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

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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 staphylococcus (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.

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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 staphylococcus (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

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

Background

The population of new patients with end-stage kidney disease (ESKD) 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 ESKD 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 (1). Pathogens primarily responsible for CVC-related infections are *Staphylococcus* , 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 (2). 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 staphylococcus (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; 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 (3, 4). 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 (5-7).

P. aeruginosa is a major cause 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 (8, 9).

We conducted a 5-year single-institution study to (1) investigate the prevalence of bacterial species from VAI (2) determine the molecular characteristics of different bacterial species isolated from various types of VAIs, and (3) establish the correlation between bacterial species, sequence types (STs), and VAI types.

Materials and Methods

Ethical approval

This study was approved by the Institutional Review Board (IRB) of Chang Gung Memorial Hospital (IRB Numbers: IRB101-41888 and IRB104-8482B). Written consent was obtained from patients, and the study was performed in accordance with approved guidelines.

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 79 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 prior to performing the procedures. 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 by using the bioMérieux system.

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 in accordance with the guidelines of the Clinical and Laboratory Standards Institute (10).

Genomic DNA extraction

A single colony from a clinical isolate was inoculated in tryptic soy broth (TSB) for 16 h, and 1 mL of 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.

Molecular characterization

CoNS species determination

To further determine CoNS species, we performed a multiplex polymerase chain reaction (PCR) assay using previously described primer sets (11, 12). 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.

Burkholderia cepacia complex species identification

We conducted *recA* sequencing to identify *Burkholderia cepacia* complex (BCC) species. We performed PCR amplification using specific primers and conditions described by Fehlberg et al. (13). 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.

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 (14). We also performed a multiplex PCR assay using four primer pairs to identify SCC*mec* types I–V (15).

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 (16-19). When *aroE* of *S. aureus* was not detected, alternative primers were used: aroE745-up, 5'-TTATCACCGTCGATGCATAGTGCA-3'; aroE255-down, 5'-CGGAGTAGTATTTATCACAATATC-3' (20). 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 advanced cluster analysis to define clonal complexes (CCs) by using BioNumerics software ver. 7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

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

For the *S. aureus* and *S. argenteus* isolates, the polymorphic region of the staphylococcal protein A (*spa*) gene was amplified using previously described primer pairs and sequenced (21, 22). We determined *spa* types using BioNumerics software.

Results

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: (1) from 2013 to 2014 and (2) from 2015 to 2018. The total number of collected isolates decreased in the second interval; however, the prevalence of *S. aureus* infection increased by approximately 20% (Table 1). 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.

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) (23, 24). 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 nontypable *spa* type, were methicillin susceptible, were *mecA* negative; however, one isolate carried the SCC*mec* type I structure.

Among 59 *S. aureus* isolates, we identified 12 STs. 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. In addition, ST59, along with ST30 and ST239, was also frequently isolated from contaminated implant devices. 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.

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 3). 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.

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 4). 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.

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 *B. cepacia* , of which *B. cepacia* was the predominant species (Table 5). 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.

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 (25). 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 (26), 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.

In patients undergoing hemodialysis, *S. aureus* infection is common, especially MRSA infection, the incidence of which was reported to be higher than that observed in the general population by 100-fold (27). 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,(4) the present study revealed that ST45, ST59, and ST239 were common in other diseases or surgical infections, indicating that these are major clones in our institution and warrant more attention. Notably, we also found the *S. aureus* ST239—an emerging multidrug-resistant MRSA 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 (28). *S. argenteus* comprising several CCs with many STs, especially ST2250, is the most commonly reported lineage with an extensive geographic distribution, including France, Belgium, Thailand, Taiwan, Japan, and China, indicating a global spread (29-35). 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 (33).

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 (36) 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* has been found to be a contaminant in manufactured products, including pharmaceuticals and disinfectants (37, 38). 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 on the basis of 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 (39). Nevertheless, the growing trend of *S. argenteus* in VAIs indicates the potential and importance of this novel, difficult-to-delimit species in healthcare-associated infections. Therefore, infection prevention and control measures that can be applied for *S. aureus* can be adopted for *S. argenteus* .

