The Microbe 4 (2024) 100163

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

Harnessing next-generation sequencing to monitor unculturable pathogenic bacteria in the indoor hospital building

Nor Husna Mat Hussin [a], Darren Dean Tay [a], Ummu Afeera Zainulabid [b], Mohd Norhafsam Maghpor [c], Hajar Fauzan Ahmad [a,d,*,1]

[a] Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, Pahang 26300, Malaysia
[b] Department of Internal Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang 25200, Malaysia
[c] Chemical Hazardous to Health Laboratory, Consultation Research and Development Department, National Institute of Occupational Safety and Health, Bandar Baru Bangi, Selangor 43650, Malaysia
[d] Group of Environment, Microbiology and Bioprocessing (GERMS), Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Kuantan, Pahang 26300, Malaysia

ARTICLE INFO

Keywords:
Indoor microbiome
Core microbiome
Culture-independent
Next- generation sequencing

ABSTRACT

The hospital indoor air microbiome, a diverse range of microorganisms, gains prominence amid the COVID-19 pandemic. Elevated awareness underscores implications for patient and staff well-being. Concerns about risks to indoor air quality persist due to prolonged indoor exposure, necessitating further research on specific threats within the hospital environment. In this study, an independent culture-based approach was used to analyze the baseline core microbiome present in hospital environments, utilizing amplicon sequencing on the next-generation sequencing technology to target the V3 region of the 16S rRNA gene. Firmicutes, Proteobacteria, and Actinobacteria were the main bacterial phyla that were most isolated from the wards and clinics with different orders of abundance; Firmicutes being associated more in clinics and Actinobacteriota in wards. The bacteria *Niallia taxi*, *Methyloversatilis universalis*, unclassified *Rummeliibacillus*, unclassified *Clostridium*, and unclassified *Sphingomonadaceae* dominated the clinic area while ward areas reported *Pseudonocardia bannensis*, *Rubrobacter aplysinae*, unclassified *Brachybacterium*, unclassified *Bradyrhizobium*, and unclassified *Mycobacterium* to be the top five features. While the alpha-diversity index showed no significant differences, the beta-diversity analysis showed a significant difference between clinic and ward areas ($p<0.05$). Certain bacterial species associated with opportunistic pathogens as well as normal skin flora such as *Methylobacterium* spp., *Cutibacterium* spp., unclassified *Sphingomonadaceae*, and *Anoxybacillus B* spp., were also identified across all samples. The methods described in this research aim to establish a rapid and sensitive screening process that could be valuable for disease surveillance within the healthcare setting, shedding light on the potential impacts of the hospital microbiome on human illness.

1. Introduction

The outbreak of the SARS-CoV-2 virus had caused more than a million deaths worldwide. The COVID-19 pandemic has significantly increased people's attention to microbial exposure in indoor air due to its implications for human health. People spend 80–90 % of their time indoors, where the air contains a complex mixture of volatile and non-volatile organic compounds, particulate matter, and microbes (Horve et al., 2020). The collective microbial community within the indoor air is known as the indoor air microbiome, which includes bacteria, fungi, viruses, protozoa, and algae (Carrazana et al., 2023). The composition of the indoor air microbiome within a hospital is influenced by several key factors, including the presence of patients and healthcare staff, potential interaction with therapy animals, the operation of heating, ventilation, and air-conditioning (HVAC) systems, and the external environment (Sibanda et al., 2021; Prussin and Marr, 2015). Understanding how

* Corresponding author at: Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, Pahang 26300, Malaysia.
E-mail address: fauzanahmad@umpsa.edu.my (H.F. Ahmad).
[1] ORCID ID: 0000–0003-2985–2496

https://doi.org/10.1016/j.microb.2024.100163
Received 2 July 2024; Received in revised form 21 August 2024; Accepted 6 September 2024
Available online 12 September 2024

these elements impact the hospital's indoor microbiome is crucial for ensuring a safe and healthy environment for patients and medical personnel.

The scrutiny of microbial exposure within the indoor air of hospitals has garnered significant medical interest, primarily owing to its capacity to serve as a reservoir for a diverse range of pathogenic microorganisms (Li et al., 2019). Such exposure has been correlated with the occurrence of secondary bacterial infections in immunocompromised patients and individuals admitted with COVID-19. This phenomenon would prolong hospital stays up to weeks and add extra costs (Sebastian et al., 2022). Besides, the air microbiome is also a significant contributing factor to the increase in the prevalence of nosocomial infections or hospital-acquired infections (HAIs). Nosocomial infections are a global issue affecting not only patients but healthcare workers and visitors (Baviskar et al., 2019; Zainulabid et al., 2022). However, the profiling of airborne bacteria that potentially impact health has not yet been fully elucidated. Studies on indoor air microbiomes may help understand the potential causative agents contributing to nosocomial infections.

In many occasions, microbial communities in an environment, especially the indoor air of a building, were primarily identified using culture-dependent methods (Hassan et al., 2020; Sarah Fatihah Tamsi et al., 2022; Adhikari et al., 2017). These techniques involved collecting samples and culturing bacteria on agar plates following various methods, including the NIOSH Manual Analytical Method (NMAM) 0800 protocol (Haig et al., 2016; Cox et al., 2020a). While effective, these methods faced limitations, such as the inability to culture some microbes and time-consuming. With advancements in DNA sequencing technology, culture-independent methods have gained prominence. These methods, which do not require culturing, involve DNA extraction, amplification of the 16S rRNA gene, and sequencing (Mat-Hussin et al., 2024). Next-generation sequencing (NGS) has emerged as a widely used tool for profiling microbial diversity in indoor dust samples, and it can detect both live and dead organisms. However, NGS techniques also have their limitations. They cannot distinguish between viable and non-viable microbes, and analyzing NGS data is complex, requiring specialized bioinformatics tools and expertise (Boers et al., 2019). Despite these challenges, NGS's rapid and comprehensive profiling capabilities make it a valuable approach for studying live and dead microbial communities in various environments.

Furthermore, sequencing technology allows for the direct analysis of the different types of collected environmental samples (Mucci et al.,). This study used surface swabs and settled dust from the supply and return ventilation unit for analysis. Supply and return vents located in each clinic and ward are part of the HVAC systems that circulate and filter the air in a building but could also distribute microorganisms, making them critical components for microbial dispersion (Božić et al., 2019). Sampling dust from these vents provides insights into the indoor microbiome and helps identify microbial accumulation and transmission within healthcare settings (Kwan et al., 2020; Bonadonna et al., 2021 Jun). Passive sampling methods, like surface swabs and settled dust collections, are commonly used as they are less complex and do not disrupt the surrounding air (Cox et al., 2020b). Dust samples represent an accumulation of particles over time, which include bacteria that were once airborne. These integrated samples can provide a broader picture of the bacterial community, including transient and less abundant species that might not be captured in short-term air samples commonly using the active sampling method (Villanueva et al., 2022; Efthymiopoulos et al., 2021).

Dust samples can complement air samples by providing additional information about the indoor microbiome. While air samples give a snapshot of the airborne community, dust samples offer a cumulative perspective, enhancing the overall understanding of the indoor bacteria ecosystem (Hanson et al., 2016; Sun et al., 2020). This temporal integration offered by dust samples can be particularly valuable in understanding long-term exposure and trends. In addition, dust sampling is more sampling efficient as it is more straightforward, less time-consuming, inexpensive, and does not require specialized equipment (Mucci et al., 2020). This efficiency is significant because this study was conducted in many wards and clinics with limited time frame concerns. Researchers were given limited time for sampling as the hospital management was concerned the sampling procedure might disturb the patients' and healthcare officers' jobs and duties. Future studies could combine dust and air sampling to provide a more comprehensive understanding of the bacterial communities in indoor environments.

This study examines the method that could be an alternative option to help in collecting indoor air samples in a more convenient way, minimize disturbances due to the sampling process, and offer fast as well as reliable results at the same time – over the dependent-culture approach. In addition, this study also examines the pathogenic and commensal microflora that are normally related to humans. Moreover, we also examined the microbial distribution and investigated the differences in bacterial community profiling between sampling locations such as clinics and wards in the hospitals. We hypothesized that there would be a significant difference in bacterial composition between wards and clinics in the same hospitals.

