Biofilm Microbiome in Extracorporeal Membrane Oxygenator Catheters

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Research

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Abstract

Background : To assess the relationship between bacteremia and biofilms in extracorporeal membrane oxygenation (ECMO) catheters.

Methods: We evaluated the biofilm microbiome of ECMO catheters from adults with (n = 6) and without bacteremia (n = 15). Microbiomes of the catheter biofilms were evaluated by profiling the V3 and V4 regions of bacterial 16s rRNA genes using the Illumina Miseq sequencing platform.

Results: In total, 2,548,172 reads, with an average of 121,341 reads per sample, were generated. Although alpha diversity was slightly higher in the non-bacteremic group, it was not statistically significant. Also, there was no difference in beta diversity between the two groups. At the genus level, *Delftia* was more abundant in the non-bacteremic group, but *Bacillus*, *Flavobacterium*, *CL0-1, Candidatus, and Xiphinematobacter* were more abundant in the bacteremic group. In particular, *Arthrobacter*, *SMB53*, *Neisseria*, *Candidatus*, *Ortrobactrum*, *Candidatus, Rhabdochlamydia*, *Deefgae*, *Dyella*, *Paracoccus*, and *Pedobacter* were more abundant in the bacteremic group. In a network analysis, compared to the non-bacteremic group, the microbiome of the bacteremic group was very complex. Notably, there was a significant elevation in the secretion system of the non-bacteremic group.

Conclusions: Biofilm characteristics in ECMO catheters varied according to the presence or absence of bacteremia.

Introduction

Bacteria adapt to life on catheter surfaces through a number of metabolic changes, including the production of an extracellular substance and the regulation of specific genes [1]. They form networks, termed biofilms, which enable multicellular functions. Bacterial biofilms provide beneficial survival systems to community members [2]. Biofilms on intravascular catheters are the most common cause of hospital acquired septicemia and catheter-related bloodstream infections (CRBSI) [3]. Despite efforts to maintain sterility, catheters can easily become contaminated with bacteria. Biofilms present a significant challenge as far as their sampling, diagnosis, and treatment to prevent infection [4].

Extracorporeal membrane oxygenation (ECMO) requires vascular cannulation to support critically ill patients. Cannulas are maintained for several days to weeks depending on the patient's condition, and exchanging cannulas is almost impossible. Blood stream infections (BSI) are significant complications of ECMO, and they are associated with mortality, morbidity, and increased healthcare costs in ECMO patients [5]. Bacteremia during ECMO has been associated with catheter colonization, and in a previous study we found biofilm related infections in ECMO catheters. However, despite the formation of biofilms on the catheters, some patients did not develop bacteremia [6], and it is unclear why this did not occur. To better understand the specific linkage between biofilms and bacteremia, a deeper understanding of the microbial community within catheter biofilms is necessary. Catheter microbiomes may play an important role in the development of bacteremia from biofilms. To determine the microbiome characteristics related to biofilms in patients who developed bacteremia from ECMO catheters, we compared the differences in the catheter biofilm microbiome from patients with and without bacteremia.

Materials And Methods

Study Design and Clinical Examination

This single center, retrospective, cohort study was conducted using prospectively collected ECMO catheter specimens. Patients over the age of 18 years who experienced ECMO support between November 2016 and April 2017 were included. The catheter specimens were collected from patients who gave their consent and were stored in the Pusan National University Yangsan Hospital (PNUYH) Biobank. The biobank provided samples and clinical data collected from consented patients. We retrospectively compared the clinical information and microbiome data between patients with (n = 6) and without (n = 15) bacteremia. This study was approved by the Institutional Review Board of PNUYH (No. 05-2020-146).

Sample collection and preparation

ECMO catheters were aseptically collected from the patients upon their removal at the completion of the ECMO run. Sections of the catheter that had been within the intravascular space (5 cm) were cut, split longitudinally, and transported immediately to the laboratory for standard catheter culture and also sent to the biobank for RNA sequencing.

