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ARTICLE

# Molecular investigation of bacterial communities on the inner and outer surfaces of peripheral venous catheters

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**Abstract** Peripheral venous catheters (PVCs) are some of the most widely used medical devices in hospitals worldwide. PVC-related infections increase morbidity and treatment costs. The inner surfaces of PVCs are rarely examined for the population structure of bacteria, as it is generally believed that bacteria at this niche are similar to those on the external surface of PVCs. We primarily test this hypothesis and also study the effect of antibiotic treatment on bacterial communities from PVC surfaces. The inner and outer surfaces of PVCs from 15 patients were examined by 454 GS FLX Titanium 16S rRNA sequencing and the culture method. None of the PVCs were colonised according to the culture method and none of the patients had a bacteraemia. From a total of 127,536 high-quality sequence reads, 14 bacterial phyla and 268 diverse bacterial genera were detected. The number of operational taxonomic units for each sample was in the range of 86–157, even though 60 % of patients had received antibiotic treatment. *Stenotrophomonas maltophilia* was the predominant bacterial species in all the examined PVC samples. There were noticeable but not statistically significant differences between the inner and outer surfaces of PVCs in terms of the distribution of the taxonomic groups. In addition, the bacterial communities on PVCs from antibiotic-treated patients were significantly different from untreated patients. In conclusion, the surfaces of PVCs display complex bacterial communities. Although their significance has yet to be determined, these findings alter our perception of PVC-related infections.

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## Introduction

Peripheral venous catheters (PVCs) are vital for the administration of fluids and medications and for multiple blood access. In the USA, it is estimated that 25 million PVCs are inserted each year, accounting for 90 % of all venous catheters [1]. PVC-associated infection is considered to be implicated in 5 % of cases of nosocomial bacteraemia, which has a prevalence of 670 per 100,000 patients with PVCs [2]. Although PVC-related infections may not result in increased mortality, they contribute considerably to the burden of the health-care system by increasing morbidity and treatment costs [1, 3, 4].

Intravenous catheter insertion provides a portal of entry for bacteria to cross from an unsterile external environment to the normally sterile blood [5]. The precursor to bloodstream infection (BSI) is usually device colonisation; bacteria found

adhering to the extra- or intra-luminal surfaces of catheters are the principal source and cause of BSIs [6, 7]. Post-attachment, many microbial communities form biofilms [8, 9]. Because of increased resistance to antibiotics, the bacteria within biofilms are difficult to treat effectively [10].

The detection and identification of the bacteria found on intra-vascular catheters, including PVCs, has relied on culture-dependent techniques, such as the 'roll-plate' method [11]. This method involves culturing the external surface of the catheter tip by rolling a segment (2–3 cm) of the intra-vascular catheter four times over the surface of a blood agar plate with sterile forceps. However, the 'roll-plate' technique fails to detect bacteria on the inner surface of catheters and does not identify fastidious bacterial species. Moreover, bacteria identified from the positive cultures are rarely found to be associated with BSI [12–14]. As for the inner surfaces of short-term catheters, they are generally not examined for colonisation, as it is presumed that bacteria first colonise the outer surface before colonising the inner surface, and that the compositions of the bacterial population at these two surfaces are similar. However, the availability of nutrition, temperature, oxygen tension, exposure to antibiotics and infusion fluids, and fluid dynamics are factors that could contribute to differences in the micro-environment on the inner and outer surfaces of PVCs. This may reflect changes in the bacterial composition on both PVC surfaces. The aim of this study was to provide data to test this hypothesis and to examine the effect of antibiotic treatment on the microbial communities on PVC surfaces.

## Materials and methods

### Hospital setting and study population

After obtaining institutional ethics approval and patients' informed consent, the study was conducted from April 2008 to October 2009 at three teaching hospitals in Queensland, Australia. PVCs (Insyte™ Autoguard™, BD Medical, Franklin Lakes, NJ, USA) were inserted by hospital nurses or doctors and cared for in accordance with usual hospital practice. Skin was decontaminated with 2 % chlorhexidine gluconate in 70 % ethanol before insertion of the PVC. Transparent, semi-permeable dressings at the insertion site (Tegaderm™ 1624W, 3M, St. Paul, MN, USA) were maintained by nurses according to standard hospital protocol.

When the PVC was no longer required, the nursing staff removed the PVC after treating the insertion site with 2 % chlorhexidine in 70 % ethanol. Fifteen PVC samples were taken by qualified registered nurses with experience in the preparation of specimens for culture. The distal 2–3 cm of the tip was cut using sterile scissors and deposited in a sterile container. All PVC tips were handled under aseptic conditions and immediately transported to the laboratory for examination, where they were cultured by the semi-quantitative method [11].

