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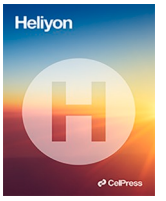

Research article

Blood microbial signatures associated with mortality in patients with sepsis: A pilot study

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A R T I C L E I N F O

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A B S T R A C T

Sepsis is a life-threatening illness caused by the dysregulated host response to infection. Nevertheless, our current knowledge of the microbial landscape in the blood of septic patients is still limited. Next-generation sequencing (NGS) is a sensitive method to quantitatively characterize microbiomes at various sites of the human body. In this study, we analyzed the blood microbial DNA of 22 adult patients with sepsis and 3 healthy subjects. The presence of non-human DNA was identified in both healthy and septic subjects. Septic patients had a markedly altered microbial DNA profile compared to healthy subjects over α- and β-diversity. Unexpectedly, the patients could be further divided into two subgroups (C1 and C2) based on β-diversity analysis. C1 patients showed much higher bacteria, viruses, fungi, and archaea abundance, and a higher level of α-diversity (Chao1, Observed and Shannon index) than both C2 patients and healthy subjects. The most striking difference was seen in the case of *Streptomyces violaceusniger*, *Phenylobacterium* sp. *HYN0004*, *Caulobacter flavus*, *Streptomyces* sp. *11-1-2*, and *Phenylobacterium zucineum*, the abundance of which was the highest in the C1 group. Notably, C1 patients had a significantly poorer outcome than C2 patients. Moreover, by analyzing the patterns of microbe-microbe interactions in healthy and septic subjects, we revealed that C1 and C2 patients exhibited distinct co-occurrence and co-exclusion relationships. Together, our study uncovered two distinct microbial signatures in the blood of septic patients. Compositional and ecological analysis of blood microbial DNA may thus be useful in predicting mortality of septic patients.

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1. Introduction

Sepsis is associated with high mortality (~30 %) and represents a substantial burden on the healthcare system around the world. Mortality rates could further increase to 60 % in the presence of septic shock [1]. Bacteria remain the most common cause of sepsis, but fungal infections are increasing rapidly [2–4]. Although early initiation of antimicrobial therapy with broad-spectrum and high-potency antibiotics remains the cornerstone of sepsis management, they are associated with the development of drug-resistant organisms and superinfections. Besides, antibiotic-induced microbiome abnormalities may lead to immune dysregulation which could further increase the risk of secondary infections and subsequent organ dysfunction [5]. Therefore, early de-escalation of antimicrobial agents is a key strategy to address this problem [6].

Blood culture remains an essential diagnostic tool in the identification of infection in sepsis [7]. However, it is not sensitive to all bacteria, owing to sampling error, slow-growing or fastidious bacteria, and prior antibiotic exposure, and is limited to the detection of blood-borne pathogens. Blood cultures also require a relatively large volume of blood samples and can take several days for growth, especially for fastidious organisms. It is poorly sensitive to fungi and not applicable to viral pathogen detection [8]. Several multicenter studies have revealed that culture-negative patients account for 28%–48 % of all cases of severe sepsis in intensive care units (ICU) around the world [9]. Contamination giving 'false-positive' results, which could vary from 0.6 % to over 6 % between institutions, is also a significant limitation of blood cultures [8]. Detection of freely circulating pathogen DNA may be more advantageous than blood cultures since infection at non-circulatory sites can be readily identified due to the shedding of pathogen DNA into circulation. Therefore, characterization of the blood microbial DNA should improve our understanding of sepsis pathophysiology and facilitate the treatment of sepsis. However, our current knowledge of the blood-microbial landscape in septic patients is still limited.

Next-generation sequencing (NGS) is a sensitive method for detecting the presence of microorganisms, including bacteria, viruses, fungi, and parasites [10]. It requires no prior knowledge of the organism(s), rendering it more advantageous than other methods to detect microorganisms. Ideally, such a method also provides comprehensive profiling of pathogenic bacteria to facilitate the management decision. In this study, we detected circulating microbial DNA by NGS in a consecutive cohort of 22 patients with severe sepsis and 3 healthy subjects. By doing this, we revealed different microbial landscapes in the blood of septic patients and healthy subjects and further identified its connection with sepsis-associated mortality. Finally, we evaluated the microbe-microbe interactions in both healthy and septic subjects.

2. Material and methods

2.1. Recruitment of patients with sepsis

Quick Sequential Organ Failure Assessment (qSOFA) score and Acute Physiology and Chronic Health Evaluation (APACHE II) score were recorded accordingly [11] to assess organ failure and disease severity respectively for adult patients admitted to the ICU at Prince of Wales Hospital of The Chinese University of Hong Kong from May 2015 to October 2016. The diagnosis of sepsis in these patients (qSOFA $\geq$2) was consistent with the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [12]. Only initial ICU admission was included. Informed consent was obtained from the patient or from a legal representative if the patient was incapable of giving consent. This study was approved by Joint CUHK-NTEC Clinical Research Ethics Committee (CREC Ref. No.: 2014.672).