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.

Conclusions

In this study, we examined 150 clinical isolates retrieved from infected VA grafts, including AVGs and TCCs, in hemodialysis patients by conducting a 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.

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Conflict of Interests

The authors have no conflict of interest to declare.

Author Contributions

Yuan-Hsi Tseng: Conceptualization (lead); methodology (lead); funding acquisition (lead); writing – original draft (lead); writing – review and editing (equal); Min Yi Wong: Conceptualization (lead); investigation (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal); Tsung-Yu Huang, Bor-Shyh Lin, Chun-Wu Tung: Investigation (supporting); formal analysis (supporting); writing – original draft (supporting); writing – review and editing (equal); Yao-Kuang Huang: Conceptualization (supporting); funding acquisition (lead); writing – original draft (supporting); writing – review and editing (equal). All authors read and approved the final manuscript.

Ethics Statement

This study was approved by the Institutional Review Board (IRB) of Chang Gung Memorial Hospital (IRB Nos: IRB101-41888 and IRB104-8482B).

Data Accessibility Statement

The authors declare that the experimental data published in this paper are made accessible upon request for interested readers.

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Tables

Table 1. Prevalence (%) of 150 isolated vascular access infection (VAI) pathogens among hemodialysis patients in Chang Gung Memorial Hospital in Chiayi

	Bacterial isolates (Total n=150)	**Bacterial isolates (Total n=150)**	**Bacterial isolates (Total n=150)**	**Bacterial isolates (Total n=150)**	**Bacterial isolates (Total n=150)**	**Bacterial isolates (Total n=150)**	**Total**
	S. aureus S. argenteus	CoNS	*P. aeruginosa*	BCC	Others G (+) [a]	Others G (-) [b]	
No.	70 (46.67%)	18 (12%)	10 (6.67%)	13 (8.67%)	17 (11.33%)	22 (14.67%)	**150**
Year							
2013-2014	35 (38.46%)	12 (13.19%)	8 (8.79%)	5 (5.49%)	14 (15.38%)	17 (18.68%)	91
2015-2018	35 (59.32%)	6 (10.17%)	2 (3.39%)	8 (13.56%)	3 (5.08%)	5 (8.47%)	59
Origin							
AVG	45	10	4	0	9	11	79
TCC	25	8	6	13	8	11	71
Isolation							
Blood	17	6	2	4	3	3	35
Others [c]	53	12	8	9	14	19	115

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

Viridans streptococcus

[b.] 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* , *Stenotrophomonas maltophilia*

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

Table 2. Distribution of MLST, *spa* types, and SCC*mec* types in different isolates of MRSA and MSSA clones according to MLST clone complex (CC).

	CC[a]	**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)

[a]CC: clonal complex

AVG: arteriovenous graft; TCC: tunneled-cuffed catheter

NT: nontypeable, no corresponding band was found in multiplex PCR for SCC*mec* typing.

NA: not applicable

Table 3. Molecular characterization of methicillin-resistant and methicillin-susceptible coagulase-negative *Staphylococcus* (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

AVG: arteriovenous graft; TCC: tunneled-cuffed catheter

ND: not determined

NA: not applicable

NT: non-typeable, no corresponding band was found in multiplex PCR for SCC*mec* typing.

Table 4. 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

ST type	Case	Isolation	Antibiotic resistance profile	No.
Total	**Total**	**Total**	**Total**	**9**

AVG: arteriovenous graft; TCC: tunneled-cuffed catheter

CIP: ciprofloxacin; GEN: gentamicin; LVX: levofloxacin; CAZ: ceftazidime; PIP: piperacillin

Table 5. 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	**Total**	**Total**	**Total**	**Total**	**13**

TCC: tunneled-cuffed catheter

CST: colistin; GEN: gentamicin; IPM: imipenem: DOR: doripenem

Figure Legend

Figure 1. Distribution of isolates from vascular access infections in hemodialysis patients.