Received: 30 June 2020 | Revised: 11 September 2020 | Accepted: 12 September 2020

DOI: 10.1002/mbo3.1126

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

Molecular characterization of clinical isolates from vascular access infection: A single-institution study

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Funding information
Chang Gung Memorial Hospital, Chiayi, Grant/Award Number: CMRPG6H0292, CMRPG6H0293, CMRPG6J0401, CMRPG6J0402 and CMRPG6J0403

Abstract

Hemodialysis requires repeated, reliable access to the systemic circulation; therefore, a well-functioning vascular access (VA) procedure is crucial for stable hemodialysis. VA infections (VAIs) constitute the most challenging complication and cause considerable morbidity, loss of access, and even death. In this study, we investigated the molecular profiles of different bacterial isolates retrieved from various types of VA grafts. We collected clinical isolates from hemodialysis patients with VAIs in our institution for the period between 2013 and 2018. We identified the bacterial isolates using standard biochemical procedures; we used a polymerase chain reaction for coagulase-negative staphylococci (CoNS) and *Burkholderia cepacia* complex (BCC) species identification. The antibiotic resistance and molecular profile were analyzed using the disk diffusion method and multilocus sequence typing, respectively. We studied 150 isolates retrieved from patients with VAI and observed that *Staphylococcus aureus* was the predominant bacterial species, followed by *S. argenteus*, BCC, and CoNS. According to multilocus sequence typing data, we identified a wide variety of sequence types (STs) in *S. aureus* isolates, with ST59, ST45, and ST239 being the predominant types. *Burkholderia cepacia* with two new ST types, namely ST1723 and ST1724, accounted for most of the BCC infections, along with ST102 *B. contaminans*, which were mainly isolated from infected tunneled-cuffed catheters. In summary, the increased incidence of *S. argenteus* and BCC infections provides insights into their potential clinical effects in VAIs. The various STs identified in different bacterial species indicate the high genetic diversity of bacterial species isolated from VAIs in our institution.

KEYWORDS
Burkholderia cepacia complex, coagulase-negative staphylococci, multilocus sequence typing, *Staphylococcus aureus*, vascular access infection

Yuan-Hsi Tseng and Min Yi Wong contributed equally to this manuscript.

1 | BACKGROUND

The population of new patients with end-stage renal disease (ESRD) receiving dialysis in Taiwan increased from 10,697 in 2013 to 11,596 in 2016. According to the 2018 Annual Report on Kidney Disease in Taiwan, the proportion of new patients with ESRD receiving hemodialysis was 88.9% in 2013, but it increased to 89.7% in 2016. The establishment of a well-functioning vascular access (VA) procedure is fundamental to enabling patients to undergo an efficient hemodialysis procedure. Although infection related to VA is not common, it is a problematic complication that may lead to access loss, sepsis, and even death. The major types of VA conduits commonly used are native arteriovenous fistulas (AVFs), prosthetic arteriovenous grafts (AVGs), and central venous catheters (CVCs; both temporary and cuffed tunneled). AVFs and AVGs are preferred over CVCs for dialysis access because CVCs expose patients undergoing hemodialysis to an increased risk of healthcare-associated infections (Lafrance et al., 2008). Pathogens primarily responsible for CVC-related infections are *Staphylococcus* spp., Gram-negative enteric bacilli, *Pseudomonas aeruginosa*, and *Candida* spp. These pathogens can form a biofilm on the CVC walls, rendering them strongly resistant to antibiotic action (Santoro et al., 2014). The mechanism underlying VA infections (VAIs) generally involves the migration of surface organisms along the external surface of the catheter from the exit-site wound or through the lumen of the catheter. *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) species are the most frequently isolated bacteria from VAIs.

S. aureus is among the most common causes of both endemic and epidemic infections acquired in hospitals. Patients undergoing hemodialysis are frequently exposed to *S. aureus* during their stay in dialysis centers, hospitals, or rest homes would have different implications, such as deficient hygiene measures. Previous studies indicated that a high proportion of hemodialysis patients occur nasal colonization of *S. aureus* (Boelaert et al., 1996; Sewell et al., 1982). The VA site for hemodialysis is a potential site of entry for the pathogen, and the risk of infection is particularly high when a CVC is used (Scheuch et al., 2019; Chu et al., 2019). Furthermore, recent research has reported that CoNS species as the most common etiology of nosocomial bloodstream infection (BSI), especially CVC-related BSI (CRBSI), in hospitalized patients (Freixas et al., 2013; Hebeisen et al., 2019; Lebeaux et al., 2014).

P. aeruginosa is one of the major causes of nosocomial infection, particularly in immunocompromised patients. It has a predilection for moist environments that serve as its natural reservoirs; therefore, *P. aeruginosa* is a common pathogen in graft infection (Chen et al., 2004; Pham et al., 2019).

We conducted a 5-year single-institution study to (a) investigate the prevalence of bacterial species from VAI, (b) determine the molecular characteristics of different bacterial species isolated from various types of VAIs, and (c) establish the correlation between bacterial species, sequence types (STs), and VAI types.

2 | MATERIALS AND METHODS

2.1 | Study setting and bacterial isolate collection and identification

This single-institution study was conducted between September 2013 and December 2018 at Chiayi Chang Gung Memorial Hospital, a territory referral hospital in Taiwan. We prospectively collected 150 bacterial isolates from blood and contaminated device samples of 78 patients with VAI who required removal of AVGs and tunneled-cuffed catheters (TCCs). We explained the study procedures to each patient and obtained informed consent before performing the procedures. Patients with poor compliance and those who declined to be part of this study were excluded. Demographic characteristics, including age and sex, were collected in addition to the following baseline characteristics: underlying cause of the end-stage renal disease (ESRD), type of VA, VAI site, and comorbidities. The bacterial isolates were cultured under laboratory standards. The samples were routinely cultured on blood agar at 37°C overnight. We performed strain identification through standard biochemical (phenotypic) procedures.

2.2 | Antibiotic susceptibility testing

We subjected all clinical isolates to antimicrobial susceptibility testing against a panel of antimicrobial agents by using the Kirby–Bauer disk diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2013).

2.3 | Genomic DNA extraction

A single colony from a clinical isolate was inoculated in tryptic soy broth (TSB) for 16 h, and 1 ml of the overnight culture was harvested using centrifugation at 16,500 × *g* for 5 min. Bacterial cells were suspended in 1 ml of ultrapure water and heated at 100°C for 15 min. The supernatant containing the DNA was stored at 4°C until further use.