2. Materials and methods

2.1. Sample collection

A passive sampling method was applied to collect dust samples from clinics and hospital wards. Prior to sample collection, a walkthrough building inspection was conducted to identify and randomly select the sampling locations. Sampling was conducted during clinics and wards' operating hours. In order to minimize the confounding effects of bacteria brought into the hospitals by visitors, no sampling activities were conducted during visiting hours, which are from 12:00 pm to 2:00 pm and 5:00 pm to 7 pm.

With the help of hospitals' technical staff, a total of 16 dust samples were collected from both the supply and return vents located within the clinics and wards using sterile cotton swabs with wooden sticks (HmbG, China) and placed in sterile conical 15 mL tubes made from polypropylene (Eppendorf) containing 5 mL phosphate buffer solution. As the supply and return vents are on the ceiling, the technical staff needs to climb the portable stairs to get the dust samples. Surface swab sampling was conducted on each component in size of a 20 cm^2 surface area. Sampling was conducted during clinic and ward operation hours that is between 8:00 am to 12:00 pm and 2:00 pm to 5:00 pm. No sampling was conducted during visiting and break hours that is from 12:00 pm to 2:00 pm to minimize bias from occupants and visitors from the outside of hospitals. Samples from eight wards were labeled as Locale A, B, C, D, E, F, G, and P, while samples from eight clinics were labeled as Locate H, I, J, K, L, M, N, and O, not to disclose the name of sampling areas as requested by the hospital management. Samples were kept at room temperature during the sampling process and brought back to the lab on the same day to be kept in a −20°C freezer before further analysis was done in a week.

2.2. DNA extraction, library preparation, and sequencing

The bacterial DNA extraction from the dust samples was conducted using QIAamp PowerFeacal Kit (QIAGEN, Hilden, Germany) with some modifications (Mat-Hussin et al., 2024). Briefly, dust samples were soaked in 2 mL centrifuge tubes containing 200 μL of phosphate buffer solutions (PBS) for 15 minutes and vortex for 5 minutes to homogenize the samples. Next, 800 μL of CD1 was placed into the samples and incubated in a water bath set at 65°C for at least one hour. After that, samples were placed in a PowerBead ProTube provided by the manufacturer in the extraction kit. Samples were then vortexed for 15 minutes for homogenization before centrifugations. After that, the rest of the procedure was performed according to the manufacturer's protocol. The

quality and quantity of extracted DNA were then examined using 1 % gel electrophoresis and Qubit 4 Fluorometer (Thermo Fisher Scientific, Invitrogen, USA). Samples were then kept at −20°C for downstream application.

For bacterial characterization, the library preparation was prepared using amplification by polymerase chain reaction (PCR) method that targeted the 16S rRNA V3 region using the primers sequences: 341 F – CCTACGGGNGGCWGCAG, and 518 R – ATTACCGCGGCTGCTGG (Hasain et al., 2022; Ayob et al., 2023). The PCR mixtures were prepared by mixing Promega GoTaq Green Mastermix with forward and reverse primers as well as the DNA template with one tube leaved without the DNA template as the negative control. This procedure was performed in a biosafety cabinet (BSC) to avoid cross contaminations. The mixtures were then subjected to an amplification process in an Eppendorf 5331 MasterCycle Gradient Thermal Cycler (Eppendorf, Germany) with initial denaturation at 95°C for 2 minutes followed by 30 cycles of denaturation at 95°C for 10 seconds, annealing at 50°C for 10 seconds, and extension at 72°C for 10 seconds. The final extension was set at 72°C for 30 seconds. The completed PCR products were visualized using 2 % gel electrophoresis and purified using SPRI bead. The index PCR was performed on the purified product to incorporate Illumina-specific barcodes and the remaining Illumina adapter sequences with initial denaturation at 95°C for 2 minutes followed by 8 cycles of denaturation at 95°C for 10 seconds, annealing at 55°C for 10 seconds, and extension at 72°C for 10 seconds and lastly the final extension was set at 72°C for 30 seconds. The concentration of final pool was examined using Qubit Fluorometric Quantification (Thermo Fisher Scientific). Finally, the amplicon libraries were sequenced on Illumina Novaseq6000 (Illumina, San Diego, CA).

2.3. Amplicon data analysis

Amplicon data was prepared by overlapping the raw paired-end reads using fastp (Chen et al., 2018) which was followed by the use of Cutadapt v4.4 (Martin, 2011) to trim out the primers. The overlapped reads were imported to QIIME2 where DADA2 (Callahan et al., 2016) for denoising and table count construction. Additionally, classification of the Amplicon Sequence Variant (ASV's) was conducted using qiime2-feature-classifier and trained on the GTDB 16S rRNA V3 region database (r207 release). The taxonomic classification and ASV table were formatted manually to be uploaded to the webserver of MicrobiomeAnalyst for data visualization.

2.4. Statistical analysis

Statistical analysis of microbiome data was performed using MicrobiomeAnalyst 2.0 to profile the bacterial community found in indoor air with adjustments. The data was filtered at a minimum count of zero and an interquartile range of 10 %. The number of features that remain after the data filtering step was 1021. The filtered data was then normalized using cumulative sum scaling (CSS) with no data transformation being performed. The taxonomy composition for samples was generated in bar plot at phylum, genus, and species level. In profiling abundance at phylum level, certain phyla were included if they reported an abundance of 0.5 % or greater within at least 25 % of all samples. While useful in indicating overall trends, analysis at the phylum level may not be able to provide enough resolution in inferring the significant differences across bacterial communities; hence the analysis at the genus level was included to circumvent such issues.

For diversity analysis, the permutational multivariate analysis of variance (PERMANOVA) was employed using the Bray-Curtis dissimilarity index to assess the significant difference between groups. Meanwhile, a mixture of different indices was used in the alpha diversity analysis to investigate the bacterial diversity within a group, with p-value <0.05 considered significant. These metrics were the Shannon diversity index, Chao1, and observed features. A community profiling approach was performed to describe the diversity of air microbiome in clinics and wards of hospitals. Alpha-diversity refers to the total species diversity of the samples and can be defined as the similarity or distance found between sample groups. In this analysis three diversity indices were used which were the observed features index, Chao1 index, and Shannon diversity index where the first two are normally used to test for richness while the latter is used to test for both evenness and richness. Beta-diversity on the other hand is a measure that can be used to compare the composition or feature similarity found between samples. In this analysis, the Bray-Curtis dissimilarity index was utilized.

3. Results

Results showed a wide variety of bacterial compositions within each sample. After quality control and sequence filtering of 16 dust samples, a total of 1052163 sequence reads, with an average of 65760 sequence reads per sample (min 186, max 248317) were created, corresponding to a total of 1135 operational taxonomic units (OTUs).

3.1. Bacterial community profiling

The profiling of bacterial community abundance in dust samples was classified at phylum, genus, and species levels. Overall, 20 bacteria phyla were found in dust samples, with 474 bacterial genera and 659 bacterial species present within the indoor air of sampled locations. The top 20 phyla, genus, and species were presented in stacked bar plots. The results showed varying compositions of bacteria in each sample at different taxonomy levels. Fig. 1 showed the top 20 bacterial phyla found within indoor air across the 16 dust samples. Figs. 2 and 3 showed the top 20 for bacterial genus and species, respectively, within the indoor air across the 16 dust samples. Features ranked lower than the top 20 were grouped together and marked as others.

At the phylum level, the most abundant phyla that dominated the indoor air were Firmicutes, Proteobacteria, and Actinobacteria, which were shown to be present at differing levels between ward and clinic locations. Within clinic areas, in order of abundance, the bacterial phyla observed were Firmicutes (72 %), Proteobacteria (27 %), and Actinobacteria (1 %). In contrast, within ward areas, the order of abundance reported Actinobacteria (64 %) to be first, followed by Proteobacteria (24 %), and Firmicutes (6 %). Samples from wards C and D were shown to have the highest presence of Actinobacteria. Investigation at the genus level showed different top seven bacterial genera between clinic and ward settings. For clinic locations, the top seven genera were reported to be *Niallia* (34 %), *Methyloversatilis* (21 %), *Rummeliibacillus* (9 %), *Clostridium* (7 %), *Methylobacterium* (3 %), unclassified *Sphingomonadaceae* (1 %), and *Pseudonocardia* (0.2 %) while ward locations reported the top features to be *Pseudonocardia* (23 %), *Rubrobacter* (12 %), *Brachybacterium* (8 %), *Bradyrhizobium* (5 %), *Mycobacterium* (4 %), *Salinisphaera* (4 %), and *Methylobacterium* (3 %).