### Culture method

This method is based on rolling a segment, usually the tip, of the removed PVCs back and forth on 5 % sheep blood agar plates (Oxoid, Adelaide, Australia). The plates were incubated at 37 °C under aerobic conditions for 2─4 days. Microorganisms were then isolated and identified according to standard microbiology protocols. The PVC tip was considered to be colonised if the plate grew ≥15 colony forming units (cfu).

### Molecular techniques

In order to separate the bacterial communities of the inner and outer PVC surfaces, 1 cm at both ends of the PVC tips were carefully heat-sealed. PVC tips were placed in 1.5-ml microcentrifuge tubes and gently sonicated to release the bacteria from the surfaces of the PVCs. Lysis buffer containing 20 mg/ml lysozyme, 20 mM Tris-HCl (pH8.0), 2 mM EDTA and 1.2 % Triton was added and incubated at 37 °C overnight. Following this, the PVC tips were removed and bacterial DNA from the outer surfaces of PVCs was collected. DNA extraction was continued using the QIAamp DNA Mini Kit (Qiagen, Brisbane, Australia), according to the manufacturer's instructions.

In order to extract bacterial DNA from the inner surface, the external surface of the PVC section was washed and disinfected with ethanol, both ends of PVC tips were cut and 200 μl of lysis buffer passed through. The PVC fragment was incubated at 37 °C overnight. Bacterial DNA from the inner surfaces was collected and extracted using the QIAamp DNA Mini Kit, according to the manufacturer's instructions. A new PVC was suspended in lysis buffer and DNA was extracted as above for a negative control. 16S rRNA genes were amplified from purified genomic DNA using the primers F (5′ AGA GTT TGA TCC TGG CTC AG 3′) and R (5′ GAG TTT GAT CCT GGC TCA G 3′), which would cover variable regions (V1, V2 and V3). Thirty different self-correction barcodes were designed and added to the primers. For each 25-μl reaction, the conditions were as follows: 3 μl of DNA template (concentration ranged from neat to $1:10^3$), 2.5 μl of 10× reaction buffer containing 20 mM $MgCl_2$, 2 μl of 25 mM dNTPs, 1 μl of each primer (10 μM), 0.1 U of Taq DNA polymerase (Qiagen, Brisbane, Australia), 5 μl of 5× BSA and 10.4 μl of sterile deionised water. Each polymerase chain reaction (PCR) run contained a negative control (sterile deionised water instead of template DNA) and a positive control (*Escherichia coli* instead of template DNA). For each DNA sample, three replicate PCRs were performed.

PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen, Brisbane, Australia). These products were sequenced unidirectionally in the reverse direction

by means of the Genome Sequencer FLX (GS-FLX) system (Roche, Sydney, Australia) at 454 Life Sciences.

### Informatics analysis

Pyrosequence reads were analysed using the QIIME package (Quantitative Insights Into Microbial Ecology) as follows [15]. Briefly, barcoded 16S rRNA gene sequences were inputted into QIIME, then sequences were classified using the Ribosomal Database Project classifier and sequences were aligned and chimera checked using PyNAST and ChimeraSlayer. UniFrac distances were calculated and data generated for summarising the proportions of taxonomy and principle coordinates analysis (PCoA) plots. All sequences were assigned to phylotypes (operational taxonomic units, OTUs) at 97 % sequence identity thresholds and 80 % confidence was used in the QIIME-based wrapper of the RDP classifier program against the RDP core set. The Chao1 microbial richness estimate and overall community diversity (Shannon–Weaver index) were computed from the OTU data. Rarefaction curves were generated by plotting the number of observed genera versus the number of sequences sampled. A heatmap was generated on the basis of the relative abundance of OTUs using R version 2.15 (The R Project for Statistical Computing). PCoA was carried out based on the theta similarities of the relative abundance of the OTUs in each sample. The top bacterial OTUs were selected after ranking their separability, measured as the variance between classes using principal component analysis with respect to instrumental variables in R version 2.15 [16]. Therefore, bacterial OTUs which contribute to the significant difference in the compared bacterial groups were shown as box plots and the two-tailed *t*-test was used to evaluate the difference between PVC groups. The Monte Carlo permutation test was used to test the statistical significance of the relationships of the bacterial communities on PVCs between patients with antibiotics and those without.