2.2. Blood collection and cultures

Blood was taken by sterile venous puncture and collected directly into blood culture bottles on the day of ICU admission. Twenty milliliters of blood distributed equally for conventional cultures in aerobic and anaerobic media was obtained each time and processed according to local standard procedures. At the same time, 10 ml of blood was collected into an EDTA tube for NGS analysis.

2.3. DNA isolation and next generation sequencing

Total genomic DNA was extracted from 2 ml of whole blood with a BioStic® Bacteremia DNA Isolation kit (Mobio; Carlsbad, CA) according to manufacturers' protocol. The NEBNext Microbiome DNA Enrichment Kit was then used to enrich microbial DNA according to the manufacturer's protocol. DNA integrity and purity were assessed by agarose gel electrophoresis. All DNA samples were stored at −20 °C until analysis. The library of microbial genomes was prepared by NEBNext Ultra DNA Library Prep Kit by Illumina (Illumina Inc., San Diego, CA). Index codes were added to attribute sequences. The DNA specimen was fragmented to 300bp by sonication. The fragments were end-polished, A-tailed, and ligated with a full-length adaptor for PCR amplification. PCR products were then purified by the AMPure XP system (Agilent, Santa Clara, CA). Clustering of index-coded samples was performed on a cBot Cluster Generation System (Illimina Inc., San Diego, CA). The resulting DNA libraries were sequenced on the Illumina HiSeq2500 platform to generate paired-end reads.

2.4. Next generation sequencing and bioinformatic analysis

Bases with Phred quality of less than 3, reads with average of quality less than 15 for every 4 bases and reads with lengths less than 36 bases were discarded using Trimmomatic software (V.0.36). Reads were then aligned against human reference genome using

Bowtie2 V.2.2.9. Taxonomic profile of the microbiota was obtained using Kraken2 (v2.0.8-beta) algorithm after host DNA removal and reads quality filtering by KneadData (v0.7.2). The standard Kraken 2 database comprising complete NCBI RefSeq genomes of bacterial, archaeal, and viral domains, and a collection of known vectors was used as reference. The differential abundance analysis of bacterial species was performed by ALDEx2, a compositional-oriented method integrating the Bayesian algorithm. Species with 1) abundance >1 % in at least one sample; 2) adjusted *P* value < .05 (FDR corrected), and 3) fold change (FC) > 1.5 in abundance between different groups were considered statistically significant. After rarefying to minimum library size of 22,372 reads, we calculated the alpha and beta diversity using phyloseq R package. Alpha diversity was measured by Observed and Chao1 index. Beta diversity was accessed by Arrhenius z distance, and the principal coordinates analysis (PCoA) was used for ordination analysis. Community dissimilarities were tested by permutational multivariate analyses of variance (PERMANOVA) with 1000 iterations using the Arrhenius z distance.

2.5. Analysis of microbial interplay

SparCC method was used to infer co-occurrence or co-exclusion relationships between microbes. Correlation coefficients between species were estimated by setting an average of inference iterations to 100, which generated 10,000 simulated datasets to calculate the corresponding empirical *P* values. *P* values were adjusted by false positive rate (FDR) and correlation coefficients with adjusted $P < 0.01$ were visualized using Cytoscape (version 3.7.1).

2.6. Statistical analysis

Mann-Whitney *U* test was performed to compare the difference between two groups. Kruskal Wallis test with FDR correction was employed for multiple comparisons. The Fisher's exact test was used for analysis of the associations between septic patients' clinicopathological characteristics and sepsis subtypes. Kaplan-Meier analysis and log-rank test were performed to evaluate the association between sepsis subtypes and septic patients' survival. The statistical tests were performed using Graphpad Prism 5.0 (GraphPad Software Inc., San Diego, CA) or SPSS 20.0 (SPSS Inc, Chicago, IL), and a two-tailed P-value of .05 was considered statistically significant (*P < 0.05; **P < 0.01; ***P < 0.001).

3. Results

3.1. Diversities of microbes in control subjects and septic patients

On average, we obtained 24,629,970 paired clean reads per sample. An average of 21,949,243, 1,288,093, 6,523, 39,725, and 1317 reads were mapped to the human, bacteria, fungi, virus, and archaea genomes, respectively (Table S1). At the species level, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* were the top 3 abundant bacteria in the healthy subjects with an average relative bacterial abundance of 30.3 %, 23.1 %, and 11.3 %, respectively (Table S2). On the other hand, *Botrytis cinerea* (25.8 %), *BeAn 58058* (33.4 %) and *Pyrolobus fumarii* (7.5 %) were the most abundant fungi, virus, and archaea in the healthy subjects, respectively, albeit in low quantity (Table S2). The average microbial abundance (ratio of microbe/human reads) in septic patients was much higher than that in control subjects, but this did not reach statistical significance, probably related to the limited sample size (Fig. 1). Culture-