2.4 | Molecular characterization

2.4.1 | CoNS species determination

To further determine the CoNS species, we performed a multiplex polymerase chain reaction (PCR) assay using previously described primer sets (Campos-Pena et al., 2014; Kim et al., 2018). Ten CoNS species, namely *S. epidermidis*, *S. haemolyticus*, *S. pasteuri*, *S. warneri*, *S. xylosus*, *S. capitis*, *S. caprae*, *S. saprophyticus*, *S. lugdunensis*, and *S. hominis*, were determined by the presence and size of the PCR product.

2.4.2 | *Burkholderia cepacia* complex species identification

We conducted *recA* sequencing to identify the *Burkholderia cepacia* complex (BCC) species. We performed PCR amplification using specific primers and conditions described by Fehlberg et al. (2013). Cycle sequencing was performed using a BigDye Terminator v3.1 cycle sequencing kit and an ABI 3730xl DNA analyzer. We further analyzed the *recA* sequences and aligned them to a database using NCBI BLASTn.

2.4.3 | Detection of *mecA* and typing of SCC*mec* for *S. aureus* and *S. epidermidis*

To confirm methicillin-resistant *S. aureus* and *S. epidermidis*, we performed *mecA* detection using PCR with the *mecA*-specific primer pairs, as described previously (Pournajaf et al., 2014). We also performed a multiplex PCR assay using four primer pairs to identify SCC*mec* types I–V (Boye et al., 2007).

2.4.4 | Multilocus sequence typing and phylogenetic analysis

For the *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and BCC isolates, we conducted multilocus sequence typing (MLST) by amplifying seven housekeeping genes using previously described primer sets (Curran et al., 2004; Enright et al., 2000; Spilker et al., 2009; Thomas et al., 2007). When *aroE* of *S. aureus* was not detected, alternative primers were used: aroE745-up, 5′-TTATCACCGTCGATGCATAGTGCA-3′; aroE255-down, 5′-CGGAGTAGTATTTATCACAATATC-3′ (Ruimy et al., 2009). Furthermore, we used an alternative forward primer for undetected *trpB* of BCC: trpE-F2, 5′-AAGGACGCGCTGAACGAAGC-3′. The alternative primers used for the undetected *tpiA* of *S. epidermidis* were as follows: tpi-DF, 5′-GCAAGTATTTGGATAAAAGC-3′; tpi-DR, 5′-CCATCTAAGATGATTAAGGC-3′. The allele numbers and STs of each isolate were assigned according to the MLST database (https://pubmlst.org/). We performed an advanced cluster analysis to define clonal complexes (CCs) by using BioNumerics software ver. 7.6 (Applied Maths).

2.4.5 | Typing of *spa* for *S. aureus* isolates

For the *S. aureus* isolates, the polymorphic region of the staphylococcal protein A (*spa*) gene was amplified using previously described primer pairs and sequenced (Schuster et al., 2017; Strommenger et al., 2008). We determined *spa* types using BioNumerics software.

3 | RESULTS

3.1 | Descriptive characteristics of hemodialysis patients with VAIs

In total, 78 hemodialysis patients with VAI were enrolled in this 5-year single-institution study. Table 1 summarizes the descriptive characteristics of patients with VAI. Cases were more female than male, and the most prevalent age distribution was between 50 to 79 years old, with the median age of 65.95 years. All patients suffered from ESRD, and most of them were hypertensive, anemic, and diabetic. Over 50% of patients were more likely to have TCCs as their hemodialysis access, and about 53% of patients with VAI were involved in *S. aureus* infections.

3.2 | Analysis of clinical isolates collected from patients with VAIs

From 2013 to 2018, we collected 150 clinical isolates from patients with VAIs—including AVG- and TCC-related infections—undergoing hemodialysis in our institution (Figure 1). To investigate the prevalence of different species of bacterial infections across time, we divided the study period into two intervals: (Lafrance et al., 2008) from 2013 to 2014 and (Santoro et al., 2014) from 2015 to 2018. The total number of collected isolates decreased in the second interval; however, the prevalence of *S. aureus* and *S. argenteus* infection increased by approximately 20% in total (Table 2). Moreover, the patients undergoing hemodialysis were mainly infected by Gram-positive bacteria, particularly *S. aureus*, *S. argenteus*, and CoNS. *P. aeruginosa* and BCC species were the main Gram-negative bacteria causing VAIs in our institution.

Regarding species isolation according to VAI types, *Staphylococcus* spp. were mostly isolated from AVG-related infections, whereas BCC species were mainly isolated from TCC-related infections.

3.3 | Molecular characterization of *S. aureus* isolates

We observed that of 70 *S. aureus* isolates, 11 were of *S. argenteus*, which is a novel staphylococcal species that is closely related to *S. aureus* genetically and has recently been defined as a part of the *S. aureus* complex (SAC) (Aung et al., 2019; Jiang et al., 2018). In this study, we identified *S. argenteus* using MLST analysis because the species cannot be distinguished from *S. aureus* through conventional microbiological identification methods. All *S. argenteus* isolates belonged to ST2250 with non-typeable *spa* type, were methicillin-susceptible, were *mecA* negative; however, one isolate carried the SCC*mec* type I structure.

TABLE 1 Clinical characteristics of hemodialysis patients with vascular access infection (VAI).

Variable	No. of patients	Proportion (%)
Sex		
Male	29	37%
Female	49	63%
Age (year)		
30–39	2	3%
40–49	6	8%
50–59	19	24%
60–69	22	28%
70–79	17	22%
80–89	9	12%
90–99	3	4%
Type of vascular access (VA)		
AVG	43	55%
TCC	35	45%
Types of bacterial infection		
Multispecies infection w/*S. aureus*	9	12%
Multispecies infection w/o *S. aureus*	10	13%
S. aureus only	32	41%
Others	27	35%
Site of bacterial isolation		
Blood	8	10%
Contaminated device	49	63%
Blood + Contaminated device	21	27%
Comorbidity		
ESRD	78	100%
HTN	71	91%
DM	52	67%
Normocytic anemia	56	72%
CHB	11	14%
CHC	29	37%
CAD	11	14%
CHF	13	17%
PAOD	8	10%
Dyslipidemia	14	18%
Carcinoma/Cancer	10	13%

Abbreviations: AVG, arteriovenous graft; CAD, coronary artery disease; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CHF, congestive heart failure; DM, diabetes mellitus; ESRD, end-stage renal disease; HTN, hypertension; PAOD, peripheral artery occlusive disease; TCCs: tunneled-cuffed catheters.

