 (100)	54 (44.3)	0.000[d]
Penicillin	22 (32.4)	0 (0)	46 (67.6)	0 (0)	0 (0)	54 (100)	100 (82.0)	0.000[d]
Rifampicin	68 (100)	0 (0)	0 (0)	53 (98.1)	0 (0)	1 (1.9)	1 (0.8)	0.260
Cotrimoxazole	56 (82.4)	0 (0)	12 (17.6)	49 (90.7)	0 (0)	5 (9.3)	17 (13.9)	0.184
Tetracycline	57 (83.8)	0 (0)	11 (16.2)	46 (85.2)	0 (0)	8 (14.8)	19 (15.6)	0.837
Vancomycin	68(100)	0 (0)	0 (0)	54 (100)	0 (0)	0 (0)	0 (0)	–
Ciprofloxacin	66 (97.1)	0 (0)	2 (2.9)	9 (16.7)	0 (0)	45 (83.3)	47 (38.5)	0.000[d]
Levofloxacin	67 (98.5)	0 (0)	1 (1.5)	9 (16.7)	0 (0)	45 (83.3)	46 (37.7)	0.000[d]
Moxifloxacin[c]	57 (98.3)	1 (1.7)	0 (0)	9 (16.7)	4 (7.4)	41(75.9)	41 (36.6)	0.000[d]
Cefoxitin	68 (100)	0 (0)	0 (0)	0 (0)	0 (0)	54 (100)	54 (44.3)	0.000[d]

[a] 1 (1.5%) were inducible resistant; [b]23 (42.6%) were inducible resistant; [c]missing information for 10 MSSA isolates, [d] $p<0.05$ indicates significant difference between MSSA and MRSA, –: not applicable

Table 2 Distribution of biofilm and slime producers among MRSA and MSSA isolates

Biofilm/slime production	No. (%) isolates		*p* value*
	MSSA ($n=68$)	MRSA ($n=54$)	
Biofilm			$p=0.241$
Strong	57 (83.8)	41 (75.9)	$p=0.405$
Moderate	7 (10.3)	8 (14.8)	
Weak	4 (5.9)	3 (5.6)	
No biofilm	0 (0)	2 (3.7)	
Slime			
No slime	15 (22.1)	15 (27.8)	$p=0.19$
Intermediate	49 (72.1)	39 (72.2)	
Strong slime producer	4 (5.9)	0	

**p* values were determined using *t* test, with $p>0.05$ indicating no significant difference between groups

Biofilm production of MRSA and MSSA isolates

Of the 122 *S. aureus* isolates tested, a majority (79.5%) were identified as strong biofilm producers. A total of 57 (83.8%) biofilm-producing isolates were MSSA and 41 (75.9%) isolates were MRSA (Table 2). In addition, 12.3% of *S. aureus* isolates were identified as moderate biofilm producers, 5.7% were identified as weak biofilm producers and 1.64% of strains did not produce biofilms.

Slime production of MRSA and MSSA isolates

Using Congo Red agar assay, most *S. aureus* isolates (72.1% MSSA and 72.2% MRSA isolates, respectively) were regarded as intermediate slime producers. There was no significant difference between MSSA and MRSA isolates in slime production ($p=0.19$). Only 4 (6.0%) MSSA isolates demonstrated strong slime production after 24 h of incubation (Table 2).

Distribution of biofilm-associated genes and agr/sar quorum sensing genes in MSSA and MRS isolates

In this study, the successful amplification of the 16S rRNA gene from all *S. aureus* isolates indicated the absence of PCR inhibitors in the bacterial DNA extracts. The amplification of biofilm-associated genes from MSSA and MRSA isolates using various singleplex and

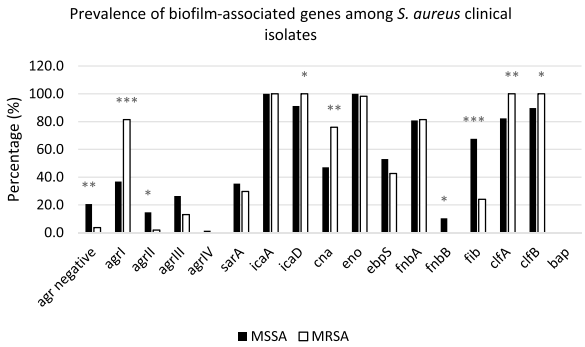

Fig. 1 Prevalence of biofilm-associated genes among MSSA and MRSA clinical isolates. Paired *t* tests were performed to determine significant difference between MSSA and MRSA isolates. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

multiplex PCR assays is shown in Additional file 1: Fig. S1.

