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Airborne microbial communities in the atmospheric environment of urban hospitals in China

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ABSTRACT

Clinically relevant antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in bioaerosols have become a greater threat to public health. However, few reports have shown that ARB and ARGs were found in the atmosphere. High-throughput sequencing applied to environmental sciences has enhanced the exploration of microbial populations in atmospheric samples. Thus, five nosocomial bioaerosols were collected, and the dominant microbial and pathogenic microorganisms were identified by high-throughput sequencing in this study. The results suggested that the dominant microorganisms at the genus level were *Massilia*, *Sphingomonas*, *Methylobacterium*, *Methylophilus*, *Micrococcineae*, *and Corynebacterineae*. The most abundant pathogenic microorganisms were *Staphylococcus saprophyticus*, *Corynebacterium minutissimum*, *Streptococcus pneumoniae*, *Escherichia coli*, *Arcobacter butzleri*, *Aeromonas veronii*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. The relationship between microbial communities and environmental factors was evaluated with canonical correspondence analysis (CCA). Meanwhile, differences in the pathogenic bacteria between bioaerosols and dust in a typical hospital was investigated. Furthermore, cultivable *Staphylococcus* isolates with multi-drug resistance phenotype (> 3 antibiotics) in the inpatient departments were much higher than those in the transfusion area and out-patient departments, possibly attributed to the dense usage of antibiotics in inpatient departments. The results of this study might be helpful for scientifically air quality control in hospitals.

1. Introduction

The emergence and persistence of clinically relevant antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the natural environment has become a worldwide public health issue [1]. Recently, an increasing number of reports have suggested that ARB and ARGs were reported in soil [2–4] and water environments [5,6]. However, few studies have reported that ARB and ARGs were found in the atmosphere [7–9]. Humans may inhale ARB or ARGs carried by air particles, which poses a substantial risk to human health [10]. The risk increases when people inhale ARGs located within pathogens.

Air inside of hospitals and dust in the air surrounding hospitals are the primary transmission routes for ARB and ARGs, which have been initially detected in hospital air. The potential for contracting a microbial pathogen is highest within the hospital environment. Abreu et al. [11] found that organisms usually associated with nosocomial infections such as *Stenotrophomonas maltophilia, Enterococcus faecalis,* and *Serratia nematodiphila* were isolated on the same equipment in hospitals. Mess et al. [12] reported that 280 bacterial strains belonging to different genera were isolated in the operating air, and only 5% of the isolates were sensitive to all of the antibiotics tested, while the remaining strains were resistant to three (13%), four (14%), five (9%) and six (10%) antibiotics. This study confirmed the high presence of antibiotic-resistant bacteria in air samples, a finding that represents the threat of the possible transfer of resistance genes to pathogenic bacteria in nosocomial air. The presence of ARGs in hospitals is primarily related to antibiotic usage. Gilbert et al. [13] detected erythromycin resistance genes but sporadically detected tetracycline resistance genes in air samples collected from the same hospital rooms, because tetracycline is rarely used for curing diseases in hospitals. In nosocomial air, more researchers focus on isolating bacteria by culture methods using selective media. However, there is little evidence available to investigate the abundance of airborne microbial communities and related ARGs, as well as how they change in indoor environments.

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High-throughput sequencing has been widely used for the study of microbial communities in water, soil, and sediment environments, partly owing to an optimized protocol for extracting airborne microbial genomic DNA from collected bioaerosols and an improved sequencing library preparation protocol. The introduction of high-throughput sequencing to environmental sciences has enhanced the exploration of microbial populations in atmospheric samples. Recent studies have provided new information about the large diversity of airborne microbial communities in nosocomial air [14,15], leading to an improved understanding linking nosocomial airborne microbial communities and diversity with human health impacts [11,16]. Additionally, high-throughput sequencing allows for hundreds to thousands of identifications per sample and thus can provide in-depth information on non-cultivable pathogens and population diversity.

In this study, bioaerosol samples were collected from five nosocomial locations in eastern and southern China. The dominant microbial and pathogenic microorganisms were identified by high-throughput sequencing, and correlations with microbial communities and environmental factors were analysed with canonical correspondence analysis (CCA). The abundance of $bla_{CTX\text{-}M}$ and *mecA* resistance genes in the hospital bioaerosols from the transfusion area, out-patient department, and inpatient department were determined by real-time quantitative PCR (RT-qPCR). This finding provides an independent scientific foundation to assess the effectiveness of hospitals at improving indoor air quality and reducing air emissions from hospital operations for the protection of community health.