The composition of bacterial genera between the two areas was shown to be quite different, with features such as *Niallia*, *Methyloversatilis*, *Rummeliibacillus,* and *Clostridium* being more abundant within clinic areas. In contrast, ward areas showed overall more diversity, with features like *Pseudonocardia, Rubrobacter A*, and *Brachybacterium* being more prevalent within the dataset. Additionally, samples obtained from locations B, C, D, and N were the most diverse across all the samples. As for bacterial species, a similar pattern was observed for the top 5 species shown between the two areas. For clinic areas, the top species observed were *Niallia taxi* (35 %), *Methyloversatilis universalis* (21 %), unclassified *Rummeliibacillus* (9 %), unclassified *Clostridium* (7 %), unclassified *Sphingomonadaceae* (1 %). In comparison, ward areas reported *Pseudonocardia bannensis* (21 %), *Rubrobacter aplysinae* (12 %), unclassified *Brachybacterium* (8 %), unclassified *Bradyrhizobium* (5 %), and unclassified *Mycobacterium* (4 %) to be the top five features. Similarly, samples obtained from wards B, C, D, and clinic N contained the most diversity compared to the other locations from clinics and wards.

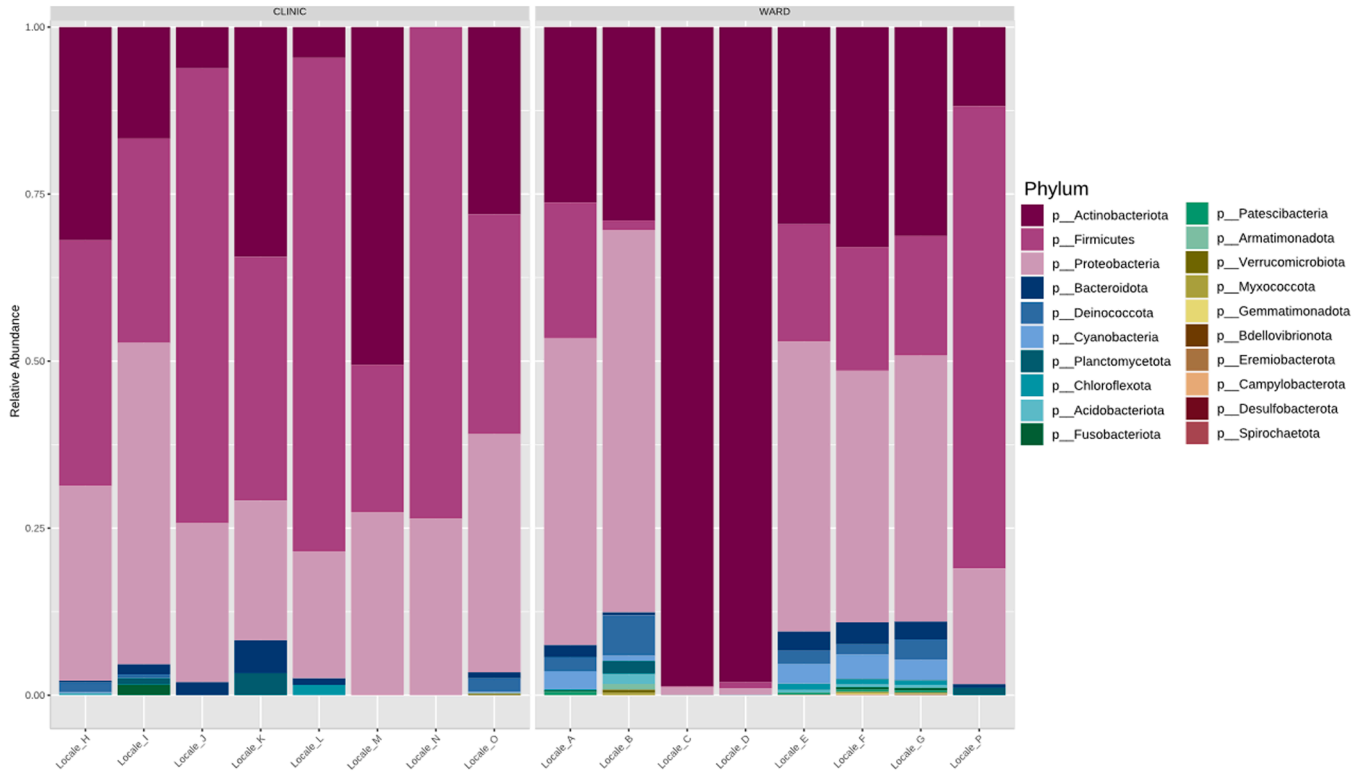

Fig. 1. Relative abundance of the 20 bacterial phyla observed across the 16 dust samples obtained from ward and clinic areas.

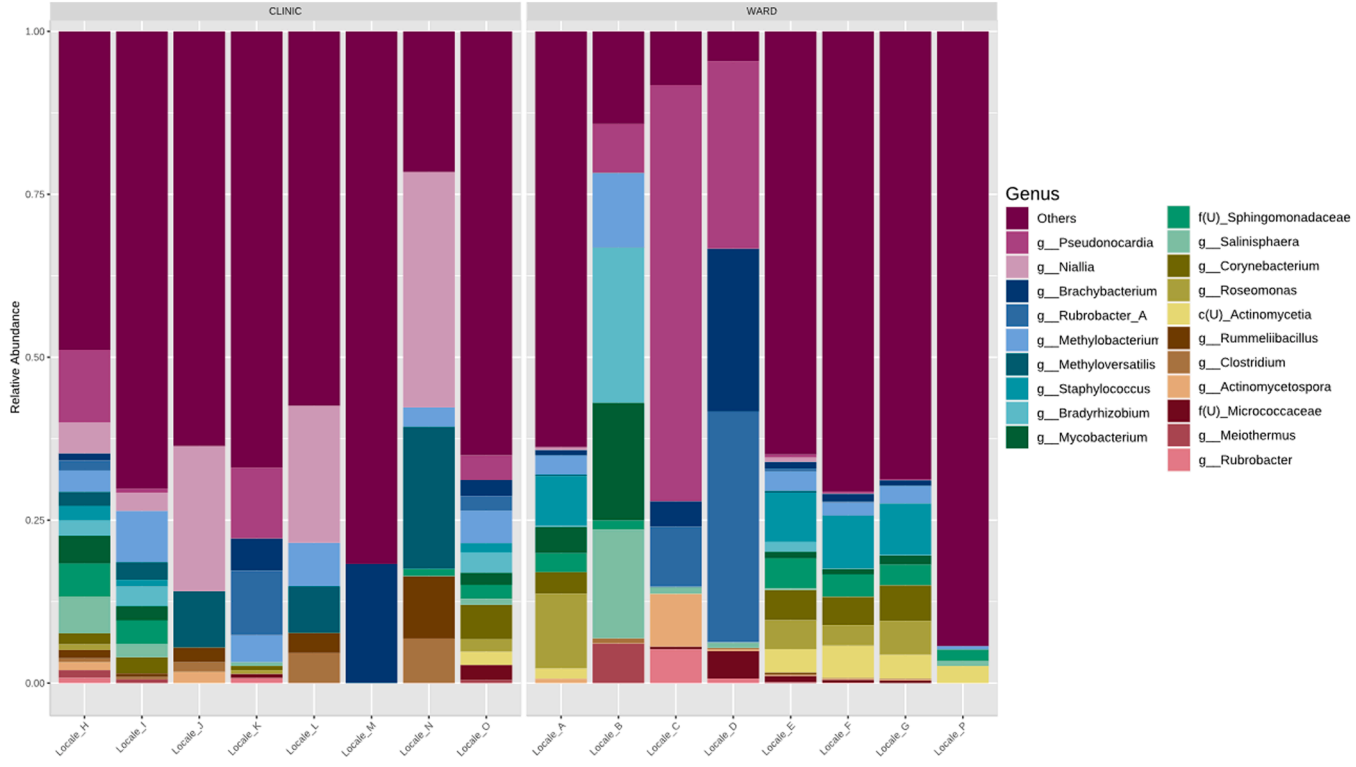

Fig. 2. Relative abundance of the 20 bacterial genera observed across the 16 dust samples obtained from ward and clinic areas.