The presence of the intracellular adhesion A (*icaA*) gene was observed in all *S. aureus* isolates (100%). There was variability in the distribution of other biofilm-associated genes in MSSA and MRSA (Fig. 1). Overall, the intracellular adhesion A and D (*icaA* and *icaD*), laminin-binding protein (*eno*), clumping factors A and B (*clfA* and *clfB*), and fibronectin-binding protein A (*fnbA*) were the most prevalent biofilm-associated genes in *S. aureus* isolates, regardless of MSSA or MRSA.

Compared to MSSA, the detection rates of *agrI, icaD, cna, clfA,* and *clfB* genes were significantly higher in MRSA, while the fibronectin-binding protein B (*fnbB*) gene was absent in all MRSA isolates. All MSSA isolates harbored the laminin-binding protein (*eno*) gene, while all MRSA isolates harbored intracellular adhesion D (*icaD)*, clumping factors A and B (*clfA* and *clfB*) genes. Intriguingly, the *bap* gene (encoding biofilm matrix protein) was not amplified from any of the isolates.

The number of biofilm-associated genes detected in *S. aureus* varied from three to eleven, with most isolates having 10 genes (including 24 MSSA and 10 MRSA isolates). However, the number of genes detected from an isolate was not significantly associated with biofilm production ($p = 0.299$, Pearson's Chi-square, Table 3). Interestingly, the presence of *agrI* in MSSA ($p = 0.018$), and *ebpS* in MRSA isolates was significantly associated with biofilm production ($p = 0.006$) (Table 4).

In this study, the most prevalent *agr* type in *S. aureus* isolates was *agrI* (56.7%), followed by *agrIII* (20.5%) and *agrII* (9.0%). The *agrI* was detected with a significantly higher rate in MRSA (81.5%) as compared to MSSA (36.8%). In contrast, higher detection rates of *agrIII* and *agrII* were found in MSSA (26.5% and 14.7%, respectively) as compared to MRSA (13% and 1.9%, respectively). The *agrIV* was only detected in only one MSSA isolate (1.5%). Sequence analyses of representative *agr* alleles in this study demonstrated 100% similarity to *agrI* (352/352, 100%, GenBank accession no. AJ617710), *agrII* (472/472, 100%, GenBank accession no. AJ6177170), *agrIII* (333/333, 100%, GenBank accession no. AJ617723) and *agrIV* (577/577, 100%, GenBank accession no. AJ617712), as reported by Goerke et al. [33]. In this study, the presence of *agrI* was significantly correlated with ciprofloxacin ($p = 0.000$), levofloxacin ($p = 0.003$), moxifloxacin ($p = 0.000$), oxacillin ($p = 0.000$) and cefoxitin ($p = 0.000$) resistance (Table 5).

Discussion

The treatment and management of *S. aureus* infection pose significant challenges and a big threat in healthcare settings worldwide due to the emergence

Table 3 Cross-tabulation between the number of *S. aureus* biofilm-associated genes and biofilm production

Number of *S. aureus* biofilm-associated genes	Number of *S. aureus* isolates					
	Non-biofilm	Weak	Moderate	Strong	Total	*p* value
3	0	0	0	3	3	$p = 0.299$
4	0	0	0	3	3	
5	0	0	2	3	5	
6	0	0	2	1	3	
7	0	0	1	13	14	
8	0	2	3	22	27	
9	0	2	6	19	27	
10	3	2	1	28	34	
11	0	1	0	5	6	
Total	3	7	15	97	122	

*p value were determined using Pearson's Chi-square

Table 4 Correlations between *agr, sarA*, and biofilm-associated genes, with biofilm production in 68 MSSA and 54 MRSA isolates investigated in this study