Among 59 *S. aureus* isolates, we identified 15 STs (Table 3). Specifically, ST239, ST45, and ST59 were predominant in methicillin-resistant *S. aureus* (MRSA) isolates, and ST15 and ST7 were predominant in methicillin-sensitive *S. aureus* (MSSA); ST45, ST59, and ST15 were dominant in blood culture. Also, ST59, along with ST30 and ST239, was frequently isolated from contaminated implant devices. Besides, isolates with ST239 were multidrug-resistant (≥3 antimicrobial classes) and showing resistance toward at least three types of non-β-lactam antibiotics (Table A1). ST8, ST15, ST30, and ST45 were more prevalent in AVG isolates than in TCC isolates. Furthermore, we assigned 25 *spa* types to the isolates, with t437, t4864, t1081, and t091 being the predominant *spa* types. We observed ST8-t008 and ST239-t4864 in both MRSA and MSSA. Moreover, we analyzed the distribution of diverse STs and *spa* types among various SCC*mec* types. ST5-SCC*mec*IV-t437 (abbreviated as ST5-IV-t437), ST59-V-t437, ST45-V-t081, and ST7-MSSA-t091 were the most prevalent clones in this study.

3.4 | Molecular characterization of CoNS isolates

Four staphylococcal species were successfully identified among the 18 CoNS isolates, namely *S. epidermidis* (*n* = 9), *S. haemolyticus* (*n* = 2), *S. hominis* (*n* = 1), and *S. lugdunensis* (*n* = 1), and five isolates were unclassified; 16 isolates were methicillin-resistant (Table 4). Besides, approximately 78% of them were multidrug-resistant (Table A2). Methicillin-resistant *S. epidermidis* (MRSE) was the predominant species that belonged to seven distinct STs: ST2, ST22, ST57, ST173, ST226, ST490, and ST810. Of the nine MRSE isolates, two carried multiple SCC*mec* types, and the predominant SCC*mec* type was type IV. For the *S. haemolyticus* isolates, the oxacillin-susceptible isolate carried *mecA* and SCC*mec* type V. Moreover, the identified *S. hominis* and *S. lugdunensis* isolates carried SCC*mec* type II from AVG- and TCC-related infections, respectively, and were methicillin-resistant. Among the five unidentified CoNS isolates, two were methicillin-resistant CoNS (MR-CoNS) that did not carry *mecA*. Moreover, of the CoNS isolates, approximately 66.67% and 33.33% were isolated from contaminated implant devices and blood culture, respectively. Nevertheless, this study revealed no correlation between ST and origin of isolation.

3.5 | Molecular characterization of *P. aeruginosa* isolates

Of nine *P. aeruginosa* isolates, we identified six STs, one of which was a new ST (ST3373). Among the six STs, five were singletons, signifying that they represented only one strain (Table 5). Among the *P. aeruginosa* isolates, nearly 77.8% were from contaminated implant devices and nearly 22.2% were from blood culture. We identified a high antibiotic susceptibility rate (77.78%; 7/9) for the VAIs, with only two of the nine strains being resistant to antibiotics. ST235, the most prevalent *Pseudomonas* spp. to have multiple-drug resistance, was resistant to aminoglycoside and fluoroquinolones in this study.

3.6 | Molecular characterization of BCC isolates

We identified a total of 13 BCC isolates from TCC-related VAIs; these isolates involved two species, namely *B. contaminans* and

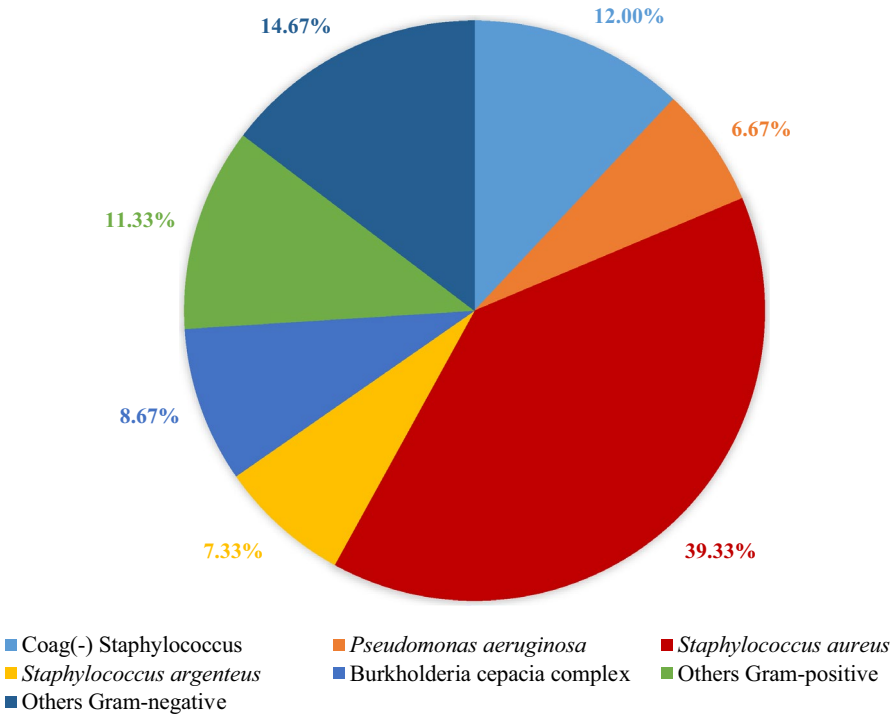

FIGURE 1 Distribution of isolates from vascular access infections in hemodialysis patients

TABLE 2 Prevalence (%) of 150 isolated vascular access infection (VAI) pathogens among hemodialysis patients in Chang Gung Memorial Hospital in Chiayi

	Bacterial isolates (Total *n* = 150)							
						Others		
	S. aureus	*S. argenteus*	CoNS	*P. aeruginosa*	BCC	G (+)[a]	G (–)[b]	Total
No.	59 (39.33%)	11 (7.33%)	18 (12%)	10 (6.67%)	13 (8.67%)	17 (11.33%)	22 (14.67%)	**150**
Year								
2013-2014	32 (35.16%)	3 (3.30%)	12 (13.19%)	8 (8.79%)	5 (5.49%)	14 (15.38%)	17 (18.68%)	91
2015-2018	27 (45.76%)	8 (13.56%)	6 (10.17%)	2 (3.39%)	8 (13.56%)	3 (5.08%)	5 (8.47%)	59
Origin								
AVG	37	8	10	4	0	9	11	79
TCC	22	3	8	6	13	8	11	71
Isolation								
Blood	14	3	6	2	4	3	3	35
Others[c]	45	8	12	8	9	14	19	115

Others G (+) included *Corynebacterium* spp., *Corynebacterium jeikeium*, *Clostridium perfringens*, *Enterococcus faecalis*, *Enterococcus faecium*, Group D *Streptococcus* (GDS), *Streptococcus agalactiae*, and *Viridans streptococcus*[a]

Others G (–) included *Acinetobacter baumannii*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Citrobacter koseri*, *Escherichia coli*, *Escherichia* spp., *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, and *Stenotrophomonas maltophilia*[b]

Others included abscess, AV-shunt, body fluid, CVP, graft, Hickman, pus, tissue, and wound.[c]

B. cepacia, of which *B. cepacia* was the predominant species (Table 6). MLST typing revealed that *B. cepacia* strains possessed new MLST types: ST1723 (*n* = 5) and ST1724 (*n* = 5). Most of the isolates that belonged to ST1723 were resistant to imipenem, whereas ST1724 isolates were resistant to gentamicin. Among the BCC isolates, approximately 70% were from contaminated implant devices and 30% were from blood culture. However, the study revealed no correlation between the origin of isolation and ST.