3.2. Alpha and beta diversity

Fig. 4 showed the results of the diversity analysis. Alpha-diversity reported no significant results, whereas beta-diversity analysis was considered significant.

In alpha diversity analysis, the boxplot showed a higher observed diversity within the ward compared to the clinic areas. However, statistical tests showed that these differences were not significant when

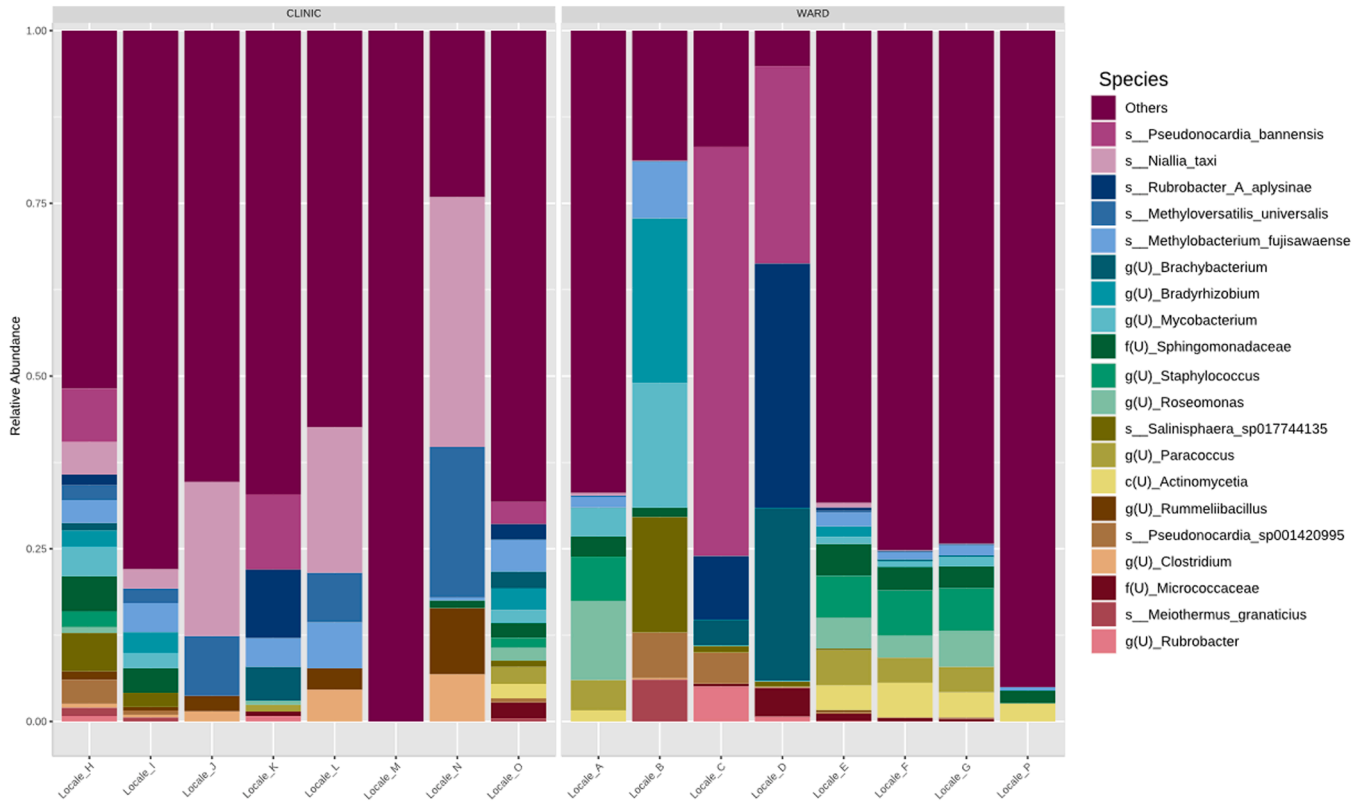

Fig. 3. Relative abundance of the 20 bacterial species observed across the 16 dust samples obtained from ward and clinic areas.

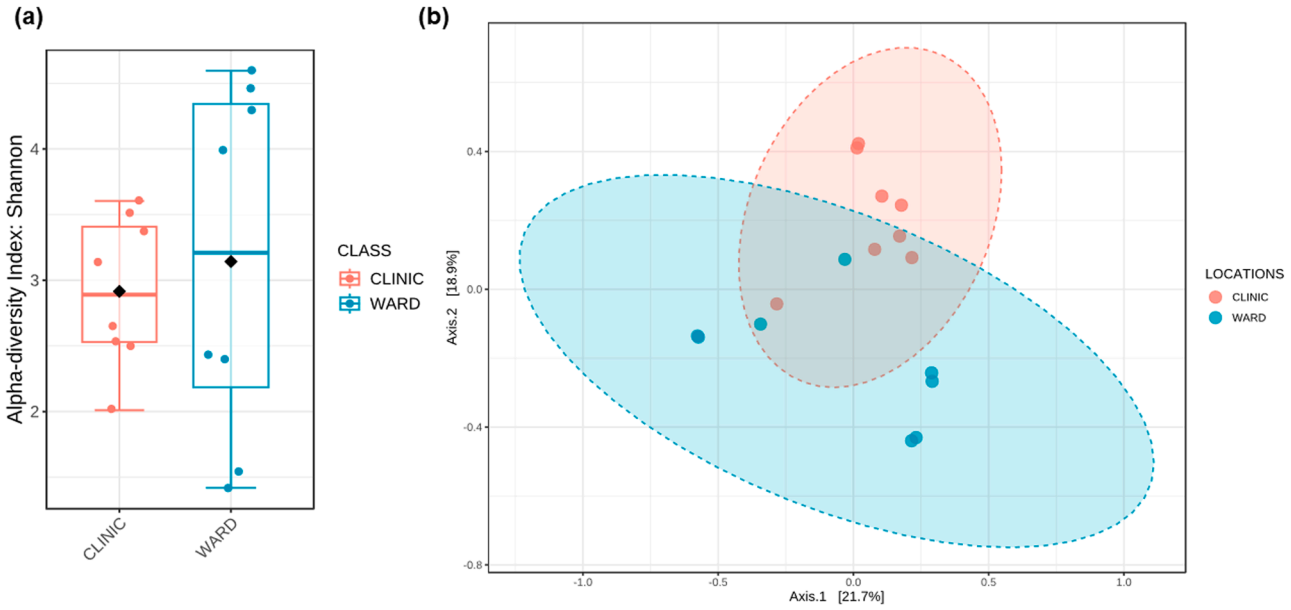

Fig. 4. Results for diversity analysis where (a) represented not significant alpha-diversity results (p =0.6681) using Shannon diversity index as boxplots while (b) represented significant beta-diversity results (p = 0.015) as 2D PCoA plots using the Bray-Curtis dissimilarity.

examined using the Shannon diversity index, suggesting that the overall community diversity is similar between the two areas. In contrast, we found significant statistical differences when we evaluated species richness through the Chao1 index and the observed feature index. This may indicate that while the evenness of species may be comparable, the species richness in the ward and clinic areas differ significantly. Furthermore, our beta diversity analysis showed statistically significant results, demonstrating that the bacterial composition between the clinic and ward areas is distinct and unique. These findings highlight the complexity of microbial communities and underscore the importance of considering both richness and evenness when interpreting alpha diversity metrics.

3.3. *Common and location-specific core indoor microbiome*

The core microbiome refers to microbes that are consistently found

in all dust samples. A feature needs to be present at least at an abundance of 1 % at a minimum within at least 75 % of all samples used for the microbiome analyses to be defined as a part of the core microbiome [Cox et al., 2020b]. Results for the core microbiome analysis can be found in Fig. 5 which showcased the bacterial phyla that dominate the indoor air across all samples and Fig. 6 which showcased the prominent members of indoor air bacteria at genus and species levels.

Core microbiome results showed the bacterial phyla Proteobacteria, Actinobacteriota, and Firmicutes to be quite prevalent within the indoor air, being relatively present as high as 21 % within all samples. At the genus level, features such as *Methylobacterium*, *Cutibacterium*, unclassified *Sphingomonadaceae*, and *Anoxybacillus B* were shown to be core members, with the latter being the highest with a prevalent up to 4 % across all samples. Other notable members at the genus level included *Bacillus M* and *Brachybacterium*. At the species level, core members included *Cutibacterium acnes*, unclassified *Sphingomonadaceae*, *Methylobacterium fujisawaense*, and *Anoxybacillus vitaminiphillus* with the latter being the most prevalent up to 4 % across all samples. Another notable species member is *Bacillus M halodurans*.