Extraction of Total Genomic DNA

DNA was extracted from buccal and subgingival plaque using a Gram positive DNA purification kit (Lucigen, Biosearch Technology, Novato, CA) following the manufacturer's instructions. The final concentration was measured with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA) and stored at − 80 °C until use.

PCR Amplification and Sequencing Analysis

Each sequenced sample was prepared according to Illumina 16S Metagenomic Sequencing Library protocols. Quantification of DNA and DNA quality was measured by PicoGreen and Nanodrop. The 16S rRNA genes were amplified using 16S V3-V4 primers, and the primer sequences are as follows: 16S V3-V4 primers

16S Amplicon PCR Forward Primer

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG16S Amplicon PCR Reverse Primer

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

Input genomic DNA was amplified with 16S V3-V4 primers and a subsequent limited cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. The final products were normalized and pooled using PicoGreen, and the size of the libraries was verified using TapeStation DNA screentape D1000 (Agilent). Sequencing was then performed using the MiSeq™ platform (Illumina, San Diego, USA).

Raw reads were processed with QIIME2 v.2019.10 using the DADA2 plugin to decrease noise in the quality filter reads, call amplicon sequence variants (ASVs), and generate a feature table of ASV counts and host metadata [7]. In the quality filtering step, datasets were truncated to a read length of 265 to 280 base pairs for the forward and reverse reads, and a chimera removal step was performed. Following quality filtering, bacterial taxonomies were assigned to the ASV feature table using the Naïve Bayesian Q2 classifier feature in QIIME2. The data obtained was compared to the Greengenes reference sequence database of the V3-V4 region of the 16S rRNA gene.

Bioinformatic Analysis, Statistical Analysis, and Visualization

To evaluate alpha diversity, sample microbiota were estimated by the Chao1 and Shannon indices. The Wilcoxon-rank-sum test was used to compare significant differences of the alpha diversity indices between different groups ($P < 0.05$). Similarity of the microbial community structure among all samples was evaluated using a principal coordinates analysis (PCoA) at the operational taxonomic unit (out) level. An analysis of similarities was calculated to compare the intra- and inter-group similarities based on Bray-Curtis dissimilarity, which was calculated by QIIME2.

LEfSe and PICRUSt analysis

Linear discriminant analysis (LDA) effect size (LEfSe) analyses were performed on the samples [8]. LDA was performed to determine features (taxa) differentially represented between bacteremia and non-bacteremia samples. LEfSe combines Kruskal-Wallis test or pairwise Wilcoxon rank-sum test with LDA. It ranks features by effect size, ranking features that explain more of the biological differences higher. The cut off value was an absolute LDA score (log10) > 2.5. Functional capacity of oral microbiota were predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Galaxy Version 1.0.0) [9]. The closed reference OTU table was generated from quality control reads in QIIME2 against the Greengenes reference sequence database. The closed OTU table drawn by QIIME2 was compared with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to obtain functional predictions. PICRUSt predictions were categorized as levels 1–3 relative to KEGG pathways.

Network analysis

SparCC was developed to calculate correlations between OTU frequencies in the microbiome data while accounting for their inherent sparseness and composition [10]. The microbiome network was constructed sing SparCC to identify links involved in co-abundance with an absolute pairwise correlation > 0.7. The resulting correlations among taxa were graphed as a network using the igraph package [11] in R and visualized in Cytoscape [12].

Results

Patient characteristics and clinical outcomes

Patient characteristics and clinical outcomes are summarized in Table 1. The median age was 55 years (IQR 47–62.5), 71.4% (n = 15) of the patients were male, and the median ECMO duration was 9 days (IQR 6–14.5). There were no significant differences in patients'

characteristics between the two groups except for APACHE II scores (median 13 vs 10, $P = 0.019$) and the percentage of pneumonia (66.7% vs 20%, $P = 0.040$) being higher in the bacteremic group. There were no significant differences in clinical outcomes.