Genes	Biofilm production									
	No. MSSA isolates ($n=68$)				*p* value	No. MRSA isolates ($n=54$)				*p* value
	None	Weak	Moderate	Strong		None	Weak	Moderate	Strong	
agrI	0	4	1	20	0.018*	1	2	5	36	0.154
agrII	0	0	0	10	0.407	0	0	0	1	1.000
agrIII	1	0	3	14	0.175	1	1	2	3	0.090
agrIV	0	0	0	1	1.000	0	0	0	0	–
sarA	0	1	1	22	0.530	1	1	0	14	0.202
icaA	1	4	7	56	–	2	3	8	41	–
icaD	1	4	7	50	0.756	2	3	8	41	–
cna	1	1	3	27	0.740	2	3	6	30	0.635
eno	1	4	7	56	–	2	3	7	41	0.241
ebpS	1	3	4	28	0.782	2	3	5	13	0.006*
fnbA	1	4	4	46	0.304	2	2	5	35	0.307
fnbB	0	0	0	7	0.655	0	0	0	0	–
fib	1	3	5	37	1.000	1	2	2	8	0.267
clfA	1	4	5	46	0.633	2	3	8	41	–
clfB	1	4	6	50	1.000	2	3	8	41	–

*$p<0.05$ indicates significant difference between groups; –: not applicable

of antibiotic-resistant strains. In comparison with the Malaysia National Surveillance of Antimicrobial Resistance (NSAR) 2022 report [34], higher resistance rates to clindamycin (12.4% vs 5.9%), erythromycin (33.6% vs 9.9%) and gentamicin (13.1% vs 3.2%) were reported from a collection of clinical *S. aureus* isolates in this study. No linezolid-resistant strain was identified in this study, consistent with the very low percentage of linezolid resistance (0.4%) documented in the latest national report [34]. So far, the highest linezolid resistance rate was reported in a previous NSAR study (2010) whereby 7.7% in MRSA and 3.3% in MSSA were linezolid resistant [1], while there have been no studies documenting *S. aureus* resistance to vancomycin in Malaysia [35, 36].

In addition to antibiotic resistance, almost 80% of S. *aureus* isolates (MSSA and MRSA) in this study exhibited slime and biofilm production. However, no correlation was found between slime and biofilm production among staphylococcal isolates investigated in this study (Table 2). Similar observations have been reported for *S. aureus* human and animal isolates in earlier investigations [21, 37]. The lack of correlation between slime and biofilm production in *S. aureus* may be attributed to different measurement methods, i.e., Congo red agar method versus microtiter plate-based crystal violet assay, leading to disparities in the results. In addition, the complex nature of biofilm formation, possibly affected by bacterial genetic diversity, environmental factors, and regulatory mechanisms, may be attributed to the limited correlation between slime and biofilm production in *S. aureus.*

The most prevalent biofilm-associated genes detected in MRSA isolates in this study were intracellular adhesion A and D (*icaA* and *icaD*), laminin-binding protein (*eno*), clumping factors A and B (*clfA* and *clfB*), and fibronectin-binding protein A (*fnbA*), as shown in Fig. 1. The *agrI, icaD, cna, clfA,* and *clfB* genes were detected at significantly higher rates amongst MRSA isolates, while *fnbB* was detected at a significantly higher rate in MSSA isolates. The variability observed in the frequencies of biofilm-associated genes could be attributed to strain-to-strain difference [22, 38], source of isolation [39], and geographical settings [40]. Amongst the biofilm-associated genes, the elastin-binding protein (*ebpS)* gene has been significantly associated with biofilm production amongst MRSA isolates in this study (Table 3). Elastin-binding protein facilitates *S. aureus*-binding to elastin-rich tissues and promotes bacterial colonisation on mammalian tissues [41]. It has been significantly associated with strong biofilm production in *S. aureus* food isolates in two previous studies [38, 42].

The distribution of *agr* types is variable in *S. aureus* from different geographical regions [43]. In this study, the most prevalent *agr* type identified from *S. aureus* isolates was *agrI* (56.7%), followed by *agrIII* (20.5%) and *agrII* (9.0%), while *agrIV* (0.8%) has a low occurrence rate.

Table 5 Association between the presences of *agrI* with antibiotic susceptibility in *S. aureus* clinical isolates

Antibiotics	Susceptibility	No of isolates		*p* value
		agrI	Non *agrI*	
Clindamycin	S	41	42	0.002*
	R	7	8	
	IR	21	3	
Erythromycin	S	41	40	0.082*
	R	28	13	
Gentamicin	S	61	41	0.259
	R	7	9	
	IN	1	3	
Oxacillin	S	25	43	0.000*
	R	44	10	
Penicillin	S	10	12	0.342
	R	59	41	
Rifampicin	S	68	53	1.000
	R	1	0	
Cotrimoxazole	S	61	44	0.437
	R	8	9	
Tetracycline	S	64	39	0.005*
	R	5	14	
Ciprofloxacin	S	31	44	0.000*
	R	38	9	
Levofloxacin	S	31	45	0.000*
	R	38	8	
Moxifloxacin	S	27	39	0.000*
	R	35	6	
	IN	3	2	

