Original research article

The microbial biofilm composition on peripherally inserted central catheters: A comparison of polyurethane and hydrophobic catheters collected from paediatric patients

Maddie Higgins[1] ⑩, Li Zhang[2,3], Rebecca Ford[1], Jeremy Brownlie[1], Tricia Kleidon[4,5], Claire M. Rickard[3,4] and Amanda Ullman[3,4]

Abstract

Background: Peripherally inserted central catheters are susceptible to microbial colonisation and subsequent biofilm formation, leading to central line–associated bloodstream infection, a serious peripherally inserted central catheter–related complication. Next-generation peripherally inserted central catheter biomaterials, such as hydrophobic materials (e.g. Endexo®), may reduce microbial biofilm formation or attachment, consequently reducing the potential for central line–associated bloodstream infection.

Methods: Within a randomised controlled trial, culture-dependent and culture-independent methods were used to determine if the biomaterials used in traditional polyurethane peripherally inserted central catheters and hydrophobic peripherally inserted central catheters impacted microbial biofilm composition. This study also explored the impact of other clinical characteristics including central line–associated bloodstream infection, antibiotic therapy and dwell time on the microbial biofilm composition of peripherally inserted central catheters.

Results: From a total of 32 patients, one peripherally inserted central catheter was determined to be colonised with *Staphylococcus aureus*, and on further analysis, the patient was diagnosed with central line–associated bloodstream infection. All peripherally inserted central catheters ($n = 17$ polyurethane vs $n = 15$ hydrophobic) were populated with complex microbial communities, including peripherally inserted central catheters considered non-colonised. The two main microbial communities observed included *Staphylococcus* spp., dominant on the colonised peripherally inserted central catheter, and *Enterococcus*, dominant on non-colonised peripherally inserted central catheters. Both the peripherally inserted central catheter biomaterial design and antibiotic therapy had no significant impact on microbial communities. However, the diversity of microbial communities significantly decreased with dwell time.

Conclusion: More diverse pathogens were present on the colonised peripherally inserted central catheter collected from the patient with central line–associated bloodstream infection. Microbial biofilm composition did not appear to be affected by the design of peripherally inserted central catheter biomaterials or antibiotic therapy. However, the diversity of the microbial communities appeared to decrease with dwell time.

[1] School of Environment and Science, Griffith University, Brisbane, QLD, Australia
[2] School of Dentistry and Oral Health, Gold Coast Campus, Griffith University, Brisbane, QLD, Australia
[3] Alliance for Vascular Access Teaching and Research (AVATAR), Menzies Health Institute Queensland, Griffith University, Brisbane, QLD, Australia
[4] School of Nursing and Midwifery, Griffith University, Brisbane, QLD, Australia
[5] Vascular Assessment and Management Service, Queensland Children's Hospital, Brisbane, QLD, Australia

Corresponding author:
Maddie Higgins, School of Environment and Science, Griffith University, Brisbane, QLD 4122, Australia.
Email: maddiejhiggins@bigpond.com

Introduction

Peripherally inserted central catheters (PICCs) are commonly used for short- and long-term access in patients for the delivery of intravenous therapy, including but not limited to, the administration of antibiotic therapy, chemotherapy, blood products and parenteral nutrition.[1,2] The use of PICCs has significantly increased over the previous decade due to assumed reliability, ease of insertion, cost-effectiveness, outpatient suitability and safety, when compared with centrally inserted catheters.[3–5] However, PICCs are susceptible to complications, such as occlusion and venous thrombosis.[6–8] In addition, PICCs are susceptible to microbial colonisation and subsequent biofilm formation, leading to central line–associated bloodstream infection (CLABSI), which may occur with the administration of parenteral nutrition, prolonged dwell time and patient immunosuppression.[6–8] Previous research has demonstrated that microbial colonisation may occur within 24 h of PICC insertion, with microbial biofilm formation evident within 48–72 h.[9] These microbial biofilms may become resistant to antibiotic therapy, making them difficult to eradicate and therefore becoming a recurrent source of CLABSI.[9–11]