Table 1
Patient characteristics

Characteristic	Bacteremic (n = 6)	Non-bacteremic (n = 15)	*P*
Age	56.5 (46.3–62.5)	55 (47–63)	0.668
Male	4 (66.7)	11 (73.3)	0.760
BMI	25.5 (21.7–26.4)	22.8 (20.4–27.9)	0.697
SOFA	14 (10.3–16.3)	12 (10–15)	0.531
APACHE II	13 (11.8–18)	10 (8–12)	0.019
Vasopressor	5 (83.3)	11 (73.3)	0.627
RRT	2 (33.3)	4 (26.7)	0.760
Pre ECMO PF ratio	62.5 (56.5–68.5)	66 (57–81)	0.640
ECMO duration (d)	12 (7.8–23.5)	9 (4–11)	0.227
ICU day	21.5 (15.1–48.0)	42.0 (19.5–70.9)	0.436
Pneumonia	4 (66.7)	3 (20)	0.040
Antibiotic use	6 (100)	15 (100)	1.000
ECMO complications			
Bleeding	2 (33.3)	1 (6.7)	0.115
Thrombosis	0	1 (6.7)	0.517
Weaning success from ECMO	5 (83.3)	14 (93.3)	0.481
Survival to discharge	5 (83.3)	14 (93.3)	0.481
Data are presented as median (interquartile range) or n (%).			
BMI. body mass index; SOFA, sequential organ failure assessment; APACHE II, acute physiology and chronic health evaluation II; RRT, renal replacement therapy; ECMO, extracorporeal membrane oxygenation; PF ratio, PaO2/FiO2; ICU, intensive care unit			

Blood culture isolates

The most common organism isolated by blood culture within 3 days of catheter removal was S. epidermidis (4/6, 66.7%), followed by A. baumanii (1/6, 16.7%), and E.coli (1/6, 16.7%).

Diversity of bacterial community in ECMO

In total, 2,548,172 reads, with an average of 121,341 reads per sample were generated after initial quality filtering and chimera removal (Supplementary Table 1). Initially, we measured alpha diversity of the bacterial community in each group. The total bacterial diversity was estimated by Chao1 index. The evenness of microbiota was estimated by the Shannon index (Fig. 1A). Although it was slightly higher in the non-bacteremic group, there was no statistically significant difference by Wilcoxon-rank-sum-test.

In order to examine the microbial community variability between the two groups, we calculated beta diversity by decomposing microbiome variability onto major components using PCoA on Bray-Curtis dissimilarity. (Fig. 1B) There was no significant difference between the two groups.

Differences of microbiome composition according to the presence of bacteremia

Microbiota play an important role in the pathophysiology of many diseases. In order to identify microbiome profiles of the two groups, we examined the microbiota's taxonomic composition and the relative abundance of bacteremia at different taxonomic levels. At the phylum level, we found a 47 different phyla, 11 of which were present in all samples. The major phyla were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia*, and *Firmicutes*, which were present, on average, in 10% or more of the samples. (Fig, 2A)

We found 367 different genera, 8 of which were present in all samples. Among them, the major genera were *Limnohabitans*, *Flavobacterium*, *Delftia*, *Massilia*, *Bacillus*, *Candidatus, Xiphinematobacter*, and *CL0-1* which were present, on average, in 1% or more of the samples. *Delftia* was more abundant in the non-bacteremic group; however, *Bacillus*, *Flavobacterium*, *CL0-1, Candidatus, and Xiphinematobacter* were more abundance in the bacteremic group (Fig. 2B)

Difference in dominant microbiota between the groups

We used LEfSe to determine the taxa that most likely explain differences between the two groups. In this study, a P value < 0.05 and LDA score > 2.5 were considered to be significant. Differentially abundant taxa and their predominant bacteria are shown in Fig. 3. Significant differences were found between the two groups. LEfSe analysis revealed 10 discriminative genera, all of which were more abundant in the bacteremic group (Fig, 3A): *Arthrobacter*, *SMB53*, *Neisseria*, *Candidatus*, *Ortrobactrum*, *Candidatus, Rhabdochlamydia*, *Deefgae*, *Dyella*, *Paracoccus*, and *Pedobacter*. (Fig. 3B)

