Major Article

Skin colonization at peripheral intravenous catheter insertion sites increases the risk of catheter colonization and infection

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Background: Peripheral intravenous catheters (PIVCs) break the skin barrier, and preinsertion antiseptic disinfection and sterile dressings are used to reduce risk of catheter-related bloodstream infection (CRBSI). In this study, the impact of PIVC skin site colonization on tip colonization and the development of CRBSI was investigated.
Methods: A total of 137 patients' PIVC skin site swabs and paired PIVC tips were collected at catheter removal, cultured, and bacterial species and clonality were identified.
Results: Of 137 patients, 45 (33%) had colonized skin sites and/or PIVC tips. Of 16 patients with paired colonization of both the skin site and PIVC tips, 11 (69%) were colonized with the same bacterial species. Of these, 77% were clonally related, including 1 identical clone of *Pseudomonas aeruginosa* in a patient with systemic infection and the same organism identified in blood culture.
Conclusions: The results demonstrate that opportunistic pathogen colonization at the skin site poses a significant risk for PIVC colonization and CRBSI. Further research is needed to improve current preinsertion antiseptic disinfection of PIVC skin site and the sterile insertion procedure to potentially reduce PIVC colonization and infection risk.

Peripheral intravenous catheters (PIVCs) are small flexible tubes that are vital for the delivery of therapies, such as fluids, drugs, and blood transfusions that are required in up to 70% of hospitalized patients.[1-3] The PIVC is introduced through the skin into the peripheral veins of the arms, hands, or lower limbs. Despite their relatively short-term use (typically <1 week)[4] they are a potential source of catheter-related bloodstream infections (CRBSIs) implicated in up to 5% of nosocomial bacteremias, with a prevalence of 0.67%-2.4%.[5-9] Such infections increase a patient's risk of death, discomfort, and length of hospital stay.[9-11]

Bacterial colonization of a central venous catheter device is a known risk factor for CRBSIs.[12] An estimated 60% of these CRBSIs are associated with the patient's skin flora.[13] The skin is a complex

environment that provides greater space for diverse commensal and pathogenic microbes.[14,15] If aseptic technique is not adhered to during PIVC insertion, bacteria can colonize at the insertion site and develop into biofilm, allowing sustained hematogenous dissemination and infection.[13,16] Even despite skin site decontamination with antiseptic prior to PIVC insertion, bacteria may remain in the hair follicles and lower dermis, and immediately after postinsertion catheter, the bacteria begin proliferation. If conditions are particularly favorable, for example moisture from diaphoresis, blood ooze, and numerous hair follicles, bacterial growth becomes faster. Skin bacteria can progressively colonize down the insertion site along the PIVC tract.[13]

To prevent PIVC-associated CRBSIs effectively, it is important to determine the risks of skin colonization and its association with infection. However, the impact of PIVC skin site colonization on tip colonization and the development of CRBSI has never been investigated comprehensively. This study explored the distribution of bacterial species that colonized both PIVC tips and paired PIVC skin sites, and the impact of skin colonization on PIVC tip colonization through culture and molecular methods.

METHODS

Study population and samples

The study was conducted in the medical and surgical wards of one of the hospitals participating in the parent study, Royal Brisbane and Women's Hospital, a major tertiary-referral, teaching hospital in Queensland, Australia between 2013 and 2014. PIVC tips and matching skin swabs at PIVC insertion sites were collected from 174 patients aged 18 years or older. All PIVCs used in the study were placed in the forearm or wrist for <24 hours to 15 days before recovering from patients. Exclusion criteria were that the patient had an existing bloodstream infection, was non-English speaking without an interpreter, was extremely diaphoretic, and had burned or diseased skin at the PIVC site or had existing skin tears or papery skin. Data were collected for each patient: age, sex, PIVC location, antimicrobial use, parenteral nutrition, dwell-time, the reason for removal, CRBSI diagnosis, and blood culture reports.

Ethics

The study was approved by the hospital and Griffith University human research ethics committees (HREC/11/QRCH/152 and NRS/46/11/HREC). All participants provided informed written consent prior to enrollment. All patient identifiers were removed from samples with a unique study number assigned.[3]

Catheter insertion and care

Preinsertion, the skin was decontaminated with 1% chlorhexidine gluconate (CHG) in 70% alcohol (3M, Flemington, NJ) with an aseptic nontouch technique, and then 25-30 mm BD Insyte Autogard shielded IV catheters (Becton Dickinson, Sandy, UT) were inserted, with an extension set and needleless connector routinely applied.[3] Nonmedicated transparent dressings and administration sets were maintained by registered nurses using standard protocols in accordance with guidelines.[1] At the discretion of the treating clinician, PIVCs were removed when clinically indicated (eg, treatment complete, PIVC complications, or suspected infection), or routinely (72-96 hours) in some units.