Complications of PICC usage may be avoided if successful insertion and management strategies are implemented into patient care. These strategies include the use of antisepsis with 2% chlorhexidine gluconate (CHG), maximal barrier precautions, correct catheter calibre and site selection, the use of ultrasound-guided cannulation, catheter fixation and strict adherence to flushing policy.[12,13] Next-generation PICC biomaterials have also been developed to reduce complications associated with catheter thrombosis.[14] Biomaterials, such as hydrophobic materials (e.g. Endexo®), have been developed that can be combined with polyurethane in the manufacturing of PICC catheter materials.[15] This has produced a PICC (BioFlo® with Endexo® PICC, AngioDynamics Inc.; Queensbury, NY) that contains the permanent, non-eluting polymer that is more resistant to the accumulation of blood components, such as platelets and clotting factors, theoretically reducing venous thrombosis.[15] Given the thrombus-resistant properties of the BioFlo PICC there may be an associated reduction in microbial biofilm formation and accumulation, thereby reducing the incidence of CLABSI.[15] However, manufacturers of the BioFlo PICC have not made these anti-infective claims.[16]

Nested within a randomised controlled trial,[14] the aim of this study was to characterise the microbial biofilm composition on two different PICC biomaterials, traditional polyurethane PICCs and hydrophobic PICCs, from paediatric patients. In addition, this study also explored the impact of clinical characteristics, including CLABSI, antibiotic therapy and dwell time, on the microbial biofilm composition of PICCs.

Method

Ethical approval

Clinical PICC samples were supplied from 32 in-patients at the Queensland Children's Hospital (QCH), Queensland, Australia, as a substudy of a pilot randomised controlled trial comparing traditional polyurethane PICCs (Turbo-Ject® Power-Injectable PICCs (Cook Medical; Bloomington, IN)) and hydrophobic PICCs (Bioflo® with Endexo PICCs (Angiodynamics Inc; Queensbury, NY)).[14] Ethical approval for the study was granted by Queensland Health Human Research Ethics Committee (HREC/15/QRCH/164) and Griffith University Human Research Ethics Committee (HREC/2016/077), and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615001290583).

Study inclusion criteria

Patients were eligible to participate in the study if they were less than 18 years of age; required a PICC inserted for treatment for more than 24 h; did not have a bloodstream infection at time of recruitment; and written informed consent from legal guardians was obtained. Patients were eligible for participation in the substudy when PICC materials could be safely collected at time of PICC removal. Other than the PICC allocation, all other aspects of PICC insertion, use and management were completed by clinical staff in accordance with local clinical practice guidelines, including the use of 2% CHG in 70% ethanol for skin antisepsis.[17]

Sample collection and culture method

When the PICC was no longer required, nursing staff removed the PICC after disinfecting the insertion site with 2% CHG in 70% ethanol. All PICCs were collected by qualified registered nurses (research nurses) with experience in preparation of specimens for culture. The distal 2–3 cm of the PICC tip was cut using sterile scissors and deposited in a sterile container. All PICCs were

handled under aseptic conditions and immediately transported to the laboratory for examination where they were cultured using the semi-quantitative roll-plate culture method.[18] Descriptive information including patient demographics, PICC utilisation, clinical characteristics and PICC complications was prospectively collected from each patient by research nurses during the trial, using an online, secure database. Incidence of CLABSI was defined in accordance with Centre for Disease Control National Health Safety Network criteria by a blinded infectious disease specialist.[19]

Genomic DNA extraction

Following the processing for microbial culture, PICCs were suspended in 200 μL of lysis buffer, which contained 20 mg/mL lysozyme, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton and Proteinase K at 37°C overnight. Genomic DNA was extracted from all PICCs using the PowerSoil DNA Isolation Kit (Mo Bio, USA). For each PICC, a control (sterile) PICC was taken from the original packaging and rolled back and forth on blood agar plates. Any potential genomic DNA was extracted from each control PICC using the PowerSoil DNA Isolation Kit (Mo Bio).