Network analysis of the ECMO microbiome

In order to find a relationship between taxa associated with the bacteremic state, we conducted a network analysis at the genus level. Compared to patients without bacteremia, the bacteremic group was very complex. In non-bacteremic patients, only genera associated with the hub were correlated (Fig. 4A). However, the microbiome of bacteremic patients showed a high correlation with genera that were not associated with the hub (Fig. 4B). Although not a hub, *Flavobacterium* and *CL0.1*, which were abundant in the bacteremic group, were considered important genera because they connected different subnetworks.

Metabolic prediction

We explored if metabolic pathways in the bacteremia state were related to the metabolic differences found in the two groups. We performed a comparative prediction analysis of the functional metagenome using PICRUSt. Among the 328 affiliated KEGG pathways, only 1 was shown to be statistically significant with P < 0.05 and LDA > 2. Notably, a significant elevation in the secretion system was found in the non-bacteremic group (Fig. 5)

Discussion

We detected significant differences in the microbiota of biofilms on ECMO catheters depending on the presence or absence of bacteremia. There was no significant difference in the diversity of the microbiomes. However, the dominant microbiota differed significantly between the two groups. Additionally, compared to the non-bacteremic group, the bacteremic group exhibited a very complex network connection within its microbiome.

To our knowledge, there have been no prior studies on the association between ECMO catheter microbiomes, biofilms, and bacteremia. In microbial communities, many microorganisms exhibit a synergistic relationship and depend on each other to survive [13]. The diversity and composition of the microbial communities within biofilms may contribute to the biofilm's dispersal and BSI [14]. In this study, there were significant differences in the biofilm's microbiota depending on the presence of bacteremia. Although there was no significant difference in diversity between the two groups, there was a difference in the dominant microbiota of each group. At the genus level, *Delftia* was more abundant in the non-bacteremic group, but *Bacillus*, *Flavobacterium*, *CL0-1, Candidatus, and Xiphinematobacter* were more abundant in the bacteremic group. This suggests that differences in the composition of the microbial community within the biofilm may be more important than its diversity for the development of BSI.

In this study, there was no correlation between the dominant microbiota and the actual bacteremic pathogens. Previously, *Bacillus* and *Flavobacterium* have been reported as causes of device-related BSI in patients with significant underlying conditions [15–22]. Specific bacteria such as *Arthrobacter*, *SMB53, Dyella, Paracoccus, and Pedobacter* have been reported as dialysis catheter-related infections or CRBSI [23–27]. Currently, the significance of the presence of other bacteria, including *CL0-1, Candidatus, Xiphinematobacter, Candidatus*, *Ortrobactrum*, *Candidatus, Rhabdochlamydia*, and *Deefgae* for biofilm formation and bacteremia is not clear. *Flavobacterium* and *CL0.1* have shown a connection with different subnetworks, highlighting the possibility of an important genus in the development of bacteremia. Further research is required on the role of such bacteria in bacteremia and biofilm production.

Conclusion

In conclusion, there were significant differences in the biofilm microbiota of ECMO catheters based on the presence of bacteremia. Each of the ECMO catheters had biofilms composed of a diverse bacterial community. There were no significant differences in diversity between the two groups, but there were significant differences in the community composition of the biofilms. The biofilm associated community was dynamic, with the bacteremic group showing a very complex network connection within the microbiome. Evidence for such a relationship is still limited, but these results may provide a critical background for studying the linkage between biofilms and bacteremia. Further research is required on the bacterial community within biofilms to better understand biofilm associated infection.