TABLE 3 Distribution of MLST, *spa* types, and SCC*mec* types in different isolates of MRSA and MSSA clones according to MLST clone complex (CC)

	CC	ST type	*spa* type	SCC*mec*	Case	Isolation
MRSA *n* = 37	CC5	5	t002(2)	II (2)	AVG (1), TCC (1)	Blood (1), Others (1)
	CC8	8	t008(1)	IV (1)	AVG (1)	Others (1)
		239	t4864(2), t3528(1), t037(2), t748(1)	II (2), III (4)	AVG (2), TCC (4)	Blood (1), Others (5)
		4798	t037(1)	III (1)	TCC (1)	Others (1)
	CC30	30	t019(4), t1836(1)	IV (5)	AVG (3), TCC (2)	Others (5)
	CC45	45	t002(1), t026(3), t1081(4), t2383(1)	II (1), IV (4), V (4)	AVG (6), TCC (3)	Blood (4), Others (5)
		508	t026(1)	NT (1)	AVG (1)	Others (1)
	Other	59	t437(7), t3513(3), t3527(2)	IV (7), V (5)	AVG (6), TCC (6)	Blood (3), Others (9)
MSSA *n* = 22	CC1	1	t2457(1)	NA (1)	TCC (1)	Others (1)
		188	t2769(1), t189(1)	I (1), NA (1)	AVG (1), TCC (1)	Others (2)
	CC8	8	t008(2)	NA (2)	AVG (2)	Others (2)
		239	t4864(2)	NA (2)	AVG (2)	Others (2)
	CC15	15	t803(2), t279(2), t547(1), t084(1)	NA (6)	AVG (5), TCC (1)	Blood (3), Others (3)
	CC30	30	t3732(1)	NA (1)	AVG (1)	Others (1)
	CC97	97	t224(1)	NA (1)	AVG (1)	Others (1)
	Other	7	t091(4)	NA (4)	AVG (2), TCC (2)	Blood (1), Others (3)
		398	t571(1)	NA (1)	AVG (1)	Others (1)
		845	t084(2)	NA (2)	AVG (2)	Blood (1), Others (1)

Abbreviations: AVG, arteriovenous graft; CC, clonal complex; NA, not applicable; NT, non-typeable, no corresponding band was found in multiplex PCR for SCC*mec* typing; TCC, tunneled-cuffed catheter.

TABLE 4 Molecular characterization of methicillin-resistant and methicillin-susceptible coagulase-negative staphylococci (CoNS) isolates from vascular access infections

Species		ST type	Case	Isolation	SCC*mec*	No.
S. epidermidis n = 9	MRSE	2	TCC	Others	IV	1
		22	AVG	Others	I	1
			TCC	Blood		1
		57	TCC	Others	IV	1
		173	AVG	Others	IV + V	1
		226	AVG	Others	IV	1
		490	AVG	Others	I + III	2
		810	AVG	Blood	IV	1
S. haemolyticus n = 2	MRSH	1	TCC	Blood	V	1
	MSSH	9	AVG	Others	V	1
S. hominis n = 1	MRSHo	ND	AVG	Blood	NT	1
S. lugdunensis n = 1	MRSL	ND	TCC	Others	II	1
Coag(-) *Staphylococcus* n = 5	MR-CoNS	ND	TCC	Blood	NT	2
		ND	TCC	Others	NA	1
		ND	AVG	Others	NA	1
	MS-CoNS	ND	AVG	Others	NA	1

Abbreviations: AVG, arteriovenous graft; NA, not applicable; ND, not determined; NT, non-typeable, no corresponding band was found in multiplex PCR for SCCmec typing; TCC, tunneled-cuffed catheter.

TABLE 5 Distribution of MLST and antibiotic resistance of *P. aeruginosa* isolated from different types of access

ST type	Case	Isolation	Antibiotic resistance profile	No.
235	TCC	Blood	CIP, GEN, LVX	1
244	AVG	Blood	NONE	1
	TCC	Others		1
303	AVG	Others	CAZ, PIP, TZP	1
381	TCC	Others	NONE	1
2682	AVG	Others		1
3373	TCC	Others		2
ND	TCC	Others		1
Total				**9**

Abbreviations: AVG, arteriovenous graft; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; LVX, levofloxacin; PIP, piperacillin; TCC, tunneled-cuffed catheter.

4 | DISCUSSION

VAIs constitute a risk factor for infection in patients undergoing hemodialysis. The pattern of microbes responsible for infection varies substantially among different types of access (Tokars et al., 2002). Pooled data show that *S. epidermidis* accounts for most CVC-related infections, whereas *S. aureus* is more common in AVF- and AVG-related infections. In our study, staphylococcal species accounted for 58.67% of VAIs, with *S. aureus* being the most commonly implicated species, followed by CoNS and *S. argenteus*. In the 150 isolates collected from patients with VAIs, *S. aureus* was the predominant pathogen in AVG- and TCC-related infections, with a rate of 37/79 (46.84%) and 22/71 (30.99%), respectively. *S. argenteus*, another in SAC species, was also more predominant in AVG-related infections than in TCC-related infections. Notably, the nine *S. epidermidis* isolates were mainly collected from AVG-related infections (6/9); this finding is not consistent with those reported by a previous study (Saeed Abdulrahman et al., 2002), which indicated that improving sterilization management procedures during hemodialysis may reduce the number of skin clones such as *S. epidermidis* on TCCs. Regarding representative Gram-negative bacteria in VAIs, *P. aeruginosa* and BCC predominantly caused TCC infections; in particular, BCC caused only TCC infections.