Bioinformatic analyses

Targeted genes were amplified from purified genomic DNA using the primers F (5' AAACTYAAAKGAATTGRCGG 3') and R (5' ACGGGCGGTGWGTRC3') which amplify the variable regions (V6-V8) of the 16S rRNA (rrs encoding gene). For each genomic DNA sample, polymerase chain reaction (PCR) was performed in triplicate. The PCR products were purified using the Qiaquick PCR Purification kit (Qiagen, Australia). Barcoded library construction and paired-end sequencing was performed using the Illumina MiSeq platform, supported and operated by the Australian Centre for Ecogenomics (Brisbane, Australia).

The raw 16S rRNA sequence data were trimmed from the 3'-prime end until their average quality score exceeded 25 and reads containing ambiguous characters were additionally trimmed at the occurrence of the first 'N'. Subsequently, low-quality reads were identified and excluded using criteria adapted from Huse et al.[20]

Sequences were subsequently analysed using QIIME, and homologous reads were grouped into operational taxonomic units (OTUs) using a threshold of 97% sequence identify. Sequences were chimera checked using ChimeraSlayer. A representative sequence from each OTU was then subjected to BLAST alignment with the Greengenes database, as implemented in QIIME, and the OTU table was generated through QIIME, including the frequency of each OTU observed in each sample. These OTU tables can be represented at Phylum, Class, Order, Family and Genus/

Species levels of assignment, and heat maps at the genus level of assignment were constructed in R, using the g-plots package (R package version 2.6.0).

Alpha (within sample) diversity metrics were computed using QIIME. The Shannon diversity and richness values from these analyses were compared by paired t test, as were the differences in individual and relative OTUs abundance values. Beta (between sample) diversity metrics, including hierarchical clustering and weighted and unweighted Unifrac distances, were also computed using QIIME and the FastUnifrac metric. The Jaccard distance was used for principal coordinate analysis, and further statistical comparisons were performed using Calypso software.[21] The two-tailed t test was used to evaluate the difference between variances. A p value of less than 0.05 was considered significant.

Results

Patient demographics and culture results

In total, 32 patients were recruited, including 17 traditional polyurethane and 15 hydrophobic PICCs. Patient's had a mean age of 7 years, and 84.3% ($n = 27$) were on systemic antibiotic therapy at the time of PICC removal. Most patients (71.9%; $n = 23$) had a PICC in situ for 11–20 days, and there was no difference in dwell time between traditional polyurethane and hydrophobic PICCs. One hydrophobic PICC was found to be colonised (over 10^3 colony-forming units (CFUs)) using the semi-quantitative roll-plate culture method,[18] and on further analysis, the patient was diagnosed with a CLABSI. Isolates from both the PICC and the bloodstream were identified as *Staphylococcus aureus*. No other CLABSI were identified within the cohort.

Regardless of the semi-quantitative roll-plate culture method producing a positive or negative result, microbial genomic DNA was extracted and quantified from all PICCs and all produced amplicon libraries for sequencing. No microbial DNA was recovered or amplified from sterile, unused PICCs, which precluded any contaminating DNA arising from the manufacture of the PICCs, or the reagents used. Therefore, no negative control samples were used for sequencing and further study.