## Results and discussion

### Sample characteristics

The 15 patients, of whom 60 % were females, had a median age of 58 years. All PVCs were short term and the duration of PVCs left in situ varied from 2 to 4 days. Approximately 80 % of PVCs were in the forearm and 60 % of patients were receiving systemic antibiotic therapy during the observation period. No local signs of inflammation were noticed. No patients were suspected or diagnosed with PVC-related BSI. According to the 'roll-plate' culture results, none of the PVCs were colonised, as no bacteria were grown on the Columbia blood agar plates. In this study, bacterial DNA was extracted from all PVC samples, although they were 'non-colonised', as defined by the 'roll-plate' method. These DNA samples were amplified and used for constructing pyrosequencing libraries. No bacterial DNA was detected and amplified from the negative control PVCs. These control results indicate that the contribution to the bacterial community from the manufacture of PVCs, DNA extraction procedures and PCR reagents is negligible.

### Features of the bacterial community on PVCs

Sequence lengths outside the bounds of 150 and 600, ambiguous bases exceeding limits, missing or mean quality score below a minimum of 25, maximum homopolymer run exceeding a limit of 6 and mismatches in primers exceeding the limits of 1 were discarded. Of the 16S rRNA gene sequences generated, 127,536 high-quality sequence reads from 30 samples (15 each on the outer and inner surfaces of PVCs) passed the set threshold, and were used for further analysis. Chimera checks showed that all sequences were unlikely to be chimeric. The average number of sequences per sample was 4,251 (range 1,413–10,957) and the average base-pair length was 496 (range 150–599).

Overall, microbial communities on PVCs had a high diversity and complex community structure. 16S rRNA gene sequences were assigned to 14 different bacterial phyla: *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, cyanobacteria, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Planctomycetes*, *Proteobacteria*, TM7, *Thermotogae* and unclassified bacteria (Fig. 1a). The predominant phylum was *Proteobacteria*, including *Alphaproteobacteria* (4 % of 16S rRNA reads), *Betaproteobacteria* (8.4 %) and *Gammaproteobacteria* (68 %). Four phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*) represented, on average, 98 % of individual bacterial communities on PVC surfaces. In all, 140 OTUs were from unclassified bacteria. All of the examined sequences were assigned to 1,026 OTUs (predominant OTUs are shown in Fig. 2). A high number of OTUs was observed for each sample, ranging from 86 to 157. Sample richness was between 119 and 268, and the overall diversity (Shannon–Weaver index) was in the range from 2.24 to 3.64.

### Bacterial diversity was very complex when examined using pyrosequencing analysis

The limitations of culture methods to determine bacterial communities from environmental and clinical samples have been reported previously [17]. Several possible explanations for the discordant results from the two methods included: (1) antibiotic usage in patients, which is common for acute hospital patients [18–21]; (2) sonication as part of the protocol in molecular methods may help the detachment of