Linezolid and vancomycin were excluded from the analysis as all *S. aureus* isolates were susceptible to these antibiotics. S: susceptible; R: resistant; IR: inducible resistant; IN: intermediate, *$p < 0.05$ indicates significant difference between groups

Remarkably, a significantly higher percentage of MRSA isolates in this study was found to harbor *agrI*, compared to MSSA. The presence of *agrI* has been significantly associated with biofilm production amongst MSSA isolates in this study (Table 3), corresponding well with another study using nonclinical isolates [42]. Kawamura et al. [44] found that MRSA isolates haboring *agrII* have a significantly greater ability to produce biofilm, however; Usun Jones et al. [21] and Cha et al. [45] found no variation in MRSA biofilm production among different *agr* groups. The difference might be attributed to variations between strains, potentially resulting from microbial adaptation and geographical influences.

As the transcription of the *agr* locus (I–IV) is autoinducing peptide (AIP)-dependent, the differentiation of staphylococcal strains based on *agr* typing may provide further insights into the epidemiology and antibiotic resistance. Studies have shown that the *mecA* gene of MRSA indirectly activates AIPs which significantly affect biofilm production, quorum-sensing and virulence, and antibiotic resistance [17, 18]. As quorum sensing is higly influenced by cell density, high-density colonies can produce numerous small molecule signals, triggering downstream processes, such as virulence and antibiotic resistance mechanisms, which poses a threat to the host and antibiotic efficacy [46]. Biofilm production has been reported to provide a niche for generation of antibiotic resistant subpopulations or persister cells through the exchange of genetic materials [47]. Recent data demonstrated a significant correlation between *agr*I with cefoxitin and erythromycin resistance [48], as well as tetracycline, erythromycin, clindamycin, and ciprofloxacin resistance in *S. aureus* [43]. Interestingly, a significant association was found between *agrI* with fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) resistance ($p < 0.05$) for the first time in this study. In addition, the high resistance (75.9%) of MRSA against fluoroquinolones especially moxifloxacin, a fourth-generation fluoroquinolone, is alarming (Table 1).

Fluoroquinolone exposure has been identified as an increased risk factor for MRSA isolation and infection [49–51]. The key mechanims to *S. aureus* fluoroquinolone resistance are through chromosomal point mutations in *gyrA/B* (DNA gyrase subunits), *grlA/B* (DNA topoisomerase IV subunits), and the promoter region of *norA* efflux pump [52]. The accumulation of such mutations may be enhanced in biofilm producing *agr1*-habouring strains, contributing to a high level of resistance to fluoroquinolones, as observed in the MRSA isolates in this study. However, more extensive studies are required to explore the linkage between *agr1*, biofilm production and fluoroquinolone resistance.

One of the limitation of this study is its confinement to a single-center setting and convenient sampling of *S. aureus* isolates, thus the ratio of MSSA to MRSA might not reflect the actual prevalence of multidrug resistant *S. aureus* in the local setting. For more comprehensive insights, future studies are recommended to include diverse sampling methods and multiple centers, to ensure a more representative analysis of the genetic diversity and prevalence of biofilm-associated genes in the Malaysian isolates. As the antibiotic susceptibility profiling of *S. aureus* isolates was limited to planktonic cells, future reserach should also include comprehensive assessment of antibiotic susceptibility within biofilm structures to enhance understanding of their impact on biofilm-associated *S. aureus* infections. In addition, the utilization of *mec* (SCCmec) typing would be beneficial for identifying distinct MRSA types and establishing correlations with other study variables. As conventional antibiotics do not work effectively against *S. aureus* biofilm infection, new therapeutic strategies

and infection control practices are urgently needed. The genetic profiling of biofilm-associated genes and quorum sensing systems of *S. aureus* isolates has provided scientific foundation for developing a more targeted approach for surveillance, and treatment against biofilm infection in our clinical setting.