The type of VA is the most significant predictor of the infection risk, with AVGs and TCCs having higher infection risk than nature fistulas (Taylor et al., 2004). In patients undergoing hemodialysis who are particularly vulnerable to *S. aureus* infections, VA is the major entry for this golden germ. Previous studies indicated that a total of 19 to 26% of all *S. aureus* bacteremia occur in patients with ESRD (Chan et al., 2012; Fowler et al., 2003; Mylotte & Tayara, 2000; Vandecasteele et al., 2009). The incidence of which *S. aureus* infection, especially MRSA infection, was reported to be higher than that observed in the general population by 100-fold (Control CfD, Prevention, 2005). In our study, MRSA and MSSA infections accounted for 62.71% and 37.29% of *S. aureus* VAIs, respectively, with ST45, ST59, and ST239 being the predominant clones. Compared with our previous study (Chu et al., 2019), ST45, ST59, and ST239 were also common in other diseases or surgical infections, indicating that these are major clones in our institution and warrant more attention. According to the previous study in two regional hospitals in Taiwan, ST59, ST45, and ST239 were also the predominant nasal MRSA of patients visiting the emergency department (Wu et al., 2019). In addition to being the dominant lineage in Taiwan, ST59 is also endemic in China, Japan, Vietnam, Singapore, and Hong Kong (Chen & Huang, 2014). Notably, we also found the *S. aureus* ST239—an emerging multidrug-resistant MRSA

TABLE 6 Distribution of MLST and antibiotic resistance of *B. cepacia* complex (BCC) isolated from different types of vascular access

Species	ST type	Case	Isolation	Antibiotic resistance profile	No.
B. contaminans n = 3	102	TCC	Others	CST	2
			Blood	CST	1
B. cepacia n = 10	1723	TCC	Others	CST, GEN, IPM	2
				CST, IPM	1
				IPM, DOR	1
			Blood	ND	1
	1724		Blood	CST, GEN, IPM	1
			Others	CST, GEN, IPM	1
			Blood	GEN	1
			Others	GEN	1
			Others	No	1
Total					**13**

Abbreviations: CST, colistin; DOR, doripenem; GEN, gentamicin; IPM, imipenem; TCC, tunneled-cuffed catheter.

clone worldwide that generally carries an SCC*mec* type III element—in methicillin-sensitive strains without *mecA*. Furthermore, a novel nonpigmented staphylococcal lineage that cannot be distinguished from *S. aureus* using routine microbiological identification methods is now formally classified as *S. argenteus*; it was initially described as part of the distinct *S. aureus* CC (CC75) that is prevalent in aboriginal communities in the Northern Territory of Australia (Ng et al., 2009). *S. argenteus* comprising several CCs with many STs, especially ST2250, is the most commonly reported lineage with extensive geographic distribution, including France, Belgium, Thailand, Taiwan, Japan, and China, indicating a global spread (Argudin et al., 2016; Chantratita et al., 2016; Dupieux et al., 2015; Moradigaravand et al., 2017; Chen et al., 2018; Ohnishi et al., 2018; Li et al., 2019). The widespread *S. argenteus* has been isolated from both humans and animals. In our institution, ST2250 was the primary and only methicillin-sensitive ST revealed in VAIs, a finding that is consistent with those for previously reported *S. argenteus*-infected bacteremia cases in Taiwan (Chen et al., 2018).

The BCC is a group of opportunistic pathogens comprising at least 20 different species that commonly cause infections in immunocompromised patients, particularly those with cystic fibrosis (CF). *B. contaminans* was first identified from a contaminated Sargasso Sea DNA sample (Mahenthiralingam et al., 2006) and is increasingly associated with CF. However, other hospitalized non-CF patients have been reported to be affected by *B. contaminans* and *B. cepacia* infections. Nevertheless, *B. contaminans* is a contaminant in manufactured products, including pharmaceuticals and disinfectants (Martin et al., 2011; Moehring et al., 2014). In our institution, we obtained all BCC isolates from infected TCCs in hemodialysis patients with VAI; this suggests that the repeated use of mechanical device detergent and hemodialyzer reprocessing may cause contamination and that BCC species can survive in a harsh environment.

In this 5-year study, we collected 150 isolates from hemodialysis patients with VAIs and analyzed the isolates based on the year of isolation (i.e., study period interval). Although the number of isolates from infected accesses was relatively low in the interval 2015–2018, the incidence of *S. aureus*, *S. argenteus*, and BCC infections increased by approximately 10% (i.e., 45.76%, 13.56%, and 13.56%, respectively). By contrast, CoNS and *P. aeruginosa* infections decreased by nearly 3%–5%. Previous studies have not addressed the spread or transmission of *S. argenteus* in the hospital environment (Becker et al., 2019). Nevertheless, the growing trend of *S. argenteus* in VAIs indicates the potential and importance of this novel species in healthcare-associated infections. Since the therapeutic and clinical implications of *S. argenteus* are similar to those of *S. aureus*; therefore, infection prevention and control measures for *S. aureus* should be adopted for *S. argenteus*.

4.1 | Study limitations

The major limitation of this study is that the examined VAIs were mainly responsible for the removal of access. By contrast, we did not include infections managed through early intervention with conservative antibiotic treatment after identification. Therefore, we could not provide an overview of VAIs in this study.

5 | CONCLUSIONS

In this study, we examined 150 clinical isolates retrieved from infected VA grafts, including AVGs and TCCs, in hemodialysis patients by conducting 5-year epidemiological surveillance at a single institution in Taiwan. The three major STs (i.e., ST239, ST59, and ST45) of MRSA with various *spa* types showed high genetic diversity in *S. aureus* VAIs. Moreover, the ST102 *B. contaminans* isolate and two newly identified STs, namely ST1723 and ST1724 *B. cepacia* isolates, were exclusively retrieved from TCC-related infections. The increased incidence of infections engendered by *S. argenteus* and BCC provides insight into the potential clinical effects of *S. argenteus* and BCC species in VAIs.

ACKNOWLEDGEMENTS

This study was supported by grants from Chang Gung Memorial Hospital, Chiayi, Taiwan (Grant numbers: CMRPG6J0401, CMRPG6J0402, CMRPG6J0403, CMRPG6H0292, and CMRPG6H0293).

CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTION

Yuan-Hsi Tseng: Conceptualization (lead); Funding acquisition (lead); Methodology (lead); Writing-original draft (lead); Writing-review & editing (equal). **Min Yi Wong:** Conceptualization (lead); Formal analysis (lead); Investigation (lead); Writing-original draft (lead); Writing-review & editing (equal). **Tsung-Yu Huang:** Formal analysis (supporting); Investigation (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Bor-Shyh Lin:** Formal analysis (supporting); Investigation (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Chun-Wu Tung:** Formal analysis (supporting); Investigation (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Yao-Kuang Huang:** Conceptualization (supporting); Funding acquisition (lead); Writing-original draft (supporting); Writing-review & editing (equal).