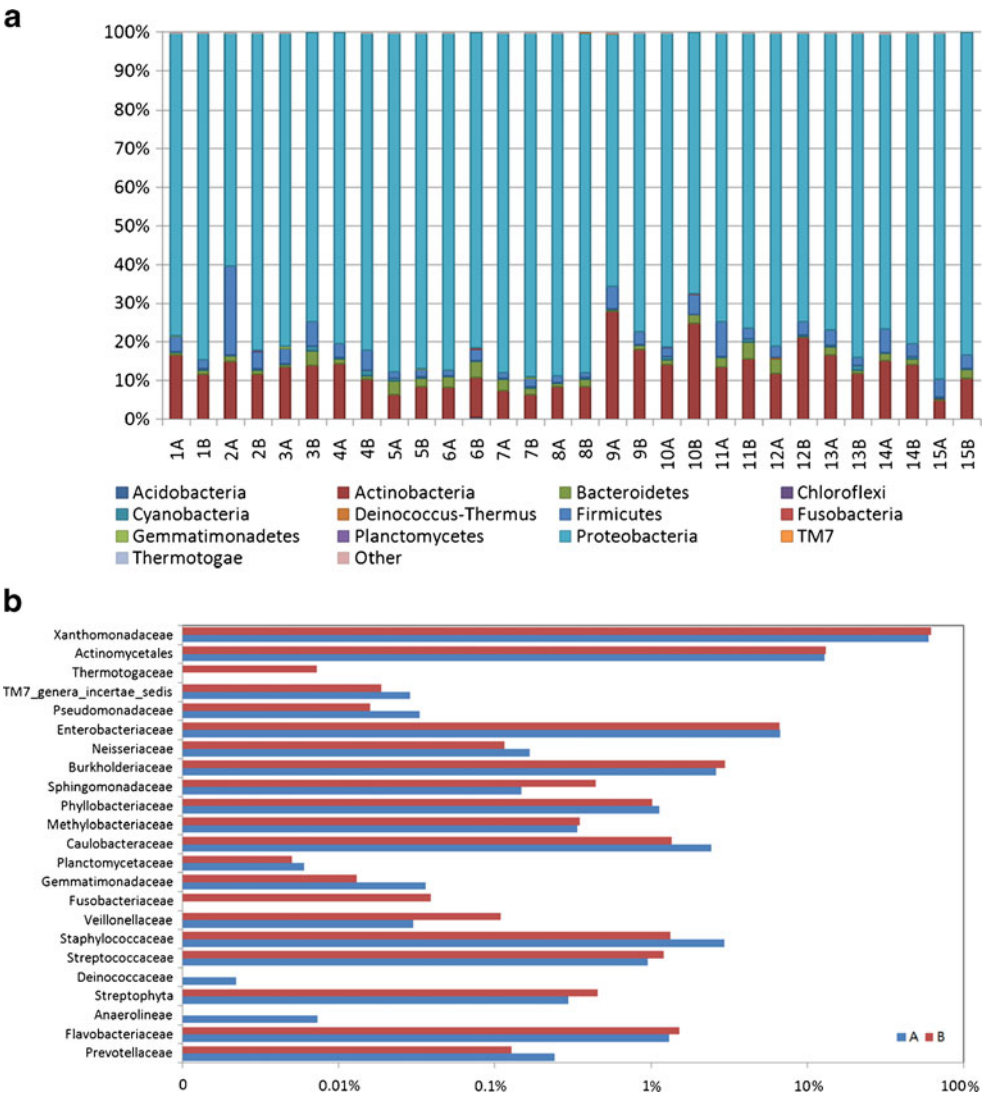



**Fig. 1** Microbial phyla detected in **a** peripheral venous catheter (PVC) samples, and **b** inner and outer surfaces of PVCs. *A* denotes the inner surface of PVCs and *B* denotes the outer surfaces of PVCs. Each column represents one sample

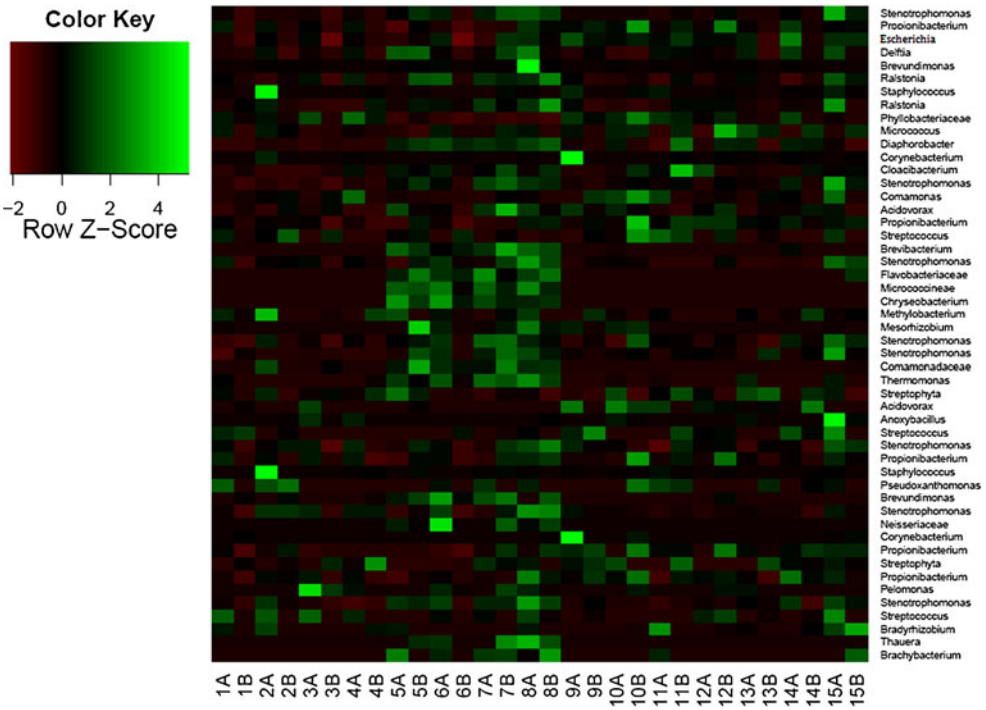
bacteria in biofilms; (3) some bacteria are more easily cultured than others; (4) DNA-based molecular methods might detect DNA from dead bacteria. Overall, the molecular methods provide much greater sensitivity than culture methods for this work.