Abbreviations

CRBSI; catheter-related bloodstream infections

ECMO; Extracorporeal membrane oxygenation

BSI; Blood stream infections

PNUYH; Pusan National University Yangsan Hospital

ASVs; amplicon sequence variants

PCoA; principal coordinates analysis

LDA; Linear discriminant analysis

LEfSe; Linear discriminant analysis effect size

PICRUSt; Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

KEGG; Kyoto Encyclopedia of Genes and Genomes

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Review Board of PNUYH (No. 05-2020-146). The catheter specimens were collected from patients who gave their consent.

Consent for publication: not applicable

Availability of data and materials: the datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no conflicts of interest

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Authors contributions

(I) Conception and design: Hye Ju Yeo (II) Provision of study patients: Woo Hyun Cho (III) Collection and assembly of data: Hye Ju Yeo (IV) Data analysis and interpretation: Yeuni Yu, Yun Hak Kim (V) Manuscript writing: Yeuni Yu and Hye Ju Yeo (VI) Final approval of manuscript: All authors

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Figures

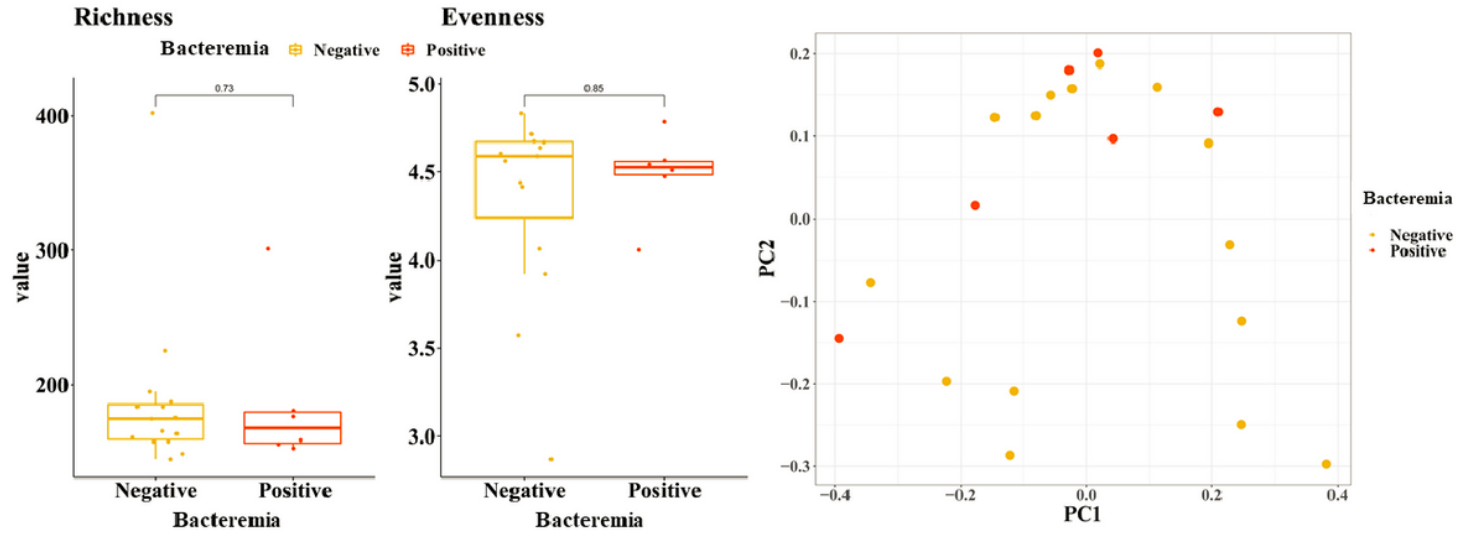

Figure 1

Taxonomic diversity of ECMO microbiomes. (A) Comparison of alpha diversity indices between bacteremic and non-bacteremic groups. (B) Principle Coordinates analysis plot based on Bray-Curtis dissimilarity.