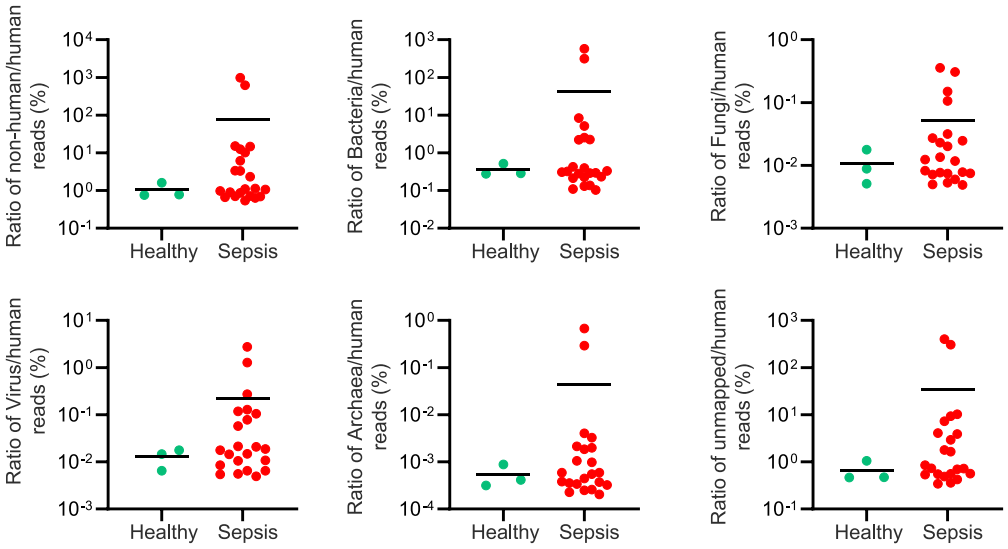

Fig. 1. The microbial abundance (ratio of microbe/human reads) in the blood of septic patients and control subjects.

negative (16/22) and -positive (5/22) patients with sepsis demonstrated similar bacteria, fungi, virus, and archaea abundance (Fig. S1).

We further explored the overall phenotypic patterns of the microbial communities and found that septic patients had a significantly higher α-diversity (Observed index) than healthy subjects, although the difference was not significant using Chao1 and Shannon index (Fig. 2A). β-diversity analysis showed a clear separation of the groups (Fig. 2B). There were two subgroups of septic patients (C1 and C2) through β-diversity analysis (Fig. 2B). Of them, C1 patients exhibited a significantly higher level of non-human DNA (Fig. 2C), and increased α-diversity (Chao1, Observed, and Shannon index) (Fig. 2D) than both C2 patients and healthy subjects. Compared with C2 patients, C1 patients presented significantly higher bacteria, viruses, fungi, and archaea abundance (Fig. S2). More importantly, C1 patients had a significantly higher risk of mortality than C2 patients (log-rank test, $P = .044$) (Fig. 3A), despite the similar demographic and clinical features (Table 1). In the C2 group, 2 out of 14 patients tested positive for blood culture (Table S8). In comparison, 3 out of 8 patients in the C1 group were blood culture positive. Although there is a slight difference in the percentage of blood culture positivity between the C1 and C2 groups, there was no difference in the survival rate between patients with sepsis who tested negative (16/22) and those who tested positive (5/22) in the blood culture (Fig. S3). Together, our data indicate the association of sepsis with increased blood microbial load and diversity and the previously unappreciated subtypes among sepsis patients.

3.2. *Microbial species associated with sepsis*

To determine microbes that were present in septic patients, we compared the bacterial reads at species levels between sepsis cases