## Comparisons between bacterial communities on the inner and outer surfaces of PVCs

The difference in Shannon–Weaver diversity indices between bacterial communities on the inner and outer surfaces of PVCs was not significant, with $p=0.365$ ($H_{\text{Inner surface}}=3.05\pm0.35$, $H_{\text{Outer surface}}=2.95\pm0.22$). Similarly, the results from PCoA suggested that bacterial communities on the inner and outer surfaces of PVCs had no statistically significant difference, as they did not separate. There were noticeable differences between the inner and outer surfaces of PVCs in terms of the distribution of the taxonomic groups. For example, PVC inner surfaces had higher abundance of *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes* and TM7 than the outer surfaces (Fig. 1b). More *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, cyanobacteria and *Proteobacteria* were detected on the outer surfaces of PVCs than on the inner surfaces. *Chloroflexi* and *Deinococcus-Thermus* were only present on the inner surfaces, and, conversely, *Fusobacteria* and *Thermotogae* were only present on the outer surfaces of PVCs. It is unknown whether those low-abundance bacteria play a significant role in PVC-related infections or not and future research is required in order to verify this. Although these results indicate variations in bacterial diversity, they are at very low abundances. No significant difference was found between the two groups.

## Effect of antibiotic treatment on the bacterial communities of PVCs

There were 12 PVC samples (inner and outer surfaces of PVCs) from six patients without antibiotic treatment. We



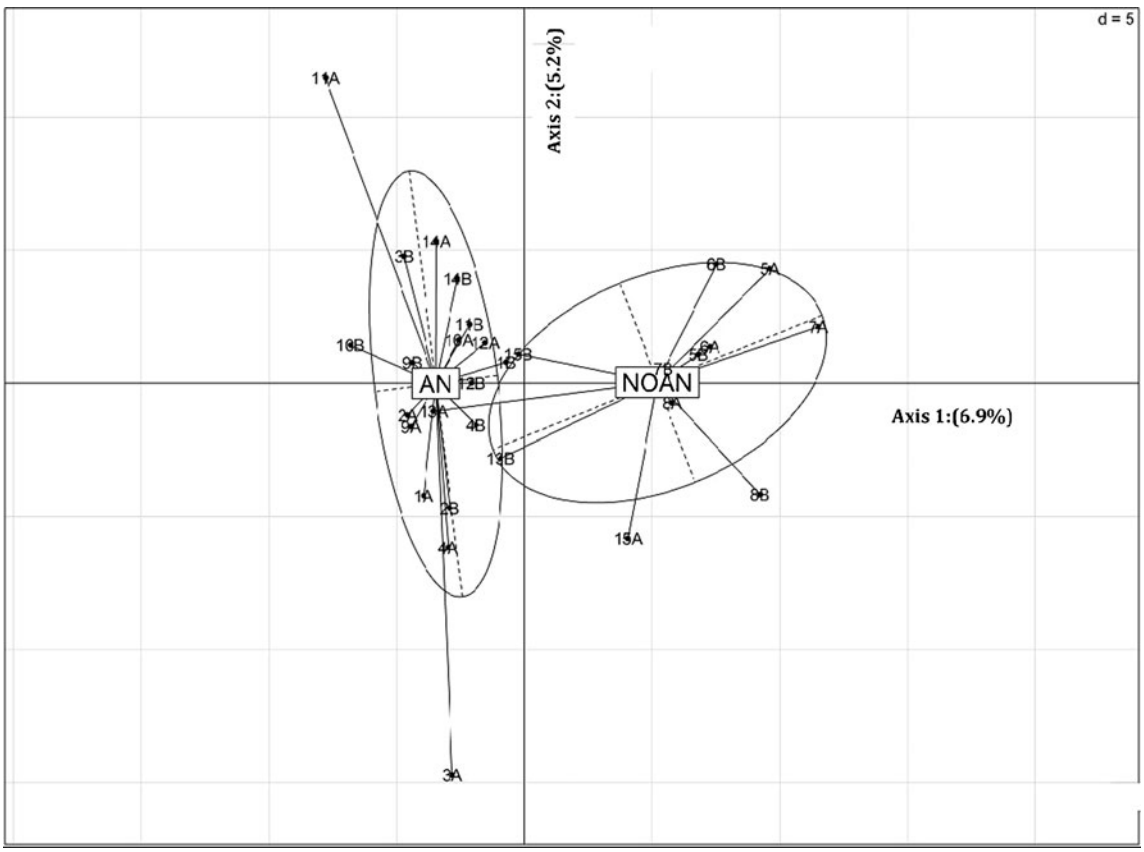




**Fig. 2** Heatmap showing the relative abundance of bacterial genera across the PVC samples of 15 patients. *A* denotes the inner surface of PVCs and *B* denotes the outer surfaces of PVCs. Each column represents one sample