4. Discussion

In this study, dust samples from the return and supply vents of HVAC systems from clinics and wards were collected using culture-independent techniques. Bacterial DNA was successfully extracted and analyzed using next-generation sequencing to profile the bacterial community of indoor air within a healthcare setting. Complex and unique interactions of numerous microbial communities have been observed in the hospital air space as some of the microbes present are pathogenic to humans, and some act as opportunistic pathogens, especially to immunocompromised patients, causing harmful effects and potential agents for nosocomial infections, while others exist as commensal or normal microflora (Fakunle et al., 2023; Chandika et al., 2021). In contrast, exposure to certain microbes during childhood may also have protective and beneficial effects in lowering the risk of getting asthma (Fu et al., 2020).

Our study identified Proteobacteria, Actinobacteria, and Firmicutes as the top core microbiome bacterial phyla found in the collected dust samples from the ventilation systems in wards and clinics. This aligns with findings from previous studies conducted in various indoor environments, including hospitals, homes, and schools across different geographical regions (Fakunle et al., 2023; Fu et al., 2021; Kirjavainen et al., 2019). The consistent presence of these bacteria phyla suggests a shared core microbiome of indoor air, potentially influenced by their presence in the pulmonary microflora (Salonen et al., 2020; Matys et al., 2024). The presence of these bacteria in ventilation systems of healthcare settings is particularly concerning given their potential role in nosocomial infections, such as lower tract respiratory infections, which remains a significant source of morbidity and mortality (Baviskar et al., 2019; Rozaidi et al., 2001). This underscores the urgent need to profile the taxonomic composition of bacterial communities in indoor air, especially in hospitals where the spread of nosocomial infections is a critical concern.

Interestingly, while certain bacterial strains within Actinobacteriota phyla have been associated with protective effects against childhood asthma (Fu et al., 2022), others may serve as opportunistic pathogens, particularly in immunocompromised patients (Fakunle et al., 2023). This dual role underscores the complexity of microbial ecology in hospital environments and highlights the importance of taxonomic profiling to better understand the pathogenic potential of these bacteria in developing effective strategies for preventing and controlling nosocomial infections.

Further analysis of the dataset at the species level showed *Cutibacterium acnes*, unclassified *Sphingomonadaceae*, *Methylobacterium fujisawaense*, and *Anoxybacillus B vitaminiphilus* were moderately prevalent across the samples. *Cutibacterium acnes* is the bacterial commensal present in the healthy skin flora, which belongs to the Actinobacteriota phylum (Dréno et al., 2018). The discovery of *C. acnes* in the ventilation system in wards and clinics attracts a closer examination of its potential role in nosocomial infection and prosthetic joint infections. There is concern that *C. acnes* from the airspace can contaminate the surgical sites, medical devices, or open wounds, especially during procedures involving implants like prosthetic joints. *C. acnes* pathogenicity is attributed to several virulence factors, including its ability to form biofilm on the surfaces, which enhances its survival and resistance to antibiotics (Salar-Vidal et al., 2021). Besides, the diagnosis of C. *acnes*-related infections is often complicated by the bacterium's slow growth rate, which can take up to 14 days for culture to give a result. This delay can lead to misdiagnosis or underdiagnosis, as *C. acne* is commonly regarded as a contaminant rather than a pathogen. Immunocompromised patients are especially at risk, as *C. acne* can also lead to respiratory or systemic infections if inhaled or if in contact with mucous membranes (Abdullah et al., 2021). Considering the challenges and costs associated with managing such infections, it becomes increasingly important to investigate and ensure stringent infection control measures in healthcare settings.

In addition, the presence of *Sphingomonas* spp. is worrying for healthcare personnel and patients as it is an opportunistic pathogen previously found in hospitals' indoor air and water tanks (Gao et al., 2018). *Methylobacterium fujisawaense* is an opportunistic gram-negative rod bacteria that is ubiquitous within multiple environments. *M. fujisawaense* rarely causes infections in healthy individuals but can cause secondary bacterial infections among immunosuppressive patients (Fanci et al., 2010). Thus, the presence of this bacteria species

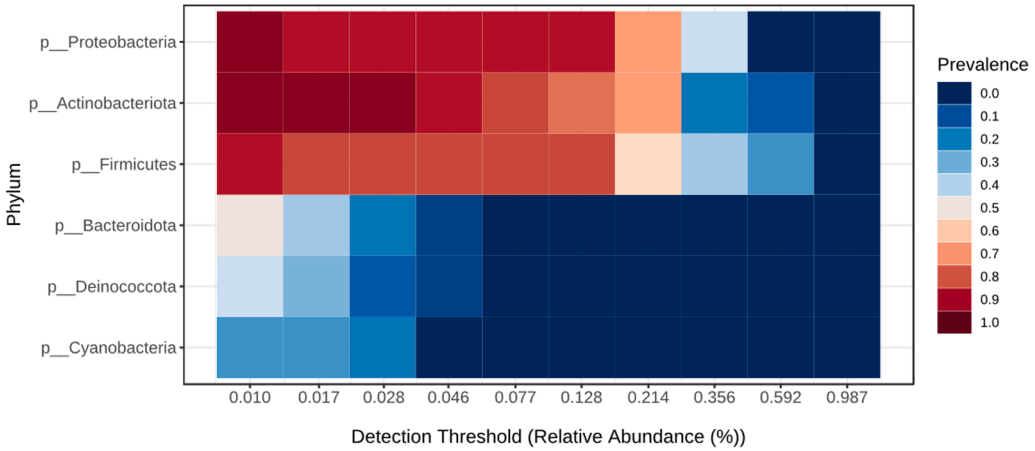

Fig. 5. Results for core microbiome showing core bacteria members for indoor air across all 16 dust samples for phylum level.

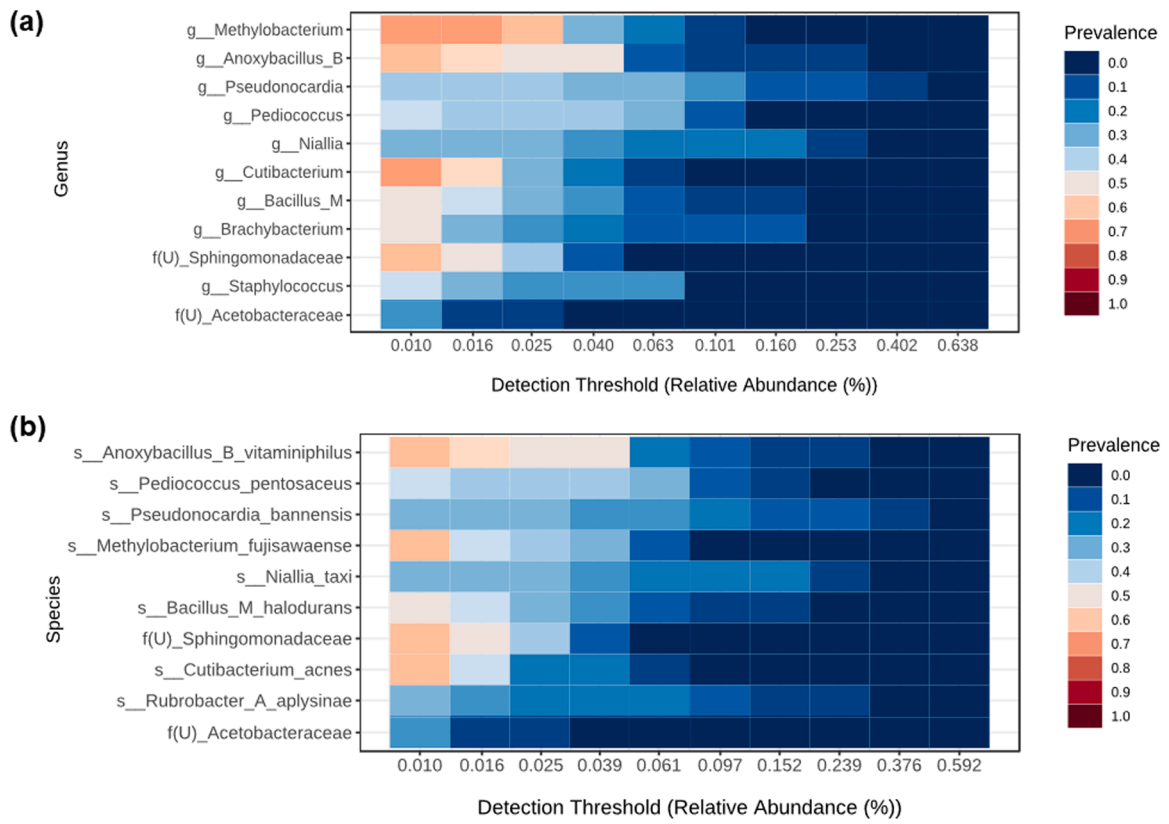

Fig. 6. Results for core microbiome showing core bacteria members for indoor air across all 16 dust samples for (a) genus and (b) species level.