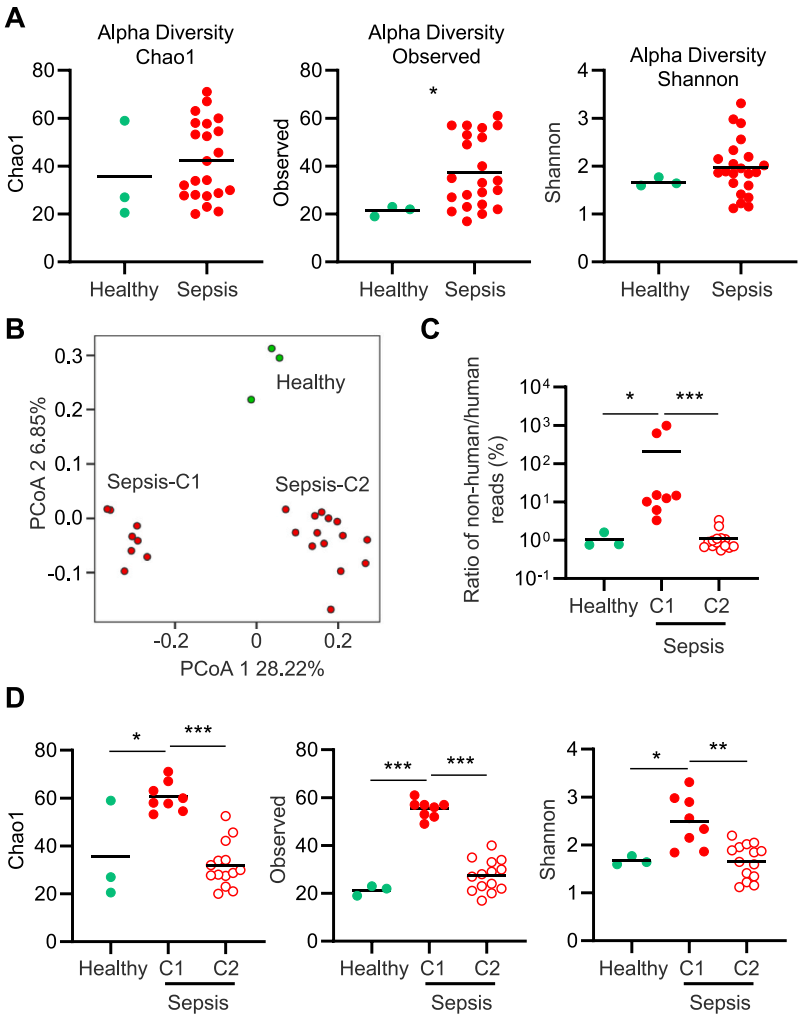

Fig. 2. Overall features of microbial community in the septic patients and healthy subjects. (A) α-diversity (Chao1, Observed and Shannon index) of blood microbial DNA. Significance of α-diversity was accessed by 2-tailed Mann-Whitney *U* test. **(B)** β-diversity analysis of blood microbial DNA. **(C)** C1 patients exhibited significantly higher level of non-human DNA than C2 patients and healthy subjects. **(D)** C1 patients exhibited increased α-diversity (Chao1, Observed and Shannon index) than C2 patients and healthy subjects. For **C** and **D**, Kruskal Wallis test with false discovery rate (FDR) correction was employed for multiple comparisons. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

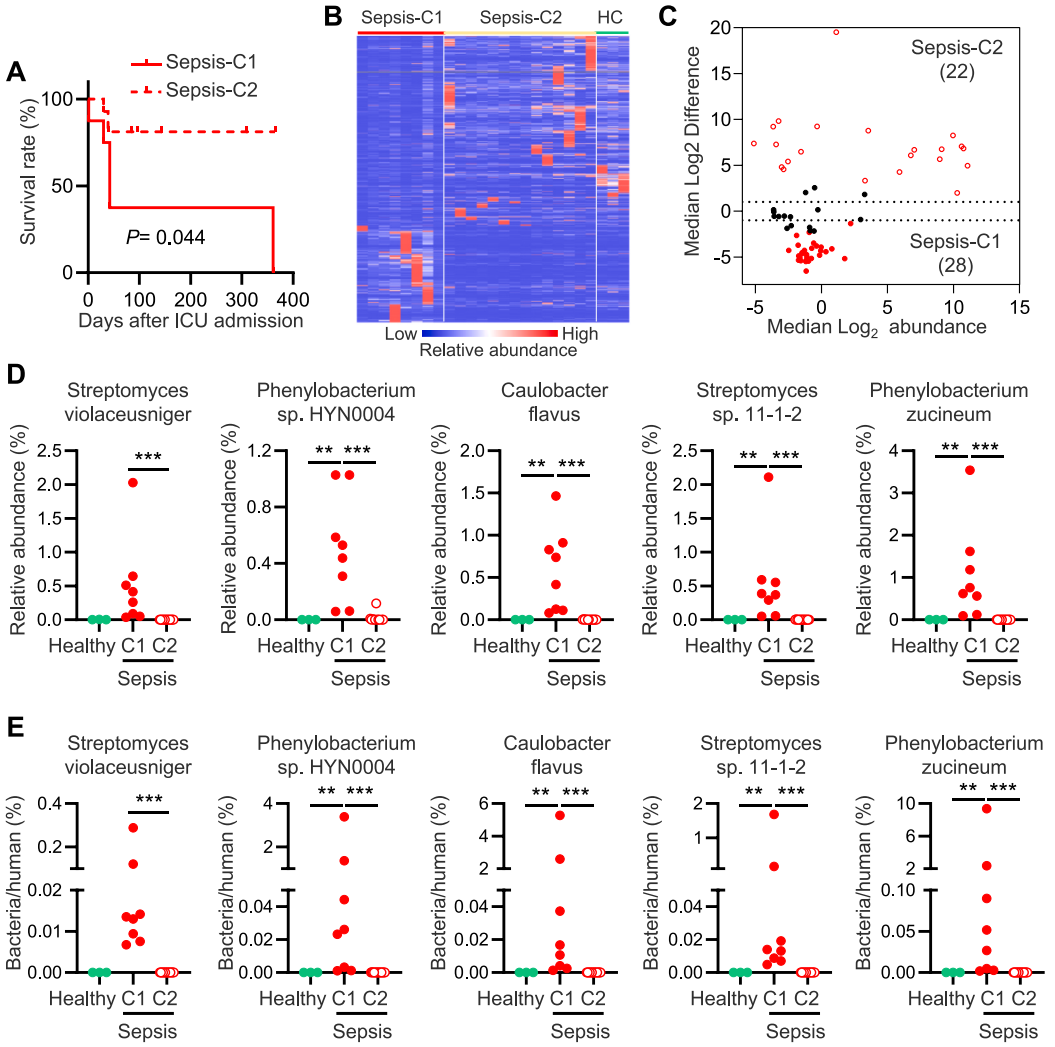

Fig. 3. Identification of microbial species associated with sepsis. (A) ICU mortality for C1 and C2 septic patients. **(B)** Heatmap analysis of bacterial species across different samples. **(C)** The differential abundance analysis of bacterial species between C1 and C2 patients. **(D)** Relative abundance of individual bacterial taxa between C1 and C2 patients. **(E)** Ratio of bacteria/human reads of individual bacterial taxa between C1 and C2 patients. For **D** and **E**, Kruskal Wallis test with false discovery rate (FDR) correction was employed for multiple comparisons. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