Conclusion

The emergence of multidrug-resistant *S. aureus* strains has been driven by the use of multiple antibiotic classes over the years. The high rates of resistance against clindamycin, erythromycin, and fluoroquinolones as reported in this study have called for more judicious use of antibiotics for treatment of MRSA infection in this region. More importantly, the identification of prevalent biofilm-associated genes and *agr* types associated with antibiotic resistance in this study has shed valuable genetic insights into *S. aureus* biofilm formation, which are important to tailor more focused surveillance and treatment strategies against *S. aureus* biofilm infection in our setting.

Abbreviations

AIP	Auto-inducing peptide
CFU	Colony forming unit
CLSI	Clinical Laboratory Standard Institute
MIC	Minimum inhibitory concentration
MIC_{50}	Lowest concentration of the antibiotic at which 50% of the isolates were inhibited
MIC_{90}	Lowest concentration of the antibiotic at which 90% of the isolates were inhibited
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
OD	Optical density
S. aureus	*Staphylococcus aureus*
UMMC	Universiti Malaya Medical Centre

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-024-01831-6.

Additional file 1: Table S1. Source of *S. aureus* clinical isolates. **Table S2.** Nucleotide sequences of primers and thermal cycling conditions used in this study. **Table S3.** MIC range, MIC_{50} and MIC_{90} values of 112 *S. aureus* isolates against various classes of antibiotics. **Figure S1.** Agarose gel electrophoresis results for amplified biofilm associated gene fragments of *S. aureus*.

Acknowledgements

We thank staff and students of the Department of Medical Microbiology, Universiti Malaya for their assistance and support in this study.

Author contributions

All authors were involved in conceptualising, data analysis, writing and editing of the manuscript. Isolate collection and laboratory investigations were conducted by SNT, and YLC, respectively. All authors read and approved the final manuscript.

Funding

This work was partially supported by Impact Oriented Interdisciplinary Grant (IIRG003C-19FNW) provided by Universiti Malaya.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author onreasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from Universiti Malaya Medical Centre Medical Ethics Committee (2022218-11004) prior to data collection.

Competing interests

No potential conflict of interest was reported by the author(s).

Author details

[1]Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia. [2]Nanotechnology and Catalysis Research Centre, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

Received: 22 October 2023 Accepted: 5 April 2024
Published online: 22 April 2024

References

1. Che Hamzah AM, Yeo CC, Puah SM, Chua KH, Chew CH. *Staphylococcus aureus* infections in Malaysia: a review of antimicrobial resistance and characteristics of the clinical isolates, 1990–2017. Antibiotics. 2019;8(3):128.
2. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–61.
3. Centers of Disease Control and Prevention C. *Staphylococcus aureus* in healthcare settings 2011. https://www.cdc.gov/hai/organisms/staph.html.
4. Davis JL. Chapter 2 - Pharmacologic principles. In: Reed SM, Bayly WM, Sellon DC, editors. Equine internal medicine (Fourth Edition). Philadelphia: W.B. Saunders; 2018. p. 79–137.
5. Barber M. Methicillin-resistant *staphylococci*. J Clin Pathol. 1961;14(4):385.
6. (WHO) WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.
7. Sahoo A, Swain SS, Behera A, Sahoo G, Mahapatra PK, Panda SK. Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm: a review. Front Microbiol. 2021;12: 661195.
8. Leshem T, Schnall BS, Azrad M, Baum M, Rokney A, Peretz A. Incidence of biofilm formation among MRSA and MSSA clinical isolates from hospitalized patients in Israel. J Appl Microbiol. 2022;133(2):922–9.
9. Lade H, Park JH, Chung SH, Kim IH, Kim J-M, Joo H-S, et al. Biofilm formation by *Staphylococcus aureus* clinical isolates is differentially affected by glucose and sodium chloride supplemented culture media. J Clin Med. 2019;8(11):1853.
10. Piechota M, Kot B, Frankowska-Maciejewska A, Grużewska A, Woźniak-Kosek A. Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. Biomed Res Int. 2018;2018:4657396.
11. Arciola CR, Campoccia D, Ravaioli S, Montanaro L. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Front Cell Infect Microbiol. 2015;5:7.
12. Nguyen D, Joshi-Datar A, Lepine F, Bauerle E, Olakanmi O, Beer K, et al. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Sci. 2011;334(6058):982–6.
13. Cucarella C, Tormo MA, Ubeda C, Trotonda MP, Monzón M, Peris C, et al. Role of biofilm-associated protein bap in the pathogenesis of bovine *Staphylococcus aureus*. Infect Immun. 2004;72(4):2177–85.
14. Mukherjee S, Bassler BL. Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol. 2019;17(6):371–82.