**Fig. 3** The principle coordinates analysis (PCoA) plot of bacterial communities on individual PVC samples as determined using UniFrac. *AN* represents PVC samples from patients with antibiotic treatment. *NOAN* represents PVC samples from patients without antibiotic treatment

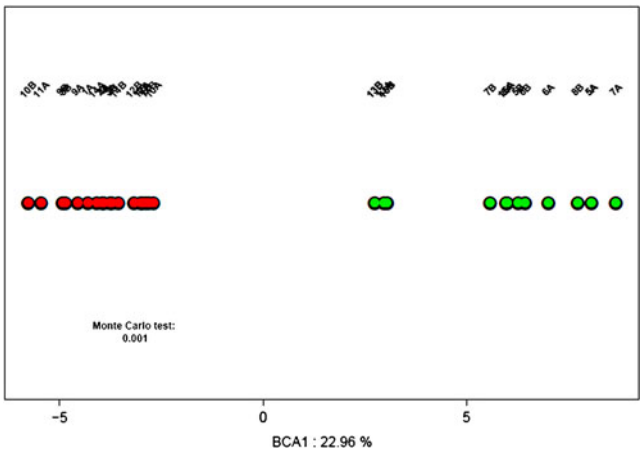



**Fig. 4** Monte Carlo permutation analysis of bacterial communities on PVCs from patients with (*red dots*) and without (*green dots*) antibiotic treatment

compared the bacterial communities on these samples to the 18 PVC samples from nine patients treated with intravenous antibiotics. The results from the PCoA (Fig. 3), Shannon–Weaver diversity index ($H_{\text{Antibiotic}} = 2.85 \pm 0.30$, $H_{\text{Noantibiotic}} = 3.13 \pm 0.21$; $p = 0.005$) and Monte Carlo permutation test (Fig. 4) showed significantly different bacterial communities on PVCs from patients that were untreated and those who were treated with antibiotics in the 2 weeks prior to PVC collection. The analysis found that PVCs from patients without antibiotic use were associated with a significantly higher abundance of *Thermomonas fusca*, *Flavobacteriaceae*, *Stenotrophomonas maltophilia*, *Brevibacterium* spp., *Chryseobacterium* spp., *Streptococcus suis* and *Leucobacter* spp. *Stenotrophomonas* were the predominant bacteria on all the examined PVCs (Fig. 5). However, *Stenotrophomonas* abundance in patients with antibiotic treatment was dramatically decreased compared with patients who were not treated with antibiotics, as shown in Fig. 5. PVCs from patients with antibiotic treatment had a significantly higher abundance of *Phyllobacterium* spp., *Propionibacterium acnes*, *Acidovorax* spp. and *Escherichia coli* (Fig. 5). The results from this study show that antibiotic therapy reduces bacterial diversity and alters the structure of bacterial communities, reducing some bacteria while selecting for others. More attention should be paid to the bacterial communities of PVCs from patients with antibiotic treatment, since they have great potential to resist antibiotics currently used and might be difficult to manage in future infections. Larger sample numbers generally have greater statistical power. In practice, however, considering the time and money required, it could be extremely difficult to include a huge number of samples in a study. In this study, 30 samples were reported to indicate the statistical trends that larger clinical trials might exhibit.

## Bacteria identified in PVCs

Bacterial species such as *Staphylococcus* spp., *Streptococcus* spp. and *Pseudomonas* spp. are well known as the major causes of colonisation and related BSI [22, 23], and they were also present on the examined PVCs in this study. Nikkari et al. examined the bacteria in venous blood from four healthy individuals [24]. They examined 192 clones and found *Pseudomonas fluorescens* (77 %), *Acidovorax defluvii* (17 %), *Propionibacterium acnes* (2 %), *Staphylococcus epidermidis* (2 %), *Stenotrophomonas maltophilia* (1 %) and *Riemerella anatipestifer* (1 %) [24]. The origins of these bacteria may be skin and/or blood [24]. The above study, despite some limitations, suggests the presence of bacterial communities in the blood of healthy people. All the bacterial species found in blood microbiota were also