within the healthcare setting may be dangerous, especially for cancer patients and those undertaking immunosuppressive therapy. Besides, bacteria *Anoxybacillus B vitaminiphilus* are motile, anaerobic spore-forming, intermediate thermophilic bacteria (Zhang et al., 2013). The finding of these anaerobic bacteria shows the advantage of using culture-independent methods in analyzing bacteria, as most culture-dependent methods use a procedure that incubates agar plates with aerobic conditions. Thus, leaving the anaerobic bacteria, especially the obligatory anaerobic bacteria, unidentified. Table 1

Interesting findings were discovered after further analyzing the composition of bacterial genera between clinic and ward areas, where significant differences and unique bacterial community compositions were observed between these two areas. *Niallia spp.*, *Methyloversatilis spp.*, *Rummeliibacillus spp.*, and *Clostridium spp.,* were more abundant within clinic areas while *Pseudonocardia spp.*, *Rubrobacter A spp.*, and *Brachybacterium spp.,* were more prevalent within the ward area. Both *Niallia spp*., and *Clostridium spp.,* belongs to the Firmicutes phyla (Gupta et al., 2020) while *Methyloversatilis spp.,* and *Rummeeliibacillus spp.,* are from phyla Proteobacteria and Bacillota respectively. Most of these bacterial genera originated from soil and water, which suggests that the bacteria that dominated the air space at sampling locations come from the environment. This can be due to the clinic and ward setting area being open to the outpatients, visitors, and healthcare practitioners, suggesting influences from human traffic moving in and out of the locations and bringing inside the environmental microbes from their clothes (Yousefzadeh et al., 2022). This suggests that dust can act as a reservoir for many environmental microbes. This would explain the higher presence of environmental microbes within the indoor air of hospitals rather than human-associated microbes or human flora. Besides, this finding also brings a new insight as most of the studies conducted in the indoor air found bacterial genera that dominated those environments to be closely related to human flora (Hanson et al., 2016; Yousefzadeh et al., 2022). A summary of the bacteria most identified in both clinics and wards and their pathogenicity was presented in Table 2.

Table 1
Table alpha-diversity results using different diversity indices.

Diversity Indices	P-value
Observed feature index	0.0398
Chao 1	0.0398
Shannon diversity index	0.6681

The presence of *Rubrobacter spp.,* in the ward is worrying as some species like *Rubrobacter radiotolerans* RSPS-4 are extremely resistant to gamma and UV radiation (Egas et al., 2014). This resilience could potentially allow these bacteria to survive and persist in critical areas like operation theater (OT) and Intensive Care Unit (ICU) wards where other bacteria might be eradicated, posing a risk to both patients and healthcare workers. Thus, thorough identification and characterization of the *Rubrobacter* spp. present is crucial to mitigate the potential threat of nosocomial infections and ensure the safety and sterility of the ward.

In addition, these bacteria have not yet been reported in other studies on indoor air in schools, houses, and hospitals. Studies that focus on bacteria exposure do not mention *Niallia spp., Methyloversatilis spp*., *and Rummeeliibacillus spp*. This suggests that this culture-independent method can profile wider bacteria compared to the culture-dependent method of sampling and analysis. These findings were expected as by utilizing different methods, different conclusions may be made. However, this finding suggests that culture-independent methods can profile more bacteria. Further research should be conducted in the future to compare both methods at the same locations and at the same time as the bacterial community may be varies by the factors of temporal and seasonal variations, time of sampling, and building occupants (Núñez and García, 2023; Naumova and Kabilov, 2022).

The B, C, and D wards had the most diverse bacterial community as compared to clinics, suggesting high humans traffic, including patients,

Table 2
Summary of the bacteria most commonly isolated in clinics and wards and their pathogenicity.

Identified bacteria	Location		Origin		Pathogenicity
	Wards	Clinics	Environment	Humans	
Core microbiome					
Cutibacterium acnes	√	√		√	Opportunistic pathogen (Salar-Vidal et al., 2021) (Cros et al., 2023)
Sphingomonas spp.	√	√	√		Opportunistic pathogen (Rohilla et al., 2021)
Methylobacterium fujisawaense	√	√	√		Opportunistic pathogen (Kovaleva et al., 2014)
Anoxybacillus B vitaminiphilus	√	√	√		Non-pathogenic (Zhang et al., 2013)
Top 3 high abundance of bacteria isolated in different areas					
Niallia taxi		√	√		Opportunistic pathogen (Hossain et al., 2023)
Methyloversatilis universalis		√	√		Non- pathogenic (Oren, 2014)
Pseudonocardia bannensis	√		√		Opportunistic pathogen (Riahi et al., 2022)
Rubrobacter A aplysinae	√				Non-pathogenic (Kämpfer et al., 2014)
Unclassified *Rummelibacillus*		√	√		Non-pathogenic (Lee et al., 2023)
Unclassified *Brachybacterium*	√		√		Opportunistic pathogen (Tak et al., 2018)

visitors, healthcare workers, and caregivers, as well as medical students, into the wards. Clinics receive less human traffic as most of the cases are scheduled by appointment, and there are few walk-in cases. Furthermore, the arrangement and sanitation for maintaining the cleanliness in the clinics are frequently and regularly made while in the wards, due to the high number of people contributed to the sanitation and cleanliness process less effective (Yousefzadeh et al., 2022). Besides, the clinics were operated during office hours, unlike wards that operated for 24 hours, thus lowering the effectiveness of cleaning frequency.

5. Conclusions

This study suggests a new sampling technique, which is collecting dust to successfully profile the bacterial community in the indoor air by performing DNA extraction and next-generation sequencing. The bacterial phyla Actinobacteria, Firmicutes, and Proteobacteria dominated the collected dust from the ventilation system of healthcare settings. New bacterial species were found by using this technique suggesting an improvement in using the culture-independent method as compared to the culture- dependent method in terms of sampling and analysis. In addition, significant differences were found in bacterial composition between clinics and wards, with a high divergence of bacteria found in wards.

Funding

This work was supported by the National Institute of Occupational Safety and Health (NIOSH) Malaysia with grant number 03.16/03/HEALTHCARE (E)/2022/01- RDU 220703 and the University Malaysia Pahang al- Sultan Abdullah (UMPSA) with grant Postgraduate Research Scheme (PGRS 230369).

CRediT authorship contribution statement

Nor Husna Mat Hussin: Writing – review & editing, Writing – original draft, Investigation. **Darren Dean Tay:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Ummu Afeera Zainulabid:** Writing – review & editing, Writing – original draft, Data curation. **Mohd Norhafsam Maghpor:** Writing – review & editing, Writing – original draft, Funding acquisition. **Hajar Fauzan Ahmad:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used QuillBot in order to utilize Grammar Checker features. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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.

Data Availability

The amplicon sequencing data was deposited at NCBI under bioproject PRJNA1042893, biosample accession number SAMN38324353 - SAMN38324368 and SRA accession number SRR26926907 - SRR26926892.

Acknowledgments

We would like to thank for those who contributed in this study.

Supplementary information

The abundance table for the features at the species level can be found under Supplementary Table.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2024.100163.

References

Horve, P.F., Lloyd, S., Mhuireach, G.A., Dietz, L., Fretz, M., MacCrone, G., et al., 2020. Building upon current knowledge and techniques of indoor microbiology to construct the next era of theory into microorganisms, health, and the built environment (Available from:). J. Expo. Sci. Environ. Epidemiol. 30 (2), 219–235. https://doi.org/10.1038/s41370-019-0157-y.