15. Yarwood JM, Schlievert PM. Quorum sensing in *Staphylococcus* infections. J Clin Invest. 2003;112(11):1620–5.
16. Ganesh PS, Veena K, Senthil R, Iswamy K, Ponmalar EM, Mariappan V, et al. Biofilm-associated *Agr* and *Sar* quorum sensing systems of *Staphylococcus aureus* are inhibited by 3-hydroxybenzoic acid derived from Illicium verum. ACS Omega. 2022;7(17):14653–65.
17. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. 2013;26(2):185–230.
18. Dehbashi S, Tahmasebi H, Zeyni B, Arabestani MR. The relationship between promoter-dependent quorum sensing induced genes and methicillin resistance in clinical strains of *Staphylococcus aureus*. J Adv Med Biomed Res. 2018;26(116):75–87.
19. Shopsin B, Mathema B, Alcabes P, Said-Salim B, Lina G, Matsuka A, et al. Prevalence of agr specificity groups among *Staphylococcus aureus* strains colonizing children and their guardians. J Clin Microbiol. 2003;41(1):456–9.
20. Niek WK, Teh CSJ, Idris N, Thong KL, Ngoi ST, Ponnampalavanar SSLS. Investigation of biofilm formation in methicillin-resistant *Staphylococcus aureus* associated with bacteraemia in a tertiary hospital. Folia microbiol. 2021;66(5):741–9.
21. Usun Jones S, Kee BP, Chew CH, Yeo CC, Abdullah FH, Othman N, et al. Phenotypic and molecular detection of biofilm formation in clinical methicillin-resistant *Staphylococcus aureus* isolates from Malaysia. J Taibah Univ Sci. 2022;16(1):1142–50.
22. Atshan SS, Nor Shamsudin M, Sekawi Z, Lung LTT, Hamat RA, Karunanidhi A, et al. Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. J Biomed Biotechnol. 2012;2012:1–10.
23. Niek WK, Teh CSJ, Idris N, Thong KL, Ponnampalavanar S. Predominance of ST22-MRSA-IV clone and emergence of clones for methicillin-resistant *Staphylococcus aureus* clinical isolates collected from a tertiary teaching hospital over a two-year period. Jpn J Infect Dis. 2019;72(4):228–36.
24. Clinical and Laboratory Standards Institute C. M100: Performance standards for antimicrobial susceptibility testing 2022.
25. Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007;115(8):891–9.
26. Moghadam SO, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* isolated from burn patients. Iran J Infect Dev Ctries. 2014;8(12):1511–7.
27. Freeman D, Falkiner F, Keane C. New method for detecting slime production by coagulase negative *staphylococci*. J Clin Pathol. 1989;42(8):872–4.
28. Thilakavathy P, Priyan RV, Jagatheeswari P, Charles J, Dhanalakshmi V, Lallitha S, et al. Evaluation of ica gene in comparison with phenotypic methods for detection of biofilm production by coagulase negative *staphylococci* in a tertiary care hospital. J Clin Diagn Res. 2015;9(8): DC16.
29. Gumaa MA, Idris AB, Bilal N, Hassan MA. First insights into molecular basis identification of 16 s ribosomal RNA gene of *Staphylococcus aureus* isolated from Sudan. BMC Res Notes. 2021;14(1):240.
30. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J Clin Microbiol. 2003;41(9):4465–7.
31. Vancraeynest D, Hermans K, Haesebrouck F. Genotypic and phenotypic screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for biofilm formation and MSCRAMMs. Vet Microbiol. 2004;103(3–4):241–7.
32. Gowrishankar S, Kamaladevi A, Balamurugan K, Pandian SK. In vitro and in vivo biofilm characterization of methicillin-resistant *Staphylococcus aureus* from patients associated with pharyngitis infection. Biomed Res Int. 2016;2016:1–14.
33. Goerke C, Esser S, Kümmel M, Wolz C. *Staphylococcus aureus* strain designation by *agr* and cap polymorphism typing and delineation of *agr* diversification by sequence analysis. Int J Med Microbiol. 2005;295(2):67–75.
34. Ministry of Health Malaysia M. National Antibiotic Resistance Surveillance Report 2022. 2022. https://imr.nih.gov.my/MyOHAR/index.php/site/archive_rpt.