and control subjects. Other microbial reads (fungi, viruses, and archaea) were not included for analysis because of the low quantity. At the species level, a heatmap of hierarchical clustering of bacteria profiles among patients and healthy controls depicted the differential abundance of bacterial taxa (Fig. 3B). The bacterial composition among individuals of septic patients was diverse (Fig. 3B), inferring that there may be no single core blood microbiome for septic patients. In comparison to healthy subjects, a modest expansion of the bacterial species *Stenotrophomonas maltophilia* was observed in the blood of both C1 and C2 patients (Fig. S4A, both P < .05). It is important to note that the sample size of healthy subjects in this study was limited (n = 3), which underscores the need for future studies with a larger sample size to validate these findings. We further compared the bacterial composition between C1 and C2 patients by the ALDEx2 approach. In C1 patients, 28 and 22 bacterial species had significantly higher and lower relative abundance (% of total bacterial reads) than in C2 patients, respectively (wi.eBH ≤0.05) (Fig. 3C and Table S3). The most striking difference was seen in *Streptomyces violaceusniger*, *Phenylobacterium* sp. *HYN0004*, *Caulobacter flavus*, *Streptomyces* sp. *11-1-2*, and *Phenylobacterium zucineum*, the abundance of which was the highest in the C1 group among septic patients and healthy subjects (Fig. 3D and E). On the other hand, *Listeria monocytogenes*, *Acinetobacter* sp. *WCHA45*, *Acinetobacter baumannii*, *Ralstonia solanacearum*, and *Pasteurella multocida* had higher relative abundance in the C2 group than in C1 group (Fig. S4B).

To investigate the potential source of the bacteria DNA in the septic patients, we used Disbiome database, which collected published microbiota-disease information in a standardized way [13], to analyze 11 bacterial species (*Achromobacter xylosoxidans*, *Bradyrhizobium* sp. *SK17*, *Caulobacter flavus*, *Caulobacter mirabilis*, *Caulobacter* sp. *K31*, *Caulobacter vibrioides*, *Delftia acidovorans*,

Table 1
Clinical features of septic patients at admission to the intensive care unit.

Variable	C1 (n = 8)	C2 (n = 14)	ALL (n = 22)	*p* (C1 vs. C2)
Age (years)[a]	56 (15)	54 (30)	56 (21)	0.404
Sex				
Male, no (%)	6 (75.0 %)	7 (50.0 %)	12 (57.1 %)	0.251
Co-exiting diseases[b], no (%)				
Malignancy	0 (0 %)	1 (7.7 %)	1 (4.8 %)	0.421
Hypertension	7 (87.5 %)	7 (53.8 %)	14 (66.7 %)	0.112
Diabetes	4 (50.0 %)	4 (30.8 %)	8 (38.1 %)	0.378
Coronary artery disease	1 (12.5 %)	3 (23.1 %)	4 (19.0 %)	0.549
Heart failure	2 (25.0 %)	3 (23.1 %)	5 (23.8 %)	0.920
Renal impairment	2 (25.0 %)	8 (61.5 %)	10 (47.6 %)	0.104
Lactate (mmol/L)[ab]	3.0 (0.7)	2.8 (2.1)	2.9 (1.3)	0.536
qSOFA score[ab]	2.0 (0.3)	2.0 (0.0)	2.0 (0.0)	0.920
APACHE II score[ab]	21.0 (6.3)	25.0 (4.0)	24.0 (5.0)	0.077
Primary site of infection[b], no (%)				0.859
Pulmonary	5 (62.5 %)	9 (69.2 %)	14 (66.7 %)	
Abdominal	2 (25.0)	2 (15.4 %)	4 (19.0 %)	
Others	1 (12.5 %)	2 (15.4 %)	3 (14.3 %)	

The clinical features of C1 and C2 patients were compared by Student's *t*-test (Age, Lactate and APACHE II score), or Chi-square test (Sex, Co-exiting diseases, qSOFA score, and Primary site of infection).
[a] Median (interquartile range).
[b] the record of one patient was missing.

Phenylobacterium zucineum, *Stenotrophomonas maltophilia*, *Streptomyces hygroscopicus*, and *Streptomyces* sp. *11-1-2*) that were enriched in C1 group compared to healthy subjects (wi.eBH ≤0.05). We classified the potential body site origins for each microbial species (Table S4). Bacteria could be detected at various human body sites (blood, gastrointestinal tract, genitourinary tract, respiratory tract, skin, and oral). For example, *Delftia acidovorans* and *Stenotrophomas maltophila* were reported to be present in oral and skin, respectively (Table S4). This suggests that the source of systemic leakage of bacteria DNA in septic patients could be derived from different sites of the human body.