ETHICS STATEMENT

This study was approved by the Institutional Review Board (IRB) of Chang Gung Memorial Hospital (IRB Nos: IRB201204188B0 and IRB201508482B0). Written consent was obtained from patients, and the study was performed following approved guidelines.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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REFERENCES

Control CfD, Prevention (2007). Invasive methicillin-resistant Staphylococcus aureus infections among dialysis patients–United States, 2005. *MMWR Morbidity and Mortality Weekly Report*, *56*(9), 197.

Argudin, M. A., Dodemont, M., Vandendriessche, S., Rottiers, S., Tribes, C., Roisin, S., de Mendonça, R., Nonhoff, C., Deplano, A., & Denis, O. (2016). Low occurrence of the new species *Staphylococcus argenteus* in a *Staphylococcus aureus* collection of human isolates from Belgium. *European Journal of Clinical Microbiology and Infectious Diseases*, *35*(6), 1017–1022.

Aung, M. S., San, T., San, N., Oo, W. M., Ko, P. M., Thet, K. T., Urushibara, N., Kawaguchiya, M., Sumi, A., & Kobayashi, N. (2019). Molecular characterization of *Staphylococcus argenteus* in Myanmar: identification of novel genotypes/clusters in staphylocoagulase, protein A, alpha-haemolysin and other virulence factors. *Journal of Medical Microbiology*, *68*(1), 95–104.

Becker, K., Schaumburg, F., Kearns, A., Larsen, A. R., Lindsay, J. A., Skov, R. L., & Westh H. (2019). Implications of identifying the recently defined members of the *Staphylococcus aureus* complex *S. argenteus* and *S. schweitzeri*: a position paper of members of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS). *Clinical Microbiology & Infection*, *25*(9), 1064–1070.

Boelaert, J. R., Van Landuyt, H. W., Gordts, B. Z., De Baere, Y. A., Messer, S. A., & Herwaldt, L. A. (1996). Nasal and cutaneous carriage of *Staphylococcus aureus* in hemodialysis patients: the effect of nasal mupirocin. *Infection Control and Hospital Epidemiology*, *17*(12), 809–811.

Boye, K., Bartels, M. D., Andersen, I. S., Moller, J. A., & Westh, H. (2007). A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. *Clinical Microbiology & Infection*, *13*(7), 725–727.

Campos-Pena, E., Martin-Nunez, E., Pulido-Reyes, G., Martin-Padron, J., Caro-Carrillo, E., Donate-Correa, J., Lorenzo-Castrillejo, I., Alcoba-Florez, J., Machin, F., & Mendez-Alvarez, S. (2014). Multiplex PCR assay for identification of six different *Staphylococcus* spp. and simultaneous detection of methicillin and mupirocin resistance. *Journal of Clinical Microbiology*, *52*(7), 2698–2701.

Chan, K. E., Warren, H. S., Thadhani, R. I., Steele, D. J., Hymes, J. L., Maddux, F. W., & Hakim R. M. (2012). Prevalence and outcomes of antimicrobial treatment for *Staphylococcus aureus* bacteremia in outpatients with ESRD. *Journal of the American Society of Nephrology*, *23*(9), 1551–1559.

Chantratita, N., Wikraiphat, C., Tandhavanant, S., Wongsuvan, G., Ariyaprasert, P., Suntornsut, P., Thaipadungpanit, J., Teerawattanasook, N., Jutrakul, Y., Srisurat, N., Chaimanee, P., Anukunananchai, J., Phiphitaporn, S., Srisamang, P., Chetchotisakd, P., West, T. E., & Peacock, S. J. (2016). Comparison of community-onset *Staphylococcus argenteus* and *Staphylococcus aureus* sepsis in Thailand: a prospective multicentre observational study. *Clinical Microbiology & Infection*, *22*(5), 458.e11–458.e19.

Chen, C. J., & Huang, Y. C. (2014). New epidemiology of *Staphylococcus aureus* infection in Asia. *Clinical Microbiology & Infection*, *20*(7), 605–623.

Chen, S. Y., Lee, H., Wang, X. M., Lee, T. F., Liao, C. H., Teng, L. J., & Hsueh, P.-R. (2018). High mortality impact of *Staphylococcus argenteus* on patients with community-onset staphylococcal bacteraemia. *International Journal of Antimicrobial Agents*, *52*(6), 747–753.

Chen, Y. K., Fang, H. C., Chou, K. J., Lee, P. T., & Chung, H. M. (2004). A puzzling cause of persistent *Pseudomonas aeruginosa* septicaemia in a patient on maintenance haemodialysis. *Nephrology, Dialysis, Transplantation*, *19*(9), 2400–2402.

Chu, C., Wong, M. Y., Tseng, Y. H., Lin, C. L., Tung, C. W., Kao, C. C., & Huang Y.-K. (2019). Vascular access infection by *Staphylococcus aureus* from removed dialysis accesses. *Microbiologyopen*, *8*(8), e800.

CLSI (2013). *Performance standards for antimicrobial susceptibility testing; Twenty-third international supplement*. CLSI document M100–S23. CLSI.

Curran, B., Jonas, D., Grundmann, H., Pitt, T., & Dowson, C. G. (2004). Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology*, *42*(12), 5644–5649.

Dupieux, C., Blonde, R., Bouchiat, C., Meugnier, H., Bes, M., Laurent, S., Vandenesch, F., Laurent, F., & Tristan, A. (2015). Community-acquired infections due to *Staphylococcus argenteus* lineage isolates harbouring the Panton-Valentine leucocidin, France, 2014. *Eurosurveillance*, *20*(23), 21154.

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J., & Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *38*(3), 1008–1015.

Fehlberg, L. C., Andrade, L. H., Assis, D. M., Pereira, R. H., Gales, A. C., & Marques, E. A. (2013). Performance of MALDI-ToF MS for species identification of *Burkholderia cepacia* complex clinical isolates. *Diagnostic Microbiology and Infectious Disease*, *77*(2), 126–128.

Fowler, V. G. Jr, Olsen, M. K., Corey, G. R., Woods, C. W., Cabell, C. H., Reller, L. B., Cheng, A. C., Dudley, T., & Oddone, E. Z. (2003). Clinical identifiers of complicated *Staphylococcus aureus* Bacteremia. *Archives of Internal Medicine*, *163*(17), 2066–2072.

Freixas, N., Bella, F., Limon, E., Pujol, M., Almirante, B., & Gudiol, F. (2013). Impact of a multimodal intervention to reduce bloodstream infections related to vascular catheters in non-ICU wards: a multicentre study. *Clinical Microbiology & Infection*, *19*(9), 838–844.