Sample collection, processing, and culture

Skin samples were collected by a research nurse using sterile cotton swabs moistened with 200 μL 0.9% sterile sodium chloride solution (Pfizer, New York, NY) from the PIVC skin site after removal of

the dressing and just prior to PIVC removal. After removal of the dressing, the insertion site was cleaned with 0.9% sterile sodium chloride solution, and 1-2 cm of the distal end of the PIVC was removed by sterile scissors and placed in a sterile container. Skin and tip specimens were transferred immediately to the microbiology research laboratory and placed in a 4°C refrigerator, then processed for microbiological culture within 24 hours. The skin swabs were placed into separate tubes containing phosphate buffer saline, vortexed, and centrifuged. The supernatant was then removed and resuspended into 200 μL phosphate buffer saline, and the resulting 100 μL suspension were each plated onto horse blood agar (Oxoid, Victoria, Australia) and chocolate agar (Oxoid) plates. The PIVC tip was cultured semiquantitatively using Maki methodology,[17] and roll-plated on blood and chocolate agar plates. The plates were then incubated at 37°C for 72 hours and monitored daily for bacterial growth. Bacterial colonies were counted and colony morphology was recorded for each sample. Bacterial colonies were subcultured into Müller-Hinton agar (Oxoid, Victoria, Australia) and stored in glycerol for further identification.

Bacterial identification using Vitek MS mass spectrometer

Vitek MS (bioMerieux, Brisbane, Australia), a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) was used to determine the bacterial species. Overnight bacterial cultures were spotted onto the Vitek MS target slides (bioMerieux). Vitek MS matrix (bioMerieux) was used on the target slide spots as per manufacturer's instruction.

DNA extraction and polymerase chain reaction and sequencing

The isolates that could not be identified through Vitek MS underwent molecular identification through Sanger sequencing. DNA was then extracted from freshly grown bacteria using the ultraclean microbial DNA isolation kit (MO BIO Laboratories, Carlsbad, CA) as instructed by the manufacturer's instructions. Polymerase chain reaction (PCR) was performed for 16 S ribosomal RNA genes amplified from genomic DNA using the forward 27F (5′AGAGTTTGAT CMTGGCTCAG3′) and 1492R (5′CGGTTACCTTGTTACGACTT3′), which covers all the variable regions (V1-V9). PCR reactions were carried out using 10 μL GoTaq Green Master Mix (Promega Bio Sciences, San Luis Obispo, CA), 1 μL forward primer (100 ng/μL), 1 μL reverse primer (100 ng/μL), 3 μL DNA template, and 10 μL nuclease-free water that were mixed in a 0.6 mL tube (LabAdvantage; Tingalpa, Queensland, Australia) to make the total volume of 50 μL and performed with standard cycling conditions. PCR products of each isolate were purified and the DNA was measured by a spectrophotometer. The DNA samples were then sent to Macrogen Inc, Seoul, Korea for Sanger sequencing.

Clonal relationship using rep-PCR

Isolates with the same species cultured from the pair of skin sites and the corresponding PIVC were typed for the clonal relationship using repetitive extragenic palindromic sequence PCR (REP-PCR) with primers REP-1 (5′-IIIGCGCCGICATCAGGC-3′) and REP-2 (5′-ACGTCTTATCAGGCCTAC-3′).[18] DNA extraction was performed by the ultraclean microbial DNA isolation kit (MO BIO Laboratories). All PCR reactions were performed with 10 μL 0.5 U BioTag DNA polymerase (Bioline, Memphis, TN), 1 μL forward primer (100 ng/μL), 1 μL reverse primer (100 ng/μL), 3 μL DNA template, and 10 μL nuclease-free water to make the total volume of 25 μL. The DNA fragment patterns were evaluated by electrophoresis with 1.0% agarose gels. Two strains were considered clonally related when they had both the same numbers and the same locations of DNA fragments.