3.3. Interactions of bacterial species in septic patients

We next inferred all pairwise taxonomic correlations at the species level within healthy subjects, C1 and C2 patients. We found that the bacterial networks from healthy subjects and septic patients were quite different (Fig. 4A). The bacterial networks in septic patients were inclined to be co-occurrence (75.2 % and 84.5 % for C1 and C2 groups, respectively); in contrast, the bacterial interactions in

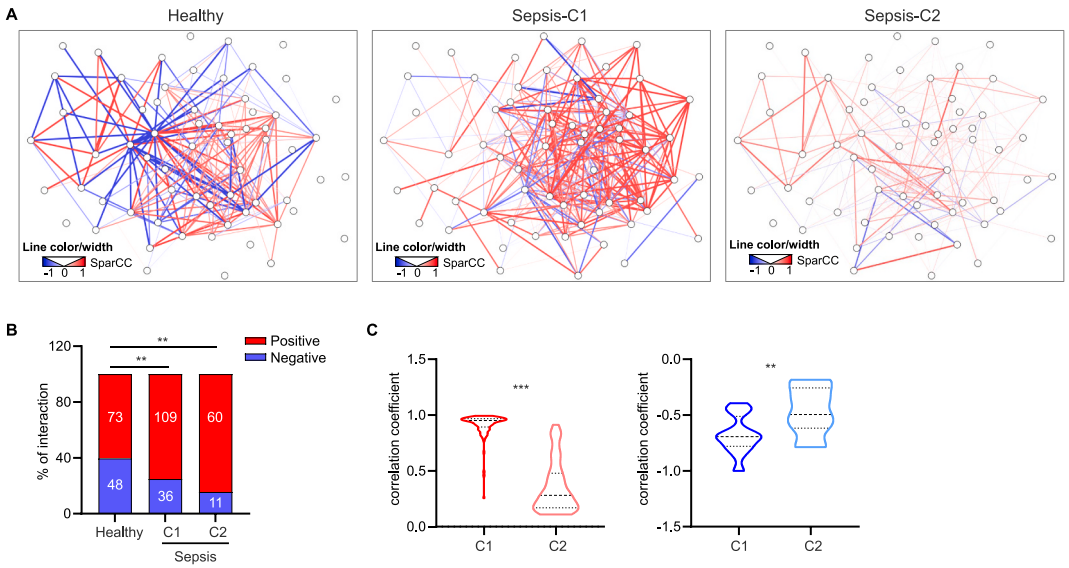

Fig. 4. Interactions of bacterial species among septic patients and healthy subjects. (A) Bacterial interactions (adjusted $P < 0.01$) were visualized using Cytoscape. **(B)** The percentage of bacterial co-occurrence (positive) and co-exclusion (negative) relationships (adjusted $P < 0.01$) in septic patients and healthy subjects. Chi-Square (X^2) test was used for comparisons. **(C)** The strength (correlation coefficient) of bacterial relationships (adjusted $P < 0.01$) in C1 and C2 septic patients. 2-tailed Mann-Whitney *U* test was conducted for comparisons. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

healthy subjects were in a balanced ratio (60.3 % and 39.7 % for co-occurrence and co-exclusion associations, respectively) (Fig. 4B). Notably, we observed distinct co-occurrence and co-exclusion relationships between C1 and C2 patients. C1 patients exhibited a significantly increased number of both co-occurrence and co-exclusion associations (adjusted $P < 0.01$) with stronger interaction than C2 patients (Fig. 4C). In C1 patients, the top 10 co-occurrence pairs were *Caulobacter* species with other taxa. In contrast, the relationships between *Streptomyces* species with other taxa were mostly co-exclusion associations. Most bacterial interactions shared between C1 and C2 groups were positive relationships of *Caulobacter* species with other taxa, including *Caulobacter*, *Brevundimonas*, and *Phenylobacterium* species (Table S5). *Caulobacter* species are commonly considered non-pathogenic and present in both the oral and gastrointestinal tract (Table S4). However, a few cases reported that *Caulobacter* isolates may be associated with recurrent peritonitis of peritoneal dialysis patients [14,15].