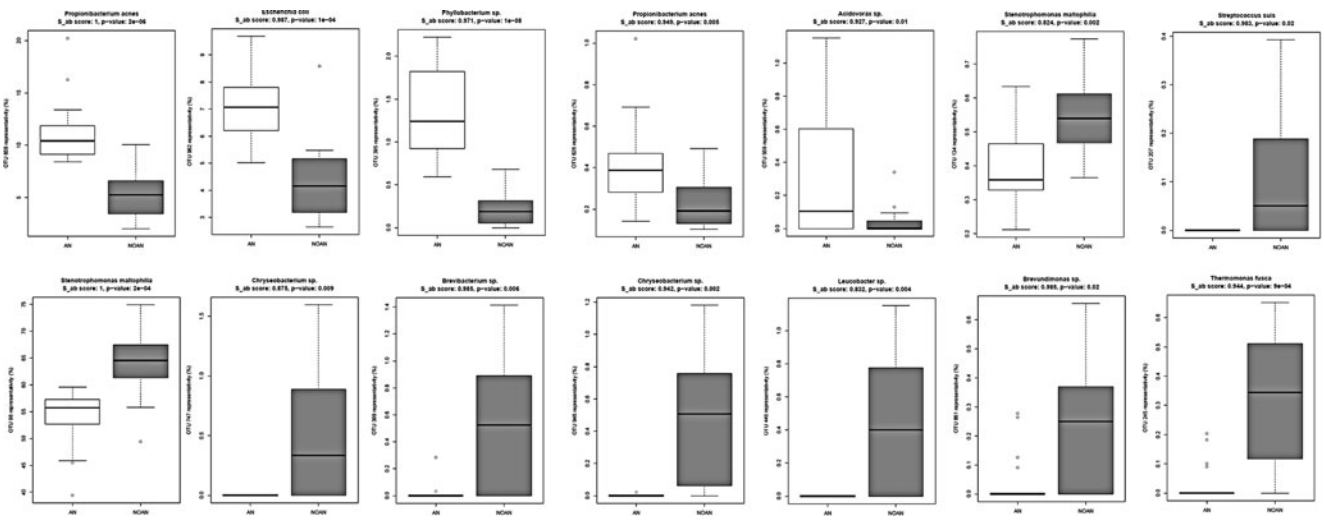


**Fig. 5** Operational taxomomic units (OTUs) are significantly different on PVCs from patients with (denoted as *AN*) and without (denoted as *NOAN*) antibiotic treatment ($p < 0.05$)

identified in our study. These species are presumably cleared efficiently by the healthy reticulo-endothelial system, but may be given the opportunity to colonise and persist on vascular catheters when they are present. Specific conditions that then may lead to the establishment of infection are yet to be determined.

The pyrosequencing library was dominated by *Stenotrophomonas maltophilia*, an opportunistic pathogen that can cause serious infection and bacteraemia in critically ill patients. The treatment of these can be difficult because *Stenotrophomonas maltophilia* is intrinsically resistant to many commonly used antimicrobial agents, including most cephalosporins, penicillins and quinolones [25–27]. *Stenotrophomonas maltophilia* has been previously reported as the predominant species on arterial catheters [28].

It was noted that PVC samples with an increase in the proportion of *Staphylococcus* (*Firmicutes*) showed a decrease of *Stenotrophomonas* (*Gammaproteobacteria*) at the same time. A similar shift of bacterial composition was also seen in *Propionibacterium* (*Actinobacteria*) and *Stenotrophomonas* (*Gammaproteobacteria*). These findings raise the possibility that low-level colonisation of PVCs is the norm and changes in bacterial composition favouring the expansion of populations of pathogens or opportunity pathogens such as *Staphylococcus* spp. might trigger the process of clinically significant bacterial colonisation and BSI. Further investigation should be undertaken to determine whether these potential pathogens represent non-pathogenic variants or simply do not cause infection, despite being present on PVC samples.

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**Conflict of interest** All authors declare no conflicts of interest relevant to this article.