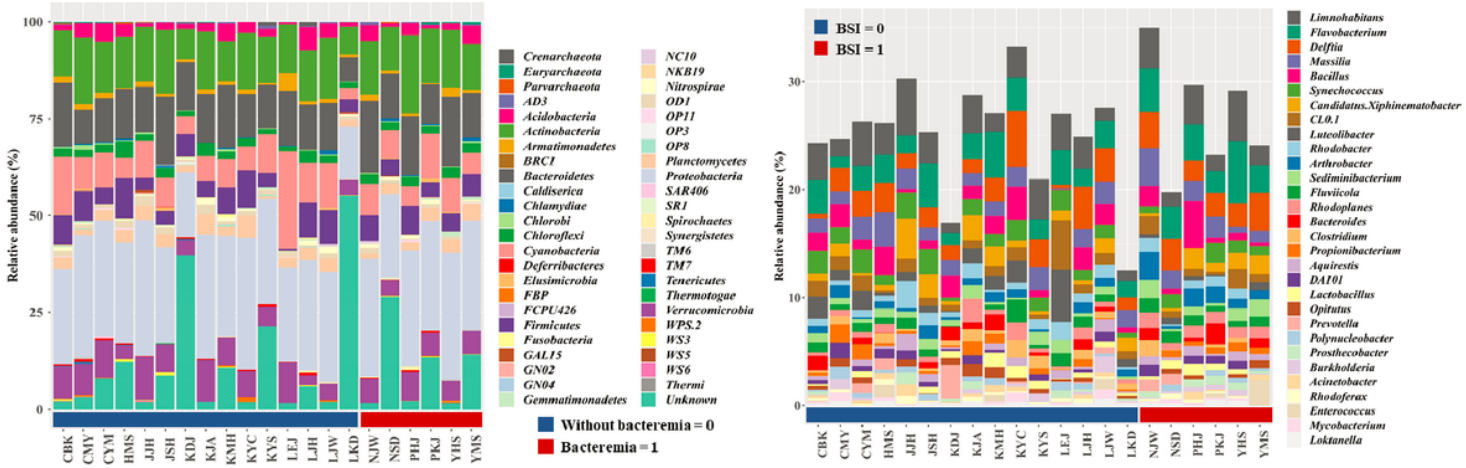

Figure 2

Taxonomic profiles of ECMO samples. (A) Phylum level taxonomic profile. (B) Genus level taxonomic profile