Microbial community profiles

A total of 1,328,160 high-quality sequence reads were produced from the 32 PICCs with an average of 41,505 reads per sample. These were from prokaryote genomes assigned to seven bacterial (Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Tenericutes and Thermi) and two fungal (Ascomycota and Basidiomycota) phyla. Almost 90% of the sequences observed were derived from just two of the phyla (Firmicutes and Proteobacteria). The OTUs were further assigned to 99 known genera including *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Propionibacterium*, *Stenotrophomonas* and *Candida*.
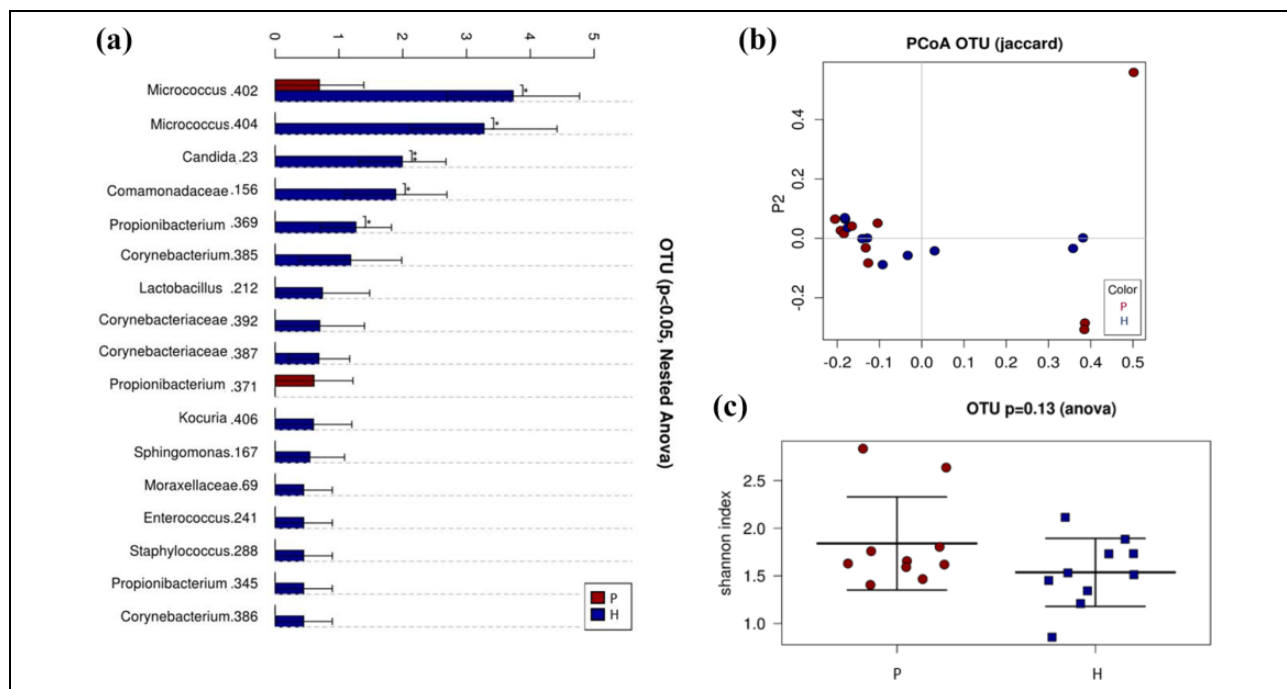
Figure 1. Comparing microbial communities on polyurethane (P) and hydrophobic (H) PICCs, (a) significant different microbial group ($p < 0.05$, ANOVA). Significant differences between groups (t test) are annotated by *$p < 0.05$, **$p < 0.01$, (b) principal coordinate analysis of phylogenetic distance among communities according to the weighted Unifrac metric and (c) Shannon index.

Catheter material impacts microbial community composition

To assess the impact of PICC biomaterials on microbial biofilm composition, both PICC dwell time and CLABSI factors first were removed. A total of 20 PICCs, inclusive of 10 polyurethane and 10 hydrophobic PICCs, with dwell times of 11–20 days were assessed. Although a slightly higher abundance of potential pathogens were detected on the traditional polyurethane PICCs, including *Candida*, *Propionibacterium*, *Micrococcus* and *Staphylococcus* sp., than on hydrophobic PICCs, these differences were not indicative of significant differences in microbial communities (Shannon–Weaver, $p = 0.13$; Richness, $p = 0.52$; Evenness, $p = 0.27$; and Simpson, $p = 0.05$). Principal coordinate analyses also suggested that microbial communities present on these PICCs could not be differentiated based on the design of the PICC biomaterials.