Carrazana, E., Ruiz-Gil, T., Fujiyoshi, S., Tanaka, D., Noda, J., Maruyama, F., et al., 2023. Potential airborne human pathogens: a relevant inhabitant in built environments but not considered in indoor air quality standards (Available from:). Sci. Total Environ. 901 (April), 165879. https://doi.org/10.1016/j.scitotenv.2023.165879.

Sibanda, T., Selvarajan, R., Ogola, H.J., Obieze, C.C., Tekere, M., 2021. Distribution and comparison of bacterial communities in HVAC systems of two university buildings: Implications for indoor air quality and public health. Environ. Monit. Assess. 193 (1).

Prussin, A.J., Marr, L.C., 2015. Sources of airborne microorganisms in the built environment. Microbiome 78, 3. Available from: ⟨https://www.scopus.com/inward/record.uri?eid=2-s2.0-85011108629&doi=10.1186%2Fs40168-015-0144-z&partnerID=40&md5=e53b79abc593f2f3bb5f0c85166bd9f1⟩.

Li, Y., Liao, H., Yao, H., 2019. Prevalence of antibiotic resistance genes in air-conditioning systems in hospitals, farms, and residences. Int J. Environ. Res Public Health 16 (5).

Sebastian, R., Gopalakrishnan, G., Sanil Kumar, P., Prasanth, L., 2022. An Epidemiological study of nosocomial infection in post-operative patients. Int J. Life Sci. Pharma Res. 12 (2), 5–9.