35. Dilnessa T, Bitew A. Antimicrobial susceptibility pattern of *Staphylococcus aureus* with emphasize on methicilin resistance with patients postoperative and wound infections at Yekatit 12 Hospital Medical College in Ethiopia. Am J Clin Exp Med. 2016;4(1):7–12.
36. Godebo G, Kibru G, Tassew H. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Ann Clin Microbiol Antimicrob. 2013;12(1):17.
37. Milanov D, Lazić S, Vidić B, Petrović J, Bugarski D, Šeguljev Z. Slime production and biofilm forming ability by *Staphylococcus aureus* bovine mastitis isolates. Acta Vet. 2010;60(2–3):217–26.
38. Chen Q, Xie S, Lou X, Cheng S, Liu X, Zheng W, et al. Biofilm formation and prevalence of adhesion genes among *Staphylococcus aureus* isolates from different food sources. Microbiologyopen. 2020;9(1): e00946.
39. Mashaly GS, Badr D. Adhesins encoding genes and biofilm formation as virulence determinants in methicillin resistant *Staphylococcus aureus* causing hospital acquired infections. Egypt J Med Microbiol. 2022;31(3):125–33.
40. Alorabi M, Ejaz U, Khoso BK, Uddin F, Mahmoud SF, Sohail M, et al. Detection of genes encoding microbial surface component recognizing adhesive matrix molecules in methicillin-resistant *Staphylococcus aureus* isolated from pyoderma patients. Genes. 2023;14(4):783.
41. Downer R, Roche F, Park PW, Mecham RP, Foster TJ. The elastin-binding protein of *Staphylococcus aureus* (EbpS) is expressed at the cell surface as an integral membrane protein and not as a cell wall-associated protein. J Biol Chem. 2002;277(1):243–50.
42. Puah SM, Tan JAMA, Chew CH, Chua KH. Diverse profiles of biofilm and adhesion genes in *Staphylococcus aureus* food strains isolated from sushi and sashimi. J Food Sci. 2018;83(9):2337–42.
43. Saedi S, Derakhshan S, Ghaderi E. Antibiotic resistance and typing of *agr* locus in *Staphylococcus aureus* isolated from clinical samples in Sanandaj, Western Iran. Iran J Basic Med Sci. 2020;23(10):1307.
44. Kawamura H, Nishi J, Imuta N, Tokuda K, Miyanohara H, Hashiguchi T, et al. Quantitative analysis of biofilm formation of methicillin-resistant *Staphylococcus aureus* (MRSA) strains from patients with orthopaedic device-related infections. FEMS Immunol Med Microbiol. 2011;63(1):10–5.
45. Cha J-O, Yoo JI, Yoo JS, Chung H-S, Park S-H, Kim HS, et al. Investigation of biofilm formation and its association with the molecular and clinical characteristics of methicillin-resistant *Staphylococcus aureus*. Osong Public Health Res Perspect. 2013;4(5):225–32.
46. Zhao X, Yu Z, Ding T. Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganisms. 2020;8(3):425.
47. Águila-Arcos S, Álvarez-Rodríguez I, Garaiyurrebaso O, Garbisu C, Grohmann E, Alkorta I. Biofilm-forming clinical Staphylococcus isolates harbor horizontal transfer and antibiotic resistance genes. Front Microbiol. 2017;8:2018.
48. Javdan S, Narimani T, Shahini Shams Abadi M, Gholipour A. *Agr* typing of *Staphylococcus aureus* species isolated from clinical samples in training hospitals of Isfahan and Shahrekord. BMC Res Notes. 2019;12(1):363.
49. Alseqely M, Newton-Foot M, Khalil A, El-Nakeeb M, Whitelaw A, Abouelfetouh A. Association between fluoroquinolone resistance and MRSA genotype in Alexandria, Egypt. Sci Rep. 2021;11(1):4253.
50. Dziekan G, Hahn A, Thüne K, Schwarzer G, Schäfer K, Daschner FD, et al. Methicillin-resistant *Staphylococcus aureus* in a teaching hospital: investigation of nosocomial transmission using a matched case-control study. J Hosp Infect. 2000;46(4):263–70.
51. Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. J Antimicrob Chemother. 2002;49(6):999–1005.
52. Jones ME, Boenink NM, Verhoef J, Köhrer K, Schmitz F-J. Multiple mutations conferring ciprofloxacin resistance in *Staphylococcus aureus* demonstrate long-term stability in an antibiotic-free environment. J Antimicrob Chemother. 2000;45(3):353–6.

Publisher's Note