The impact of CLABSI on microbial biofilm composition of PICCs

The microbial community recovered from the PICC collected from the patient with confirmed CLABSI was significantly different to the microbial communities recovered from PICCs collected from patients without CLABSI, as shown by OTU abundances, principal coordinate analysis and alpha-diversity metrics (Shannon–Weaver, $p = 0.04$; Richness, $p = 0.79$; Evenness, $p = 0.24$; and Simpson,

$p = 0.02$) (Figure 1(a).). The microbial communities recovered from the PICC collected from the patient with a CLABSI predominantly comprised *Staphylococcus* sp., while *Enterobacteriaceae* sp. dominated PICCs collected from patients without CLABSI (Figure 1(b)). The microbial communities recovered from the patient with CLABSI possessed a high abundance of *Staphylococcus*, *Propionibacterium* and *Corynebacterium,* while microbial communities recovered from PICCs collected from patients without CLABSI possessed a higher abundance of *Enterococcus*, *Desemzia* and *Rhodobacteraceae* (Figure 1(c).)

Impact of antibiotic therapy on microbial communities

The representative microbial communities recovered from PICCs collected from the patients treated with antibiotic therapy ($n = 27$) were indistinguishable from the microbial communities recovered from PICCs collected from patients who did not receive antibiotic therapy ($n = 5$). This was evident through both principal coordinate analysis and alpha-diversity metrics (Shannon–Weaver, $p = 0.04$; Richness, $p = 0.85$; Evenness, $p = 0.29$; and Simpson, $p = 0.03$).

Catheter dwell time on biofilm community composition

The microbial communities recovered from PICCs in situ for ≤10, 20, 30 or 40 days were significantly different as

shown by diversity metrics. Overall, the diversity of microbial communities decreased with PICC dwell time. Microbial communities recovered from PICCs with a dwell time of 10 days shared 78 OTUs with those recovered from PICCs with a dwell time of 20 days and shared only 15 OTUs with those recovered from a PICC a dwell time of 40 days (Shannon–Weaver, $p = 0.05$; Richness, $p = 0.85$; Evenness, $p = 0.31$; and Simpson, $p = 0.03$).

Discussion

Microbial biofilm composition did not appear to be affected by PICC biomaterial design. The two main microbial communities recovered from PICCs included *Staphylococcus* spp. recovered from the colonised PICC and *Enterococcus* recovered from non-colonised PICCs., with more diverse pathogens present on the colonised PICC. Overall, a lower microbial diversity index, but higher pathogen frequency, was recovered from the PICC collected from the patient with CLABSI, potentially triggering the process of clinically significant CLABSI. Previous studies have identified coagulase-negative *Staphylococci*, *Staphylococcus aureus*, enteric gram-negative bacilli and *Candida* as common microorganisms responsible for catheter colonisation and subsequent CLABSI.[13,22,23]

The presence of PICCs, regardless of biomaterial design, have been shown to stimulate specific immune response, therefore potentially increasing the risk of microbial attachment, colonisation, biofilm formation and subsequent CLABSI.[24–26] Within the current study, antibiotic therapy had no significant impact on microbial communities; however, the diversity of microbial communities appeared to decrease with PICC dwell time. Previous studies have identified the potential for microbial communities, present in biofilms, to have increased resistance to antibiotic therapy.[27] Possible reasons may include antibiotic therapy becoming inactive on contact with the biofilm or failing to penetrate the complex biofilm matrix.[28] Primarily, PICCs are colonised with a single species of microorganism, with multiple species of microorganisms subsequently forming to encourage microbial biofilm formation.[29] Potential reasons may include that over time, certain species of microorganisms favour environmental factors over other species, or may compete for nutrients, potentially inhibiting other species from further developing.[30]

This study had limitations. Our methods detected dead microbial genomic DNA, which may be subclinical and thereby have limited clinical impact. Also, the sample was small, and from a specific population (paediatrics), which limits its generalisability outside of this cohort. Finally, there was only one patient with confirmed PICC colonisation and subsequent CLABSI, further limiting generalisability. The study presents preliminary testing of the impact of PICC materials on biofilm formation, and a useful model for the examination of clinical factors responsible for PICC colonisation and CLABSI. However, microbial biofilm composition did not appear to be affected by the design of PICC biomaterials.

ORCID iD

Maddie Higgins 🆔 https://orcid.org/0000-0003-1317-9823

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