## References

1. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, Lipsett PA, Masur H, Mermel LA, Pearson ML, Raad II, Randolph AG, Rupp ME, Saint S; Healthcare Infection Control Practices Advisory Committee (HICPAC) (2011) Guidelines for the prevention of intravascular catheter-related infections. Clin Infect Dis 52(9):e162–e193. doi:10.1093/cid/cir257
2. Ezingeard E, Coudrot M, Guyomarc'h S, Aubert G, Blanc JL, Bertrand JC, Tardy B, Zeni F (2009) Evaluation of colonisation of peripheral venous catheters inserted by prehospital emergency service teams (SMUR) in France. J Hosp Infect 72(2):169–175. doi:10.1016/j.jhin.2009.01.007
3. Boyd S, Aggarwal I, Davey P, Logan M, Nathwani D (2011) Peripheral intravenous catheters: the road to quality improvement and safer patient care. J Hosp Infect 77(1):37–41. doi:10.1016/j.jhin.2010.09.011
4. McHugh SM, Corrigan MA, Dimitrov BD, Cowman S, Tierney S, Hill ADK, Humphreys H (2011) Preventing infection in general surgery: improvements through education of surgeons by surgeons. J Hosp Infect 78(4):312–316. doi:10.1016/j.jhin.2011.03.023
5. Zhang L, Gowardman J, Rickard CM (2011) Impact of microbial attachment on intravascular catheter-related infections. Int J Antimicrob Agents 38(1):9–15. doi:10.1016/j.ijantimicag.2011.01.020
6. Widmer AF (2005) Infections and sepsis from intravascular catheters. Internist (Berl) 46(6):643–651
7. Mermel LA (2011) What is the predominant source of intravascular catheter infections? Clin Infect Dis 52(2):211–212. doi:10.1093/Cid/Ciq108
8. Francolini I, Donelli G (2010) Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol 59(3):227–238. doi:10.1111/j.1574-695X.2010.00665.x
9. Jefferson KK (2004) What drives bacteria to produce a biofilm? FEMS Microbiol Lett 236(2):163–173
10. Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15 (2):167–193
11. Maki DG, Weise CE, Sarafin HW (1977) A semiquantitative culture method for identifying intravenous-catheter-related infection. N Engl J Med 296:1305–1309
12. Bregenzer T, Conen D, Sakmann P, Widmer AF (1998) Is routine replacement of peripheral intravenous catheters necessary? Arch Intern Med 158(2):151–156
13. Lee WL, Chen HL, Tsai TY, Lai IC, Chang WC, Huang CH, Fang CT (2009) Risk factors for peripheral intravenous catheter infection in hospitalized patients: a prospective study of 3165 patients. Am J Infect Control 37(8):683–686. doi:10.1016/j.ajic.2009.02.009
14. Malach T, Jerassy Z, Rudensky B, Schlesinger Y, Broide E, Olsha O, Yinnon AM, Raveh D (2006) Prospective surveillance of phlebitis associated with peripheral intravenous catheters. Am J Infect Control 34(5):308–312. doi:10.1016/j.ajic.2005.10.002
15. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336. doi:10.1038/nmeth.f.303
16. Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Softw 22(4):1–20
17. Rogers GB, Carroll MP, Bruce KD (2009) Studying bacterial infections through culture-independent approaches. J Med Microbiol 58(Pt 11):1401–1418. doi:10.1099/jmm.0.013334-0
18. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M (2010) The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev 23(1):235–251. doi:10.1128/CMR.00043-09
19. Woo PCY, Lau SKP, Teng JLL, Tse H, Yuen KY (2008) Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 14(10):908–934. doi:10.1111/j.1469-0691.2008.02070.x

20. Schabereiter-Gurtner C, Nehr M, Apfalter P, Makristathis A, Rotter ML, Hirschl AM (2008) Evaluation of a protocol for molecular broad-range diagnosis of culture-negative bacterial infections in clinical routine diagnosis. J Appl Microbiol 104(4):1228–1237. doi:10.1111/j.1365-2672.2007.03648.x
21. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4(4):295–305. doi:10.1038/Nrmicro1384
22. Van Donk P, Rickard CM, McGrail MR, Doolan G (2009) Routine replacement versus clinical monitoring of peripheral intravenous catheters in a regional hospital in the home program: a randomized controlled trial. Infect Control Hosp Epidemiol 30(9):915–917. doi:10.1086/599776
23. Bouza E, Burillo A, Muñoz P (2002) Catheter-related infections: diagnosis and intravascular treatment. Clin Microbiol Infect 8 (5):265–274
24. Nikkari S, McLaughlin IJ, Bi WL, Dodge DE, Relman DA (2001) Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol 39(5):1956–1959
25. Wang WS, Liu CP, Lee CM, Huang FY (2005) *Stenotrophomonas maltophilia* bacteremia in adults: four years' experience in a medical center in northern Taiwan. J Microbiol Immunol Infect 37:359–365
26. Friedman ND, Korman TM, Fairley CK, Franklin JC, Spelman DW (2002) Bacteraemia due to *Stenotrophomonas maltophilia*: an analysis of 45 episodes. J Infect 45(1):47–53
27. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat Rev Microbiol 7(7):514–525
28. Zhang L, Sriprakash KS, McMillan D, Gowardman JR, Patel B, Rickard CM (2010) Microbiological pattern of arterial catheters in the intensive care unit. BMC Microbiol 10:266–275