3.4. Pathogen identification by NGS

The most common primary infection sites of these patients were pulmonary (14/21, 66.7 %; the record of one patient was missing) followed by abdominal (4/21, 19.0 %). However, of the 22 blood specimens from septic patients, 16 (72.7 %) did not yield any bacterial growth after five days of routine culture. Of five culture positive specimens, extended-spectrum β-lactamase-producing *Escherichia coli* was detected in two patients. The remaining specimens were single isolates of coagulase-negative *staphylococci*, methicillin-resistant *Staphylococcus aureus,* and *Bacillus* spp. By NGS, the possible pathogenic bacteria [16] were identified according to bacterial/human reads >0.2 % (Table S6) and relative bacterial abundance >2 % (Table S7). Both gram-negative and gram-positive pathogenic bacteria were observed (Table S8). We found that 61.9 % (13/21) of NGS results were consistent with blood culture results (Table S8). NGS identified three polymicrobial (C1-1, −3, −8) and three monomicrobial (C1-2, −4, −6) infections. However, only one out of five culture-positive specimens processed by NGS workflow matched with the culture result (C1-3: *Escherichia coli*). Positive sputum culture of *Klebsiella* spp. was identified in C1-1 and C1-2 patients, which was concordant with NGS results (Table S8). We went further to investigate the presence of other microorganisms including viruses, fungi, and archaea. Those with microbial reads over 10, 000 were considered positive. By doing this, two, five, and one specimens were positive for fungi (C1-4: *Fusarium verticillioides*; C1-8: *Candida albicans*), virus (C1-1: *Human immunodeficiency virus 1*; C1-8: *BeAn 58058 virus*; C2-2: *Torque teno midi virus 13*; C2-4: *Torque teno virus 24*; C2-10: *Torque teno virus 24* and *Torque teno virus 20*), and archaea (C1-5: *Halorubrum ezzemoulense*) identification, respectively (Table S8). Nevertheless, none of them was identified by blood culture.

4. Discussion

In this study, we observed the presence of microbial DNA including bacteria, fungi, virus, and archaea in the blood of healthy subjects, although the latter three were in very low abundance. There are accumulating evidence supporting the existence of blood microbiome in healthy subjects by using different methods such as microscopic observation [17], blood culture [18], quantitative PCR [19,20], 16 S rRNA sequencing [21–23], as well as shotgun metagenome sequencing [24]. Stefan Panaiotov et al. recently reported the presence of a limited number of bacterial and fungal species in the blood of healthy individuals by *in vitro* culture [18]. In addition, Vasudevan Dinakaran et al. documented a relative low abundance of circulating viral and archeal DNA in the plasma of healthy human [25]. The presence of differnt eukaryotic viruses in the healthy human blood have also been reported by other teams [26–28]. Notably, our data suggest that blood-based microbial profiles appear to discriminate healthy subjects and different types of septic patients, strengthening the idea of clinical utility of NGS for sepsis diagnosis. In comparison to a published study investigating metagenomic sequencing of the blood microbiome [22], we identified several consistent results: 1) Bacterial DNA was detected in the blood samples of both healthy individuals and sepsis patients, with a significant distinction in bacterial diversity; 2) A distinct clustering pattern was observed, distinguishing individuals with sepsis from healthy subjects; and 3) a higher abundance of specific bacterial taxa at the order level was observed in sepsis patients, particularly those belonging to the C1 group, compared to healthy subjects. These taxa include *Actinomycetales*, *Aeromonadales*, *Rhizobiales*, and *Sphingomonadales* (Fig. S5). Nevertheless, at this moment we did not know whether the microbiome is blood-borne that could generate a viable ecological niche in human, or they are just a consequence of translocation from other sites, e.g., oral, skin and gastrointestinal tract.

In this study, the diagnosis of sepsis is consistent with the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) criteria [12] and presumed infection. To investigate the false positive identification of sepsis in the emergency department, Hooper et al. conducted a retrospective study involving 8267 patients meeting Sepsis-3 criteria. Their findings revealed that 8.5 % of patients did not have an infection upon final assessment, and 18.0 % had different infection sources diagnosed compared to the final assessment. However, there was no association found between false-positive infection diagnoses and 30-day mortality [29]. Our study indicates that the blood microbial DNA profiles of septic patients may exhibit distinct characteristics that could potentially be correlated with survival outcomes. The observed variable microbial DNA pattern may be a result of the microbiome originating from various tissues, indicating potential multiorgan damage. Additional research is necessary to substantiate these findings.

It is worth noting that septic patients could be separated into two subgroups in this study, of which C1 patients showed a significantly higher abundance of non-human DNA, a higher level of α-diversity, and poorer survival than C2 patients. Among the bacterial species that were enriched in the C1 group compared to healthy subjects and the C2 group (Tables S3 and S4), several have been linked to severe conditions. For instance, *Achromobacter xylosoxidans* has been documented in positive blood cultures in some high-risk cases of septicemia. These blood isolates were found to be resistant to multiple drugs but susceptible to meropenem and trimethoprim-sulfamethoxazole. This type of infection can potentially progress to fatal bacteremia, even in individuals without pre-existing health issues, and it may pose a significant risk to premature infants [30]. While *Caulobacter* species are generally considered