Statistical analysis

Statistical analysis was performed with the GraphPad Prism package (GraphPad Software, San Diego, CA). *P* values <.05 were considered statistically significant. One-way analysis of variance was used to examine the difference in the relative frequency of bacterial distribution and total colony forming units between colonized groups. A Spearman correlation test was performed to determine the correlation of bacterial distribution and relative frequency between PIVC tips and skin sites.[19]

RESULTS

Patients and PIVCs

Of the 174 patients, 137 (79%) patient PIVC tips and matching skin swabs were processed for microbiological cultures. The remaining 37 (21%) PIVC tips and matching skin swabs were excluded from culture because of prolonged storage time (>24 hours) before the microbiological processing. Only 1 patient had CRBSI. Among 137 patients, 45 (33%) were either colonized on the PIVC tip or skin insertion sites. Twenty-nine (21%) patients' PIVC tips and 34 (25%) patients' skin sites had positive cultures. *Staphylococcus capitis* (30%) and *Staphylococcus epidermidis* (28%) were the most recovered bacteria on both the skin site and matching PIVC tip. Sixteen of 45 patient skin sites and matching PIVC tips had positive cultures, of these 11 (69%) were matched species. CRBSI was diagnosed only in this patient group (Table 1).

The patient group that had colonized PIVC tips and skin with matched species were aged >50 years, 7 were men and 3 were women (Table 1). Although the average PIVC dwell-time was 4 days, 9 (82%) of these 11 patients had relatively longer (≥4 days) PIVC dwell in place than those with bacterial colonization of only PIVC tips or skin site (28%-50%). The patient group with matched bacterial species showed relatively higher colony forming unit counts (≥15) compared with the other patient groups (Table 1).

Clonal relationship of isolates recovered both the skin site and matching PIVC tip

To determine whether the bacterial species that recovered from PIVC tips were the same clone that colonized skin sites, we performed repetitive sequence-based PCR that amplified multiple fragments of various lengths. If the matching bacteria amplified multiple fragments at the same length, this represented the same bacterial clones. Of the 11 patients (26 isolates) with paired skin/tip bacterial species, 8 patients (20 isolates) had exactly the same bacterial clones. The patient who had CRBSI with *Pseudomonas aeruginosa* had identical clones of *P aeruginosa* recovered from both the skin site and matching PIVC tip.

Bacterial distribution and relative frequency

A total of 13 bacterial species colonized the PIVC tips and 12 species colonized the skin sites, representing 3 phyla. The majority were identified as Firmicutes on both PIVC tips and PIVC skin sites, followed by Proteobacteria and Actinobacteria (Table 2). Most species were gram-positive, with only 4 species of gram-negative bacteria (*P aeruginosa*, *Pseudomonas oryzihabitans*, *Roseomonas mucosa*, and *Acinetobacter lwoffi*). PIVCs were mostly colonized with skin-associated bacteria. Coagulase-negative *Staphylococcus*, *Bacillus* spp, *Pseudomonas* spp, and *Acinetobacter* spp were isolated on both skin sites and PIVC tips. *Staphylococcus aureus* was recovered on a patient's skin site but not on the matching PIVC tip. However, *A lwoffi* was recovered from 2 PIVC tip samples but not on matching skin sites. The difference in the distribution of bacterial species isolated from PIVC tips

Table 1
Demographics, clinical and culture characteristics of the 45 patients with colonized bacteria

Characteristics	Colonized on PIVC tip and skin with same bacterial species (n = 11)	Colonized on PIVC tips (n = 11)	Colonized on skin insertion sites (n = 18)
Number (%)			
Sex			
Male	7 (64)	9 (82)	8 (44)
Female	4 (36)	2 (18)	10 (56)
Age (years)			
≥50	11 (100)	10 (91)	14 (78)
<50	0	1 (9)	4 (22)
Device location			
Right	9 (82)	8 (72)	12 (67)
Left	2 (18)	3 (28)	6 (33)
Duration of the catheter in situ (days)			
≥4	9 (82)	3 (28)	9 (50)
<4	2 (18)	8 (72)	9 (50)
Clinical diagnosis			
BSI	1 (9)	0	0
Definite CRBSI	1 (9)	0	0
Complications at device removal			
Infiltration (severe swelling)	3 (27)	0	3 (17)
Phlebitis	0	1 (9)	0
Bacterial colony forming units			
≥15	4 (36)	2 (18)	3 (17)
<15	7 (64)	9 (92)	15 (83)