Hebeisen, U. P., Atkinson, A., Marschall, J., & Buetti, N. (2019). Catheter-related bloodstream infections with coagulase-negative staphylococci: are antibiotics necessary if the catheter is removed? *Antimicrobial Resistance & Infection Control*, *8*, 21.

Jiang, B., You, B., Tan, L., Yu, S., Li, H., Bai, G., Li, S., Rao, X., Xie, Z., Shi, X., Peng, Y., & Hu, X. (2018). Clinical *Staphylococcus argenteus* develops to small colony variants to promote persistent infection. *Frontiers in Microbiology*, *9*, 1347.

Kim, J., Hong, J., Lim, J. A., Heu, S., & Roh, E. (2018). Improved multiplex PCR primers for rapid identification of coagulase-negative staphylococci. *Archives of Microbiology*, *200*(1), 73–83.

Lafrance, J. P., Rahme, E., Lelorier, J., & Iqbal, S. (2008). Vascular access-related infections: definitions, incidence rates, and risk factors. *American Journal of Kidney Diseases*, *52*(5), 982–993.

Lebeaux, D., Fernández-Hidalgo, N., Chauhan, A., Lee, S., Ghigo, J.-M., Almirante, B., & Beloin, C. (2014). Management of infections related to totally implantable venous-access ports: challenges and perspectives. *The Lancet Infectious Diseases*, *14*(2), 146–159.

Li, Q., Li, Y., Tang, Y., Meng, C., Ingmer, H., & Jiao, X. (2019). Prevalence and characterization of *Staphylococcus aureus* and *Staphylococcus argenteus* in chicken from retail markets in China. *Food Control*, *96*, 158–164.

Mahenthiralingam, E., Baldwin, A., Drevinek, P., Vanlaere, E., Vandamme, P., LiPuma, J. J., & Dowson, C. G. (2006). Multilocus sequence typing breathes life into a microbial metagenome. *PLoS One*, *1*, e17.

Martin, M., Christiansen, B., Caspari, G., Hogardt, M., von Thomsen, A. J., Ott, E., & Mattner, F. (2011). Hospital-wide outbreak of *Burkholderia*

contaminans caused by prefabricated moist washcloths. *Journal of Hospital Infection*, *77*(3), 267–270.

Moehring, R. W., Lewis, S. S., Isaacs, P. J., Schell, W. A., Thomann, W. R., Althaus, M. M., Hazen K. C., Dicks K. V., Lipuma J. J., Chen L. F., & Sexton D. J. (2014). Outbreak of bacteremia due to *Burkholderia contaminans* linked to intravenous fentanyl from an institutional compounding pharmacy. *JAMA Internal Medicine*, *174*(4), 606–612.

Moradigaravand, D., Jamrozy, D., Mostowy, R., Anderson, A., Nickerson, E. K., Thaipadungpanit, J., Wuthiekanun, V., Limmathurotsakul, D., Tandhavanant, S., Wikraiphat, C., Wongsuvan, G., Teerawattanasook, N., Jutrakul, Y., Srisurat, N., Chaimanee, P., Eoin West, T., Blane, B., Parkhill, J., Chantratita, N., & Peacock, S. J. (2017). Evolution of the *Staphylococcus argenteus* ST2250 clone in Northeastern Thailand Is linked with the acquisition of livestock-associated staphylococcal genes. *Mbio*, *8*(4), e00802–17.

Mylotte, J. M., & Tayara, A. (2000). *Staphylococcus aureus* bacteremia: predictors of 30-day mortality in a large cohort. *Clinical Infectious Diseases*, *31*(5), 1170–1174.

Ng, J. W., Holt, D. C., Lilliebridge, R. A., Stephens, A. J., Huygens, F., Tong, S. Y. Currie, B. J., & Giffard, P. M. (2009). Phylogenetically distinct *Staphylococcus aureus* lineage prevalent among indigenous communities in northern Australia. *Journal of Clinical Microbiology*, *47*(7), 2295–2300.

Ohnishi, T., Shinjoh, M., Ohara, H., Kawai, T., Kamimaki, I., Mizushima, R., Kamada, K., Itakura, Y., Iguchi, S., Uzawa, Y., Yoshida, A., & Kikuchi, K. (2018). Purulent lymphadenitis caused by *Staphylococcus argenteus*, representing the first Japanese case of *Staphylococcus argenteus* (multilocus sequence type 2250) infection in a 12-year-old boy. *Journal of Infection and Chemotherapy: Official Journal of the Japan Society of Chemotherapy*, *24*(11), 925–927.

Pham, T. M., Kretzschmar, M., Bertrand, X., & Bootsma, M., Consortium C-M (2019). Tracking *Pseudomonas aeruginosa* transmissions due to environmental contamination after discharge in ICUs using mathematical models. *PLoS Computational Biology*, *15*(8), e1006697.

Pournajaf, A., Ardebili, A., Goudarzi, L., Khodabandeh, M., Narimani, T., & Abbaszadeh, H. (2014). PCR-based identification of methicillin-resistant *Staphylococcus aureus* strains and their antibiotic resistance profiles. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S293–S297.

Ruimy, R., Armand-Lefevre, L., Barbier, F., Ruppe, E., Cocojaru, R., Mesli, Y., Maiga, A., Benkalfat, M., Benchouk, S., Hassaine, H., Dufourcq, J. –B., Nareth, C., Sarthou, J. -L., Andremont, A., & Feil, E. J. (2009). Comparisons between geographically diverse samples of carried *Staphylococcus aureus*. *Journal of Bacteriology*, *191*(18), 5577–5583.

Saeed Abdulrahman, I., Al-Mueilo, S. H., Bokhary, H. A., Ladipo, G. O., & Al-Rubaish, A. (2002). A prospective study of hemodialysis access-related bacterial infections. *Journal of Infection and Chemotherapy: Official Journal of the Japan Society of Chemotherapy*, *8*(3), 242–246.

Santoro, D., Benedetto, F., Mondello, P., Pipito, N., Barilla, D., Spinelli, F., Ricciardi, C. A., Cernaro, V., Buemi, M., Pipito', N., & Barilla', D. (2014). Vascular access for hemodialysis: Current perspectives. *International Journal of Nephrology and Renovascular Disease*, *7*, 281–294.

Scheuch, M., Freiin von Rheinbaben, S., Kabisch, A., Engesser, J., Ahrendt, S., Dabers, T., Kohler C., Holtfreter S., Bröker B. M., & Stracke S. (2019). *Staphylococcus aureus* colonization in hemodialysis patients: a prospective 25 months observational study. *BMC Nephrology*, *20*(1), 153.