2. Materials and methods

2.1. Sampling and experimental setup

Bioaerosol sampling was conducted using a high-volume total suspended particle (TSP) sampler (LS2031, Qingdao, China). Bioaerosols were collected onto $20.32 \times 25.4\ cm^2$ glass fibre filters (PALL, NY, U.S.) at an average rate of $1.05\ m^3$/min for 20 h resulting in $\sim 1200\ m^3$ of flow-through volume. The bioaerosol samples collection and pretreatment strategies were followed previews studies [17–19]. The samples were collected between June 2015 and February 2016 from five urban hospitals in eastern and southern China. Sites were chosen to span subtypes in each different functional area in the hospital. Sample and site information are presented in Table 1.

2.2. DNA extraction, barcode amplification and RT-qPCR

Bioaerosol total genomic DNA was extracted from about 1/4 of the glass fibre filters (a total of $\sim 100.0\ cm^2$) using a FastDNA Spin Kit for soil (MP, CA, USA) following the manufacturer's instructions. DNA concentration and quality were checked using a Qubit 3.0 (Thermo Fisher Scientific, MA, USA). Extracted DNA was diluted to 10 ng/μL and stored at −80 °C for downstream use.

The primer pair 519F (5′- CAGCMGCCGCGGTAATWC-3′) and the reverse primer 907R (5′- CCGTCAATTCMTTTRAGTT-3′) with a unique 12 nt barcode was used to amplify the hypervariable V4 region [20,21]. A 50-μL PCR reaction solution was prepared for each sample using MightyAmp polymerase (TaKaRa, Dalian, China). The PCR amplification programme included an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. Three reactions were conducted for each sample and combined together after PCR amplification.

RT-qPCR was performed using the Quant Studio 6 Flex system (Applied Biosystems, Life Technologies, US). The primer sequence used for *mecA* and $bla_{CTX\text{-}M}$ were listed in Table S2. The PCR cycle consisted of denaturation for 5 min at 98 °C; 40 cycles of 10s at 98 °C, 15 s at annealing temperature (56 °C for *mecA* and 55 °C for $bla_{CTX\text{-}M}$ gene) and 30s at 68 °C and 1 min at 72 °C; and a final extension step of 10 min at 72 °C.

2.3. High-throughput sequencing and data analysis

The amplification products were purified using a quick-spin PCR Product Purification Kit (TaKaRa, Dalian, China) and quantified with a Qubit. All samples were pooled together with equal molar amounts from each sample. The pooled samples were applied to an Illumina MiSeq system for sequencing with the Reagent Kit v2 2×250 bp as described in the manufacturer's manual.

The Quantitative Insights into Microbial Ecology (QIIME) pipeline was used to process the raw sequences obtained through Illumina sequencing [22,23]. We assembled paired-end reads using FLASH [24]. Reads with quality scores lower than 20, ambiguous bases and improper primers were discarded before clustering. The resultant high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the UPARSE algorithm [23,25]. Simultaneously, chimaeras were checked and eliminated during clustering. Taxonomic classification of representative sequences from individual OTU was performed using the RDP Classifier [26,27]. To compare relative differences between samples, a randomly selected subset of 10,000 sequences per sample was chosen for downstream analyses.

One of the representative sequences was selected from each OTU and then compared against the human pathogen database using local BLAST with a threshold similarity of 97% with known pathogens [17,28]. These sequences were further screened and identified using online BLAST to rule out the possibility that they shared higher similarity with non-pathogenic bacteria [17,29].

In the current study, Shenzhen Second Hospital (Hospital 4) was considered a typical hospital. Samples collected at this hospital were used to investigate the relationship between dust and bioaerosol samples, the abundance of $bla_{CTX\text{-}M}$ and *mecA* resistance genes, and the cultivable methicillin resistant *Staphylococcus* (MRS) with multiple-drug resistance in bioaerosols from the transfusion area, out-patient department, and inpatient department.

2.4. Diversity analysis and statistical analysis

The downstream analysis was performed in QIIME (version 1.8.0) and R (version 3.0.2). The Alpha diversity index including Shannon index and chao1 index (change it accordingly) was calculated. Bray-Curtis dissimilarity metrics (change it accordingly) were utilized to implement a Principle coordinate analysis.

Table 1
The description of sampling hospitals.

No.	Name	Location	Level of the institution	Numbers of regular hospital beds
Hospital 1	Traditional Chinese Medicine in Fenghua	Eastern China (Fenghua)	Grade III Level B	450
Hospital 2	Cancer Center of Guangzhou Medical University	Southern China (Guangzhou)	Grade III Level A	800
Hospital 3	Ningbo Seventh People's Hospital	Eastern China (Ningbo)	Grade II Level A	528
Hospital 4	Shenzhen Second Hospital	Southern China (Shenzhen)	Grade III Level A	1500
Hospital 5	Yangzhou No.1 People's Hospital	Eastern China (Yangzhou)	Grade III Level A	1600

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