BSI, bloodstream infection; *CRBSI*, catheter-related bloodstream infection; *PIVC*, peripheral intravenous catheter.

and skin sites was not statistically significant (*P* > .05, one-way analysis of variance). *S epidermidis*, *S aureus*, *Staphylococcus hominis*, *Staphylococcus carnosus*, and *Kytococcus sedentarius* isolates were detected on skin sites more frequently than PIVC tips. However, *Staphylococcus warneri*, *Staphylococcus cohnii*, *Micrococcus luteus*, *Corynebacterium striatum*, and *P oryzihabitans* isolates were identified and detected on PIVC tips more frequently than skin sites. There was no correlation in the distribution and relative frequency of bacterial colonization of PIVC tips and skin sites (Spearman correlation coefficients = 0.2; *P* > .005). Bacterial species such as *S epidermidis*, *S capitis* and *M luteus* colonized with a higher frequency on both PIVC tips and skin sites. Other species such as *P aeruginosa*, *R mucosa*, and *Bacillus mycoides* were also colonized with a lower frequency on both PIVC tips and skin sites (Table 2).

DISCUSSION

In this study, 69% of patients in which bacteria were recovered from both skin sites and matching PIVC tips were colonized with the same species, suggesting that the skin at catheter insertion sites are the potential sources of PIVC colonization. Interestingly, a significant proportion (77%) of bacterial species that colonized skin sites and paired PIVC tips were also clonally related, which provides clear evidence that these bacteria colonized the skin first, and then migrated down the catheter to colonize the PIVC segment.

Within the study, only 1 patient was diagnosed with a CRBSI caused by *P aeruginosa*. The same clonal *P aeruginosa* isolate was also recovered on the patient's PIVC tip and skin site, which provides further evidence that the organism responsible for CRBSI was originated from the skin insertion site. To our knowledge, this is the first time has been demonstrated for PIVCs.

The bacterial species isolated from PIVC tips and skin sites were diverse, representing 3 phyla (Firmicutes, Proteobacteria and Actinobacteria), and were both gram-positive and gram-negative.

Table 2
Bacterial distribution and relative frequency on PIVC tips and paired skin swabs recovered from 137 patients

			Number (%)		
Phylum group	Organism type	Bacterial species colonized	PIVC tip and skin colonized with matched bacterial species (n = 26)	PIVC colonization only (n = 35)	Skin site colonization only (n = 40)
Firmicutes	Gram-positive	*Staphylococcus epidermidis*	6 (23)	5 (14)	11 (29)
	Gram-positive	*Staphylococcus aureus**	0	0	1 (3)
	Gram-positive	*Staphylococcus capitis*	8 (30)	4 (11)	5 (13)
	Gram-positive	*Staphylococcus warneri*†	0	2 (5)	0
	Gram-positive	*Staphylococcus haemolyticus*	0	1 (3)	2 (5)
	Gram-positive	*Staphylococcus cohnii*†	0	2 (5)	0
	Gram-positive	*Staphylococcus hominis**	0	0	3 (8)
	Gram-positive	*Staphylococcus carnosus**	0	0	1 (3)
	Gram-positive	*Bacillus cereus*	0	2 (5)	2 (5)
	Gram-positive	*Bacillus mycoides*	2 (8)	1 (3)	1 (3)
Actinobacteria	Gram-positive	*Micrococcus luteus*	6 (23)	12 (44)	11 (29)
	Gram-positive	*Corynebacterium striatum*†	0	1 (3)	0
	Gram-positive	*Kytococcus sedentarius**	0	0	1 (3)
Proteobacteria	Gram-negative	*Pseudomonas oryzihabitans*†	0	1 (3)	0
	Gram-negative	*Pseudomonas aeruginosa*	2 (8)	1 (3)	1 (3)
	Gram-negative	*Roseomonas mucosa*	2 (8)	1 (3)	1 (3)
	Gram-negative	*Acinetobacter lwoffii*†	0	2 (5)	0

PIVC, peripheral intravenous catheter.
*Bacteria colonized only on PIVC skin sites, not on PIVC.
†Bacteria colonized on PIVC, not on PIVC skin sites.

Coagulase-negative *Staphylococcus,* such as *S epidermidis*, and *S capitis* were the most commonly colonized species on PIVCs and skin sites (Table 1). All have been associated with CRBSI.[20,21] Pathogens such as *S aureus* and *P aeruginosa* that frequently cause CRBSIs[20,22,23] were also detected in this study. This is consistent with other studies[24-27] of central venous catheter that report skin colonization is one of the major risk factors for catheter colonization and subsequently increases the risk of CRBSI.