Baviskar, A.S., Khatib, K.I., Rajpal, D., Dongare, H.C., 2019. Nosocomial infections in surgical intensive care unit: a retrospective single-center study. Int J. Crit. Illn. Inj. Sci. 9 (1), 16–20.
Zainulabid, U.A., Kamarudin, N., Rus, R.M., Soffian, S.N., Yassim, A.S.M., How, S.H., et al., 2022. Hybrid de novo whole-genome assembly and annotation of SARS-CoV-2 virus from nosocomial infection in Pahang, Malaysia. Int J. Infect. Dis. 116, S19.
Hassan N.L., Yern K.K., Azimah N., Zain M. Isolation of Antibiotic Resistant Bacteria from Rivers in. 2020;3(2):291–307.
Sarah Fatihah Tamsi, N., Talib Latif, M., Othman, M., Diba Abu Bakar, F., Mohd Yusof, H., Mohd Razif Noraini, N., et al., 2022. Antibiotic resistance of airborne bacterial populations in a hospital environment. lowing Ind. Code Pract. Indoor Air 194, 629.
Adhikari, A., Kurella, S., Banerjee, P., Mitra, A., 2017. Aerosolized bacteria and microbial activity in dental clinics during cleaning procedures. J. Aerosol Sci. 114, 209–218. Available from: ⟨https://www.scopus.com/inward/record.uri?eid=2-s2.0-85029827590&doi=10.1016%2Fj.jaerosci.2017.09.019&partnerID=40&md5=7cee697cb076ce27eeb7504b793a2d09⟩.
Haig, C.W., Mackay, W.G., Walker, J.T., Williams, C., 2016. Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies (Available from:). J. Hosp. Infect. 93 (3), 242–255. https://doi.org/10.1016/j.jhin.2016.03.017.
Cox, J., Mbareche, H., Lindsley, W.G., Duchaine, C., 2020a. Field sampling of indoor bioaerosols (Available from:). Aerosol Sci. Technol. 54 (5), 572–584. https://doi.org/10.1080/02786826.2019.1688759.
Mat-Hussin, N.H., Siew, S.W., Maghpor, M.N., Gan, H.M., Ahmad, H.F., 2024. Method for detection of pathogenic bacteria from indoor air microbiome samples using high-throughput amplicon sequencing. Methods 12, 102636. Available from: ⟨https://www.sciencedirect.com/science/article/pii/S2215016124000906⟩.
Boers, S.A., Jansen, R., Hays, J.P., 2019. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. Eur. J. Clin. Microbiol Infect. Dis. 38 (6), 1059–1070.
Mucci Gianluca Gianfranceschi Cristina Cianfanelli Saverio Santucci Vincenzo Romano Spica Federica Valeriani N. Can air microbiota be a novel marker for public health? A sampling model and preliminary data from different environments. Aerobiologia (Bologna) [Internet]. 36. Available from: https://doi.org/10.1007/s10453-019-09581-3.
Božić, J., Ilić, P., Ilić, S., 2019. Indoor air quality in the hospital: The influence of heating, ventilating and conditioning systems. Braz. Arch. Biol. Technol. 62.
Kwan, S.E., Shaughnessy, R., Haverinen-Shaughnessy, U., Kwan, T.A., Peccia, J., 2020. The impact of ventilation rate on the fungal and bacterial ecology of home indoor air (Available from:). Build. Environ. 177 (January), 106800. https://doi.org/10.1016/j.buildenv.2020.106800.
Bonadonna, L., Briancesco, R., Coccia, A.M., Meloni, P., Rosa, G.La, Moscato, U., 2021 Jun. Microbial air quality in healthcare facilities. Int J. Environ. Res Public Health 18 (12).
Cox, J., Mbareche, H., Lindsley, W.G., Duchaine, C., Cox, J., Mbareche, H., et al., 2020b. Field sampling of indoor bioaerosols (Available from:). Aerosol Sci. Technol. 54 (5), 572–584. https://doi.org/10.1080/02786826.2019.1688759.
Villanueva, F., Ródenas, M., Ruus, A., Saffell, J., Gabriel, M.F., 2022. Sampling and analysis techniques for inorganic air pollutants in indoor air (Available from:). Appl. Spectrosc. Rev. 57 (7), 531–579. https://doi.org/10.1080/05704928.2021.2020807.
Efthymiopoulos S., Aktas Y.D., Altamirano H. Air sampling and analysis of indoor fungi: a critical review of passive (non- activated) and active (activated) sampling. 2021; (June):28–9.
Hanson, B., Zhou, Y., Bautista, E.J., Urch, B., Speck, M., Silverman, F., et al., 2016. Characterization of the bacterial and fungal microbiome in indoor dust and outdoor air samples: a pilot study (Available from:). Environ. Sci. Process Impacts 18 (6), 713–724. https://doi.org/10.1039/C5EM00639B.
Sun, Y., Fu, X., Li, Y., Yuan, Q., Ou, Z., Lindgren, T., et al., 2020. Shotgun metagenomics of dust microbiome from flight deck and cabin in civil aviation aircraft. Indoor Air 30 (6), 1199–1212. Available from: ⟨https://www.scopus.com/inward/record.uri?eid=2-s2.0-85087741331&doi=10.1111%2Fina.12707&partnerID=40&md5=6d9ba7e669dc9cfc809d641792e231fc⟩.
Mucci, N., Gianfranceschi, G., Cianfanelli, C., Santucci, S., Romano Spica, V., Valeriani, F., 2020. Can air microbiota be a novel marker for public health? A sampling model and preliminary data from different environments (Available from:). Aerobiology 36 (1), 71–75. https://doi.org/10.1007/s10453-019-09581-3.
Hasain, Z., Raja Ali, R.A., Ahmad, H.F., Abdul Rauf, U.F., Oon, S.F., Mokhtar, N.M., 2022. The roles of probiotics in the gut microbiota composition and metabolic outcomes in asymptomatic post-gestational diabetes women: a randomized controlled trial. Nutrients Vol. 14.
Ayob, N., Muhammad Nawawi, K.N., Mohamad Nor, M.H., Raja Ali, R.A., Ahmad, H.F., Oon, S.F., et al., 2023. The effects of probiotics on small intestinal microbiota composition, inflammatory cytokines and intestinal permeability in patients with non-alcoholic fatty liver disease. Biomedicines Vol. 11.
Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor (Available from:). Bioinforma. [Internet] 34 (17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17 (1), 10.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data (Available from:). Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.
Fakunle, A.G., Jafta, N., Bossers, A., Wouters, I.M., Kersen, W. van, Naidoo, R.N., et al., 2023. Childhood lower respiratory tract infections linked to residential airborne bacterial and fungal microbiota (Available from:). Environ. Res [Internet] 231 (P1), 116063. https://doi.org/10.1016/j.envres.2023.116063.
Chandika, A.B., Mkala, R.S., Lugoba, B., Kipilipili, B.C., Saitot, W., Kamkunguru, C.E., et al., 2021. Bacterial contaminants on exposed surfaces and their antibiotic sensitivity patterns at the Benjamin Mkapa Hospital, Dodoma-Tanzania. Asian J Res. Infect. Dis. 7 (1), 1–11.
Fu, X., Norbäck, D., Yuan, Q., Li, Y., Zhu, X., Hashim, J.H., et al., 2020. Indoor microbiome, environmental characteristics and asthma among junior high school students in Johor Bahru, Malaysia. Environ. Int 138 (December 2019).
Fu, X., Ou, Z., Zhang, M., Meng, Y., Li, Y., Chen, Q., et al., 2021. Classroom microbiome, functional pathways and sick-building syndrome (SBS) in urban and rural schools - potential roles of indoor microbial amino acids and vitamin metabolites. Sci. Total Environ. 795. Available from: ⟨https://www.scopus.com/inward/record.uri?eid=2-s2.0-85109475330&doi=10.1016%2Fj.scitotenv.2021.148879&partnerID=40&md5=b4ca40f672efbe860657b79ce51424cc⟩.
Kirjavainen, P.V., Karvonen, A.M., Adams, R.I., Täubel, M., Roponen, M., Tuoresmäki, P., et al., 2019. Farm-like indoor microbiota in non-farm homes protects children from asthma development (Available from:). Nat. Med. 25 (7), 1089–1095. https://doi.org/10.1038/s41591-019-0469-4.
Salonen, H., Salthammer, T., Morawska, L., 2020. Human exposure to air contaminants in sports environments. Indoor Air 30 (6), 1109–1129.
Matys, J., Kensy, J., Gedrange, T., Zawiślak, I., Grzech-Leśniak, K., Dobrzyński, M., 2024. A molecular approach for detecting bacteria and fungi in healthcare environment aerosols: a systematic review. Int J. Mol. Sci. 25 (8), 4154.
Rozaidi, S.W., Sukro, J., Dan, A., 2001. The incidence of nosocomial infection in the Intensive Care Unit, Hospital Universiti Kebangsaan Malaysia: ICU-acquired nosocomial infection surveillance program 1998-1999. Med J. Malays. [Internet] 56 (2), 207–222. Available from: ⟨https://europepmc.org/article/med/11771082⟩.
Fu, X., Meng, Y., Li, Y., Zhu, X., Yuan, Q., Ma'pol, A., et al., 2022. Associations between species-level indoor microbiome, environmental characteristics, and asthma in junior high schools of Terengganu, Malaysia. Air Qual. Atmos. Heal 15 (6), 1043–1055.
Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A., Roques, C., 2018. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: a brief look at the latest updates. J. Eur. Acad. Dermatol. Venereol. 32, 5–14.
Salar-Vidal, L., Achermann, Y., Aguilera-Correa, J.J., Poehlein, A., Esteban, J., Brüggemann, H., 2021. Genomic analysis of cutibacterium acnes strains isolated from prosthetic joint infections. Microorganisms 9 (7), 1–10.
Abdullah, H.M., Waqas, Q., Abdalla, A., Omar, M., Berger, P., 2021. Cutibacterium acnes pneumonia in an immunocompromised patient: a case report and review of the literature. South Dak. Med.: J. South Dak. State Med. Assoc. U. S. Vol. 74, 523–526.
Gao, X.L., Shao, M.F., Wang, Q., Wang, L.T., Fang, W.Y., Ouyang, F., et al., 2018. Airborne microbial communities in the atmospheric environment of urban hospitals in China. J. Hazard Mater. 349 (January), 10–17.
Fanci, R., Corti, G., Bartoloni, A., Tortoli, E., Mariottini, A., Pecile, P., 2010. Unusual *Methylobacterium fujisawaense* Infection in a Patient with *Acute Leukaemia* Undergoing Hematopoietic Stem Cell Transplantation: First Case Report (Available from:). Case Rep. Med. 2010, 313514. https://doi.org/10.1155/2010/313514.
Zhang, X.Q., Zhang, Z.L., Wu, N., Zhu, X.F., Wu, M., 2013. Anoxybacillus vitaminiphilus sp. nov., a strictly aerobic and moderately thermophilic bacterium isolated from a hot spring. Int J. Syst. Evol. Microbiol 63 (PART 11), 4064–4071.
Gupta, R.S., Patel, S., Saini, N., Chen, S., 2020. Robust demarcation of 17 distinct bacillus species clades, proposed as novel bacillaceae genera, by phylogenomics and comparative genomic analyses: Description of robertmurraya kyonggiensis sp. nov. and proposal for an emended genus bacillus limiting it o. Int J. Syst. Evol. Microbiol. 70 (11), 5753–5798.
Yousefzadeh, A., Maleki, A., Athar, S.D., Darvishi, E., Ahmadi, M., Mohammadi, E., et al., 2022. Evaluation of bio-aerosols type, density, and modeling of dispersion in inside and outside of different wards of educational hospital (Available from:). Environ. Sci. Pollut. Res. 29 (10), 14143–14157. https://doi.org/10.1007/s11356-021-16733-x.
Cros, M.P., Mir-Pedrol, J., Toloza, L., Knödlseder, N., Maruotti, J., Zouboulis, C.C., et al., 2023. New insights into the role of Cutibacterium acnes-derived extracellular vesicles in inflammatory skin disorders (Available from:). Sci. Rep. 13 (1), 16058. https://doi.org/10.1038/s41598-023-43354-w.
Rohilla, R., Raina, D., Singh, M., Pandita, A.K., Patwal, S., 2021. Evaluation of Sphingomonas paucimobilis as an emerging nosocomial pathogen in a teaching hospital in Uttarakhand. Iran. J. Microbiol. 13 (5), 617–623.
Kovaleva, J., Degener, J.E., van der Mei, H.C., 2014. Methylobacterium and its role in health care-associated infection. J. Clin. Microbiol 52 (5), 1317–1321.
Hossain, M.I., Saleh, N.U.A., Numan, A., Hossain, M.M., Uddin, M.A., Hossain, M.S., 2023. Bombyx mori as a model for Niallia circulans pathogenicity. Drug Discov. Ther. 17 (1), 18–25.
Oren, A., 2014. The Family Rhodocyclaceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes: Alphaproteobacteria and Betaproteobacteria [Internet]. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 975–998. https://doi.org/10.1007/978-3-642-30197-1_292.
Riahi, H.S., Heidarieh, P., Fatahi-Bafghi, M., 2022. Genus Pseudonocardia: What we know about its biological properties, abilities and current application in biotechnology. J. Appl. Microbiol. 132 (2), 890–906. Available from: ⟨https://ami-journals.onlinelibrary.wiley.com/doi/abs/10.1111/jam.15271⟩.
Kämpfer, P., Glaeser, S.P., Busse, H.-J., Abdelmohsen, U.R., Hentschel, U., 2014. Rubrobacter aplysinae sp. nov., isolated from the marine sponge Aplysina aerophoba. Int J. Syst. Evol. Microbiol. 64 (Pt 3), 705–709.

Lee, S.-J., Kim, S.H., Noh, D.-I., Lee, Y.-S., Kim, T.-R., Hasan, M.T., et al., 2023. Combination of host-associated rummeliibacillus sp. and microbacterium sp. positively modulated the growth, feed utilization, and intestinal microbial population of olive flounder (Paralichthys olivaceus). Biology 12 (11).

Tak, E.J., Kim, P.S., Hyun, D.-W., Kim, H.S., Lee, J.-Y., Kang, W., et al., 2018. Phenotypic and genomic properties of brachybacterium vulturis sp. nov. and Brachybacterium avium sp. nov. Front Microbiol [Internet] 9. Available from: ⟨https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.01809⟩.

Egas, C., Barroso, C., Froufe, H., Pacheco, J., Albuquerque, L., da Costa, M., 2014. Complete genome sequence of the Radiation-Resistant bacterium Rubrobacter radiotolerans RSPS-4. Stand Genom. Sci. 9 (3).

Núñez, A., García, A.M., 2023. The aerobiome in a hospital environment: Characterization, seasonal tendencies and the effect of window opening ventilation. Build. Environ. 230 (January).

Naumova N.B., Kabilov M.R. About the Biodiversity of the Air Microbiome. 2022;14(55): 50–56..