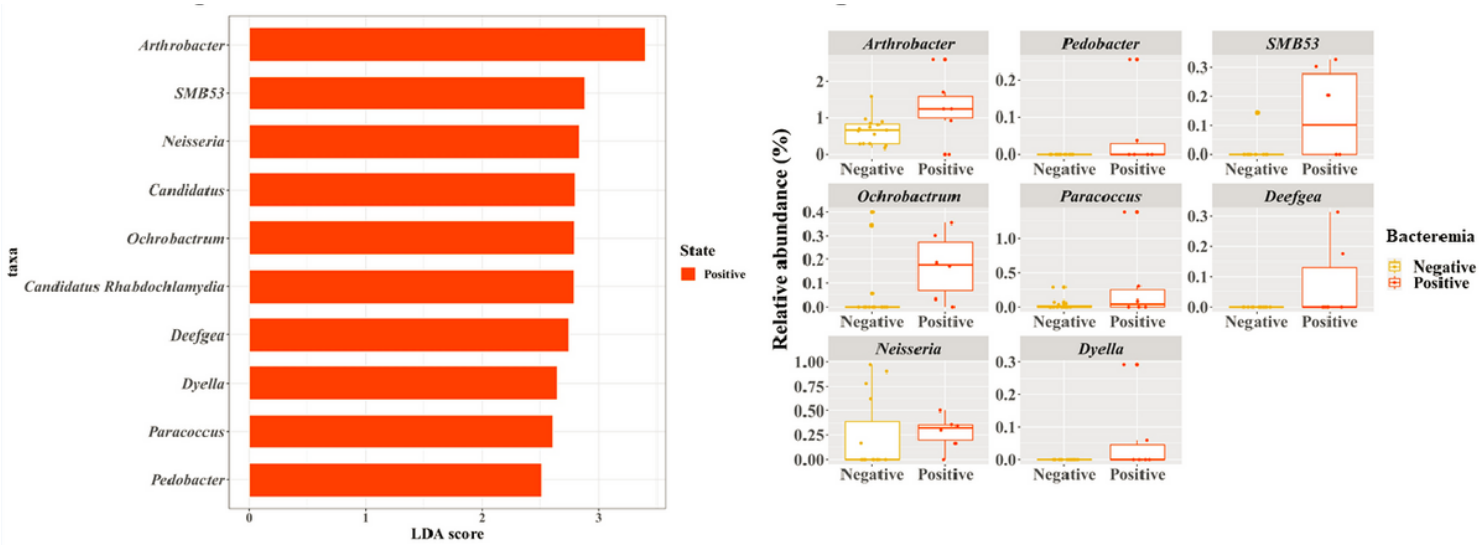

Figure 3

Differential abundance of taxa between bacteremic and non-bacteremic groups. (A) Taxa identified by linear effect size with linear discriminant analysis values of 3.0. Taxa enriched in different groups are displayed by color indicated in the key (red indicates taxa abundant in bacteremia) (B). Differential abundance of 10 discriminative genera across both groups.