Schuster, D., Rickmeyer, J., Gajdiss, M., Thye, T., Lorenzen, S., Reif, M., Josten, M., Szekat, C., Melo, L. D. R., Schmithausen, R. M., Liégeois, F., Sahl, H. -G., Gonzalez, J. -P. J., Nagel, M., & Bierbaum, G. (2017). Differentiation of *Staphylococcus argenteus* (formerly: *Staphylococcus aureus* clonal complex 75) by mass spectrometry from *S. aureus* using the first strain isolated from a wild African great ape. *International Journal of Medical Microbiology*, *307*(1), 57–63.

Sewell, C. M., Clarridge, J., Lacke, C., Weinman, E. J., & Young, E. J. (1982). Staphylococcal nasal carriage and subsequent infection in peritoneal dialysis patients. *JAMA*, *248*(12), 1493–1495.

Spilker, T., Baldwin, A., Bumford, A., Dowson, C. G., Mahenthiralingam, E., & LiPuma, J. J. (2009). Expanded multilocus sequence typing for *Burkholderia* species. *Journal of Clinical Microbiology*, *47*(8), 2607–2610.

Strommenger, B., Braulke, C., Heuck, D., Schmidt, C., Pasemann, B., Nubel, U., & Witte, W. (2008). *spa* Typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. *Journal of Clinical Microbiology*, *46*(2), 574–581.

Taylor, G., Gravel, D., Johnston, L., Embil, J., Holton, D., Paton, S. (2004). Incidence of bloodstream infection in multicenter inception cohorts of hemodialysis patients. *American Journal of Infection Control*, *32*(3), 155–160.

Thomas, J. C., Vargas, M. R., Miragaia, M., Peacock, S. J., Archer, G. L., & Enright, M. C. (2007). Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *Journal of Clinical Microbiology*, *45*(2), 616–619.

Tokars, J. I., Miller, E. R., & Stein, G. (2002). New national surveillance system for hemodialysis-associated infections: initial results. *American Journal of Infection Control*, *30*(5), 288–295.

Vandecasteele, S. J., Boelaert, J. R., & De Vriese, A. S. (2009). *Staphylococcus aureus* infections in hemodialysis: what a nephrologist should know. *Clinical Journal of the American Society of Nephrology*, *4*(8), 1388–1400.

Wu, T. H., Lee, C. Y., Yang, H. J., Fang, Y. P., Chang, Y. F., Tzeng, S. L., & Lu, M. -C. (2019). Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* among nasal carriage strains isolated from emergency department patients and healthcare workers in central Taiwan. *Journal of Microbiology, Immunology, and Infection*, *52*(2), 248–254.

How to cite this article: Tseng Y-H, Wong MY, Huang T-Y, Lin B-S, Tung C-W, Huang Y-K. Molecular characterization of clinical isolates from vascular access infection: A single-institution study. *MicrobiologyOpen*. 2020;9:e1126. https://doi.org/10.1002/mbo3.1126

APPENDIX 1

Table A1 Distribution of antibiotic resistance profile in different *S. aureus* isolates according to ST type

S. aureus	CC	ST type	*spa*	Antibiotic profile	*mecA* gene	SCC*mec*	No.
MRSA	CC30 *n* = 5	30	t019	ERY, OXA, PEN	+	IV	1
				CLI, ERY, OXA, PEN		IV	3
			t1836	OXA, PEN		IV	1
	CC45 *n* = 10	45	t002	CLI, ERY, FUS, OXA, PEN	+	II	1
			t026	OXA, PEN		IV	2
				CLI, ERY, OXA, PEN		IV	1
			t1081	CLI, ERY, FUS, OXA, PEN		V	4
			t2383	CLI, ERY, OXA, PEN		IV	1
		508	t026	OXA, PEN	−	-	1
	CC5 *n* = 2	5	t002	CLI, ERY, OXA, PEN	+	II	1
				CLI, ERY, FUS, OXA, PEN		II	1
	CC8 *n* = 8	8	t008	ERY, OXA, PEN	+	IV	1
		239	t4864	CLI, ERY, OXA, PEN, SXT		II	2
			t3528	CLI, ERY, OXA, PEN, SXT, TGC		III	1
			t037	CLI, ERY, OXA, PEN, SXT		III	1
				CLI, ERY, FUS, OXA, PEN, SXT		III	1
			t748	CLI, ERY, FUS, OXA, PEN, SXT		III	1
		4798	t037	CLI, ERY, FUS, OXA, PEN, SXT		III	1
	Other *n* = 12	59	t437	CLI, ERY, OXA, PEN	+	IV	4
				CLI, ERY, OXA, PEN		V	3
			t3513	CLI, ERY, OXA, PEN		IV	3
			t3527	CLI, ERY, OXA, PEN		V	2
MSSA	CC1 *n* = 3	1	t2457	PEN	−	-	1
		188	t2769	PEN		-	1
			t189	PEN		I	1
	CC8 *n* = 4	8	t008	PEN		-	2
		239	t4864	CLI, ERY, PEN, SXT		-	2
	CC15 *n* = 6	15	t803	PEN	−	-	2
			t279	PEN		-	2
			t547	PEN		-	1
			t084	ERY, PEN		-	1
	CC30	30	t3732	CLI, ERY, PEN	−	-	1
	CC97	97	t224	PEN	−	-	1
	Other *n* = 7	7	t091	PEN	−	-	3
				ERY, PEN		-	1
		398	t571	CLI, ERY		-	1
		845	t084	PEN		-	2

Table A2 Distribution of antibiotic resistance profile in different coagulase-negative staphylococci (CoNS) isolates according to ST type

Species	ST type	Antibiotic resistance profile	SCC*mec*	No.
S. epidermidis	2	CLI, ERY, OXA, PEN, SXT	IV	1
	22	CLI, ERY, OXA, PEN, SXT	I	2
	57	OXA, PEN	IV	1
	173	ERY, OXA, PEN, SXT	IV + V	1
	226	ERY, OXA, PEN, SXT	IV	1
	490	CLI, ERY, OXA, PEN, SXT	I + III	2
	810	OXA, PEN, SXT	IV	1
S. haemolyticus	1	CLI, ERY, OXA, PEN, SXT	V	1
	9	PEN	V	1
S. hominis	ND	ERY, OXA, PEN, SXT	NT	1
S. lugdunensis	ND	CLI, ERY, OXA, PEN	II	1
Coag(-) Staphylococcus	ND	CLI, ERY, OXA, PEN, SXT	NT	1
	ND	CLI, ERY, OXA, PEN	NT	1
	ND	CLI, ERY, OXA, PEN, SXT	NT	1
	ND	CLI, ERY, OXA, PEN, SXT	NT	1
	ND	PEN	NT	1