non-pathogenic and can be found in both the oral and gastrointestinal tract, a few reported cases have associated *Caulobacter* isolates with recurrent peritonitis in peritoneal dialysis patients [14,15]. *Delftia acidovorans* is rarely implicated as a human pathogen. Colonization or persistent infection with *Delftia acidovorans* was observed in a relatively small proportion, approximately 7 % of patients, although most of these patients had at least one underlying comorbidity. Notably, the all-cause mortality within the first year after infection was found to be high [31–33]. We also identified a significant expansion of *Streptomyces violaceusniger*, *Phenylobacterium* sp. *HYN0004*, *Caulobacter flavus*, *Streptomyces* sp. *11-1-2*, and *Phenylobacterium zucineum* in C1 patients compared to both C2 patients and healthy subjects. Nevertheless, none of them are considered pathogenic and thus are more likely passenger bacteria. *Streptomyces* species are known for their ability to produce antimicrobial substances and are rarely reported to cause infection in humans [34]. Intriguingly, *Phenylobacterium zucineum* was recently reported as an intracellular bacterium that was isolated from human erythroleukemia cell line K562 [35]. Nevertheless, no evidence of pathogenic involvement of *Phenylobacterium zucineum* was found. It is important to note that non-pathogenic bacteria can occasionally act as opportunistic pathogens in individuals with weakened immune systems or underlying health conditions. In such cases, these bacteria can take advantage of compromised immune defenses to cause infections that, if untreated, may contribute to sepsis severity [36]. Furthermore, non-pathogenic bacteria can coexist with pathogenic bacteria in polymicrobial infections. While the non-pathogenic bacteria may not directly cause harm, their interaction with pathogenic bacteria can lead to increased virulence or antibiotic resistance [37]. These interactions have the potential to worsen the severity of the infection. The distinct blood-microbial landscapes among septic patient may indicate the presence of different antimicrobial susceptibility patterns. These findings suggest that considering concomitant microorganisms is important when selecting appropriate antibiotics.

In this study, by NGS, we not only identified gram-negative (*Burkholderia pseudomallei*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia*) and gram-positive pathogenic bacteria (*Staphylococcus aureus*), but also revealed the presence of other pathogenic microorganisms including fungi (*Fusarium verticillioides* and *Candida albicans*), virus (*Human immunodeficiency virus 1, BeAn 58058 virus* and *Torque teno virus*), and archaea (*Halorubrum ezzemoulense*) in septic patients. *Candida* is the most common cause of fungal sepsis or septic shock in ICU [38]; *Fusarium* species could cause a broad spectrum of infections in humans [39,40]; Torque teno viruses are considered a significant part of the mammalian virome which is detected at high rates in both healthy and diseased individuals [41]; *BeAn 58058* is recently identified in postmortem Covid-19 patients [42]. Our study suggests that the NGS method is sensitive to detecting bloodstream pathogens, especially those that are difficult or impossible to culture.

We further investigated the microbial interactions in the blood. We found that the bacterial networks in septic patients were inclined to be co-occurrence (75.2 % and 84.5 % for C1 and C2 groups, respectively) rather than co-exclusion. In addition, different microbial interaction patterns between C1 and C2 patients were identified. Compared with C2 patients, C1 patients presented more co-occurrence and co-exclusion associations with much stronger interaction, implying that C1 patients had a more complex microbial community. In C1 patients, the top 10 co-occurrence pairs were *Caulobacter* species with other taxa while the relationships between *Streptomyces* species with other taxa were inclined to be co-exclusion. Both *Caulobacter* and *Streptomyces* species are considered non-pathogenic in humans, although a few studies reported that *Caulobacter* isolates were associated with recurrent peritonitis of peritoneal dialysis patients [19,20]. Notably, *Streptomyces* species can effectively suppress various bacteria and fungi since they are capable to produce antimicrobial substances [34].

Our study has some limitations. First, qSOFA was used for identifying septic patients in this study (from May 2015 to October 2016), which is not recommended by the recent sepsis guidelines [43]. Second, as a proof-of-concept study, the sample size is relatively small. Future larger-scale clinical trials are needed to translate our findings into biomarker applications in ICU. Third, the subjects were evaluated at a single time point. Follow-up investigations should be conducted in the future to characterize the change in the blood microbiome. Fourth, NGS analysis could not inform whether the microorganisms identified are alive or dead; however, it is useful to characterize the unculturable microbial species.

In summary, our study reveals differential blood-microbial landscapes between septic patients and healthy subjects. Importantly, two distinct blood microbial DNA profiles associated with different survival outcomes among septic patients were identified. The variable microbial DNA pattern in septic patients is probably associated with the diverse tissue origin of microbiome due to multiorgan damage. Detection of blood-microbial landscape could thus be useful to facilitate the treatment of sepsis.

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Data availability

The raw sequencing data generated in this study have been deposited in NCBI Sequence Read Archive under BioProject PRJNA1072595.

CRediT authorship contribution statement

Huarong Chen: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Weixin Liu:** Methodology, Investigation, Formal analysis. **Olabisi Oluwabukola Coker:** Methodology, Investigation, Formal analysis. **Na**

Qin: Investigation. **Hongyan Chen:** Investigation. **Yifei Wang:** Investigation. **Xiaodong Liu:** Investigation. **Lin Zhang:** Investigation. **Gordon Y.S. Choi:** Investigation. **Wai Tat Wong:** Investigation. **Czarina C.H. Leung:** Investigation. **Lowell Ling:** Investigation. **Mamie Hui:** Investigation. **Tony Gin:** Investigation. **Sunny Hei Wong:** Investigation. **Matthew Tak Vai Chan:** Writing – review & editing, Supervision, Funding acquisition. **William Ka Kei Wu:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29572.

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