It is possible that the topical antiseptic used to decontaminate the skin sites prior to insertion may have been inadequate, and thus increased the risk of bacteria regeneration and subsequent migration down the catheter. Although preinsertion antiseptic disinfection kills most of the bacteria, thereby contributing to reductions in CRBSI, some bacteria remain.[24-28] Even despite the best practice decolonization of skin at catheter sites, approximately 25% of patient's skin site was still colonized with bacteria, which is consistent with the colonization rate (21%) at PIVC insertion skin site.[29] The results indicate that current skin decolonization practice could be improved with antimicrobial dressings to prevent microbial colonization at catheter skin sites.

Inadequate skin antisepsis may result from a lack of intrinsic antimicrobial activity of the antiseptic, insufficient volume of solution applied, inadequate drying time, the presence of a resistant pathogen, or the use of a contaminated antiseptic. Reduced susceptibility to CHG in *S aureus* and *S epidermidis* and other major nosocomial pathogens has also been reported.[30-32] Importantly, antiseptics are not equally effective for all classes of bacteria. For example, chlorhexidine is only moderately effective for gram-negative bacteria.[33] Moreover, there is increasing antibiotic resistance and cross-resistance to antiseptic in gram-negative bacteria.[34-38] Interestingly, in our study, the patient diagnosed with CRBSI was colonized with gram-negative *P aeruginosa* at the skin insertion site, on the PIVC tip, and in blood culture, which suggests that CHG decolonization may not be effective to remove the gram-negative bacteria. In this study, we did not collect any skin swab prior to the CHG decolonization, so we are not certain if *P aeruginosa* was already colonized in the skin or contaminated at the skin site during PIVC insertion. Further research is needed on effective topical antiseptic regimens that can effectively eliminate bacteria including gram-negatives present at the catheter insertion site. The repeated use of antiseptic during PIVC dwell, not just at insertion,

may be effective, as well as the use of antimicrobial dressings; these all require research investigation in PIVC cohorts.

In this study, we only investigated extraluminal colonized bacteria and did not include the intraluminal catheter cultures. Therefore, the number of PIVC colonization may not be truly representative. Furthermore, we may have been unable to identify other bacterial populations that colonized PIVC tips and skin sites owing to the lack of sensitivity of culture techniques to grow diverse bacteria. Therefore, careful consideration should be taken during the interpretation of these results concerning the potential bias. Samples were taken from patients under clinical care, including the use of 2% CHG in 70% alcohol to decolonize the skin site for PIVC insertion. Thus, we could not ascertain whether skin colonization developed after being decolonized with the single-use antiseptic agent as we did not take skin swabs immediately after the decolonization of the insertion sites. Finally, the sample size was likely inadequate to detect associations in outcomes in subgroups of patients.

Previously, bacteria isolated from catheter tips were identified at a genus level with the traditional method such as Gram's staining and biochemical test.[39] In this study, bacterial isolates were identified by Vitek MS, a comprehensive bacterial identification method that provides 99% accurate identification of both gram-positive and gram-negative bacteria at the species level.[39,40] The Vitek MS identification in this study presented a detailed bacterial profile on PIVC tips and skin sites and provided confidence to the results at a species level. To our knowledge, this is the first study on PIVCs that explored the distribution, association of their relative frequency, and molecular typing of bacterial species that colonized both PIVCs and paired PIVC skin sites, and provides clear evidence that the same bacterial clones colonized on skin sites are responsible for colonization on PIVC tips. These findings highlight the importance of using an effective topical decolonizer to achieve successful suppression of skin colonization and subsequently reduce the risk of CRBSI.

CONCLUSIONS

Understanding of bacteria that colonize PIVCs and associated skin sites provide critical information on the source of bacteria that pose risk for CRBSIs and highlights the importance of optimal skin disinfection before catheter insertion. Current topical antiseptics may be

inadequate for preventing cutaneous bacterial spread. Improved topical antiseptics, skin and catheter care are crucial to prevent catheter colonization and subsequent infection. The results indicate that there is a need to research more frequent use of skin antiseptics during PIVC dwell, and the use of antimicrobial dressings to potentially reduce PIVC infection risk.

Acknowledgements

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