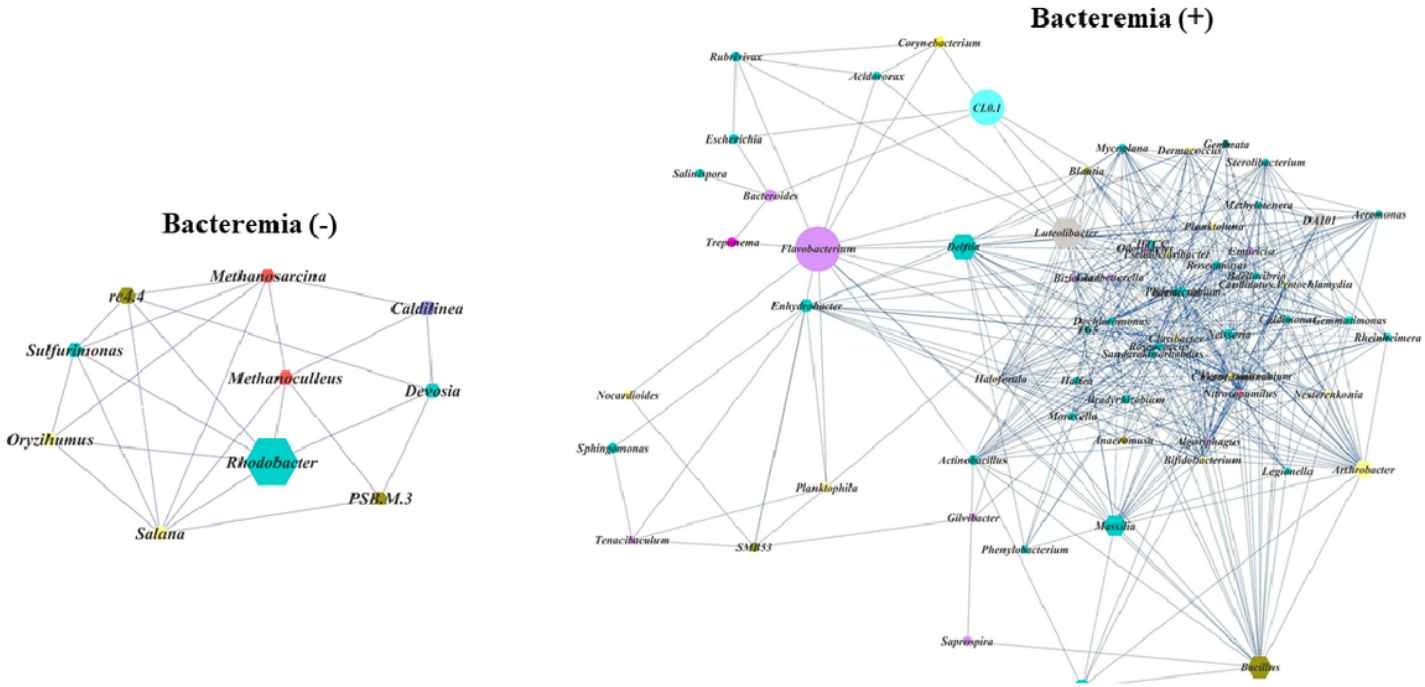

Figure 4

Identification and comparison of highly connected clusters of co-occurring networks of ECMO microbiota. (A) Non-bacteremic group. (B) Bacteremic group. Each node represents a genus and is colored by its assigned phylum. Hexagonal nodes represent hub taxa.

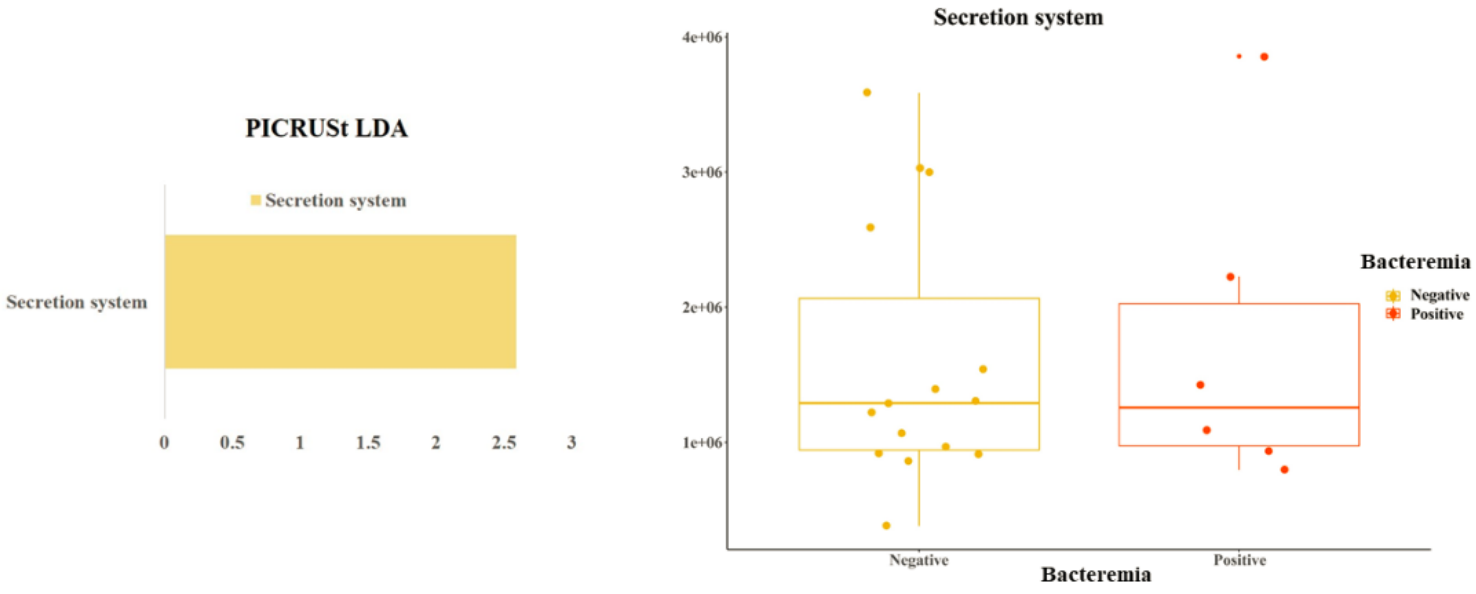

Figure 5

Differentially abundant gene functions between the non-bacteremic and bacteremic groups. (A) Gene functions enriched in the non-bacteremic group are colored green. The box plot represents the predicted value of the secretion system. Functional categories of genes on

the ECMO catheters were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States. Differentially abundant functions were identified using linear discriminant analysis coupled with effect size measurements.

Supplementary Files

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