MethodsX 12 (2024) 102636

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/methodsx

Method for detection of pathogenic bacteria from indoor air microbiome samples using high-throughput amplicon sequencing

Nor Husna Mat-Hussin[a], Shing Wei Siew[a], Mohd Norhafsam Maghpor[b], Han Ming Gan[c], Hajar Fauzan Ahmad[a,d,*]

[a] *Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, Kuantan, Pahang 26300, Malaysia*
[b] *Laboratory Division, Consultation and Research Department, National Institute of Occupational Safety and Health (NIOSH), Seksyen 15, Bandar Baru Bangi, Selangor 43650, Malaysia*
[c] *Patriot Biotech Sdn. Bhd., Bandar Sunway, Subang Jaya, Selangor 47500, Malaysia*
[d] *Group of Environment, Microbiology and Bioprocessing (GERMS), Universiti Malaysia Pahang, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, Kuantan, Pahang 26300, Malaysia*

ARTICLE INFO

Method name:
Detection of pathogens from indoor air microbiome

Keywords:
Indoor air microbiome
Pathogens
Culture-independent method
Next-generation sequencing

ABSTRACT

The exposure of the air microbiome in indoor air posed a detrimental health effect to the building occupants compared to the outdoor air. Indoor air in hospitals has been identified as a reservoir for various pathogenic microbes. The conventional culture-dependent method has been widely used to access the microbial community in the air. However, it has limited capability in enumerating the complex air microbiome communities, as some of the air microbiomes are uncultivable, slow-growers, and require specific media for cultivation. Here, we utilized a culture-independent method *via* amplicon sequencing to target the V3 region of 16S rRNA from the pool of total genomic DNA extracted from the dust samples taken from hospital interiors. This method will help occupational health practitioners, researchers, and health authorities to efficiently and comprehensively monitor the presence of harmful air microbiome thus take appropriate action in controlling and minimizing the health risks to the hospital occupants. Key features;

- Culture-independent methods offer fast, comprehensive, and unbias profiles of pathogenic and non-pathogenic bacteria from the air microbiomes.
- Unlike the culture-dependent method, amplicon sequencing allows bacteria identification to the lowest taxonomy levels.

* Corresponding author at: Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang, Kuantan, Pahang 26300, Malaysia.
E-mail address: fauzanahmad@ump.edu.my (H.F. Ahmad).
Social media: (H.F. Ahmad)

https://doi.org/10.1016/j.mex.2024.102636
Available online 27 February 2024

Specifications table

Subject area:	Immunology and Microbiology
More specific subject area:	Molecular Identification of Indoor Air Microbiome
Name of your method:	Detection of pathogens from indoor air microbiome
Name and reference of original method:	Siew et al., Gut microbiome and metabolome of sea cucumber (Stichopus ocellatus) as putative markers for monitoring the marine sediment pollution in Pahang, Malaysia. Marine Pollution Bulletin, (2022) [1]. Siew et al., Evaluation of pre-treated healthcare wastes during COVID-19 pandemic reveals pathogenic microbiota, antibiotics residues, and antibiotic resistance genes against beta-lactams. Environmental Research, (2022) [2].
Resource availability:	*NCBI SRA data* *Equipment: Illumina NovaSEQ6000 (2 × 150 bp configuration)* *Software: QIIME 2 and MicrobiomeAnalyst 2.0*

Method details

Background

Although SARS-CoV-2 has had a profound impact on the whole world [3–5], it has raised awareness that microbes, even though small, exist everywhere in the environment without us realizing it, including in the air. After the SARS-CoV-2 pandemic, significant attention has been drawn to the exposure of microbes in the indoor air of a building as people spent more than 90 % of their time indoors [6]. Health authorities urge extra attention to microbiome exposure in the indoor air of hospitals as hospital may serve as a reservoir for various pathogenic bacteria [4,7]. Patients especially those undergoing treatment for cancer, leukemia or receiving bone marrow transplants in a hospital are particularly more susceptible to infection than the general public [8]. Indoor air in hospitals can become a primary source for the dissemination of various microbes through human activities such as coughing, sneezing, and contaminated surfaces. The potential for contracting a microbial pathogen is high within the hospital environment [9]. Therefore, monitoring and profiling of pathogenic bacteria in the indoor air of hospitals are extremely important to reduce the risk of infection in patients, minimize the risk factor to healthcare practitioners and improve the efficacy of the healthcare system.

Traditionally, culture-dependent method has been used for sample collection, isolation, and identification of bacteria in the indoor air of hospitals [10–12]. However, this method had a few drawbacks, including require a large volume of air to be sampled for a period of time [13] laborious as it requires many media preparations and different types of culture media for bacteria cultivation and isolation, take longer time to obtain result as bacteria need to be cultivated and incubated prior to analysis, and accuracy of the results can be doubted because some bacteria present in indoor air could not be cultivated [14].

With the advancement of DNA sequencing technology, investigations of both known and unknown bacterial species, as well as culturable and unculturable organisms from air samples, can be done with speed and precision without the necessity of obtaining bacterial pure-culture [13,15,16] Thus, in this study a culture- independent method was applied to identify and profile bacteria from the indoor air of the hospital building using next-generation sequencing technology.

Materials, reagents and solutions

Autoclave (Hirayama HVE-50), biosafety cabinet class II,(ESCO) Eppendorf 5331 MasterCycle Gradient Thermal Cycler (Eppendorf, Germany), PCR tubes, 50 ml and 25 ml microcentrifuge tubes, tubes rack, gel electrophoresis system (MBE-LID-Y1), conical flask 250 ml, QIAamp PowerFecal Pro-DNA Kit (Qiagen, Germany), micropipette (1000 ul and 10 ul) and pipette tips, agarose powder, Tris-acetate- EDTA (TAE) buffer, 75 % ethanol, Phosphate Buffer Saline (PBS), 6x loading dye.

1. *Sample collection and processing*

About 50–70 mg of dust samples were collected from 19 supplies and return vents of air handling unit (AHU) room and 16 air conditioning units located inside the clinics and wards using sterile swab and put into sterile 15 mL collection tubes. The AHU is the core components of air conditioning systems that filters the outside air entering into the hospital buildings and keep it well ventilated with appropriate temperature and relative humidity before secondary filter and return it back to the outside [17]. All samples were kept at room temperature and brought back to the lab for bacterial DNA extraction.

2. *Procedure for isolation of DNA*

Bacterial DNA was extracted from the dust samples following the procedure manual supplied by the manufacturer of QIAamp PowerFecal DNA Kit with some modifications. Firstly, dust samples were soaked in 1 ml PBS for 10 min prior to homogenization in a 2 ml bead beating tube. These beads facilitate the lysis of microbial cells by increasing physical surface areas. The remaining procedures were followed the manufacturer guidelines and illustrated in the flowchart below (Fig. 1).

3. *Determination of quality and quantity of isolated DNA*

The quality and quantity of extracted DNA were examined using gel electrophoresis and Qubit 4 Fluorometer (Thermo Fisher Scientific, Invitrogen, USA), respectively, based on manufacturer's guidelines.

4. *Library preparation using PCR amplification and sequencing*

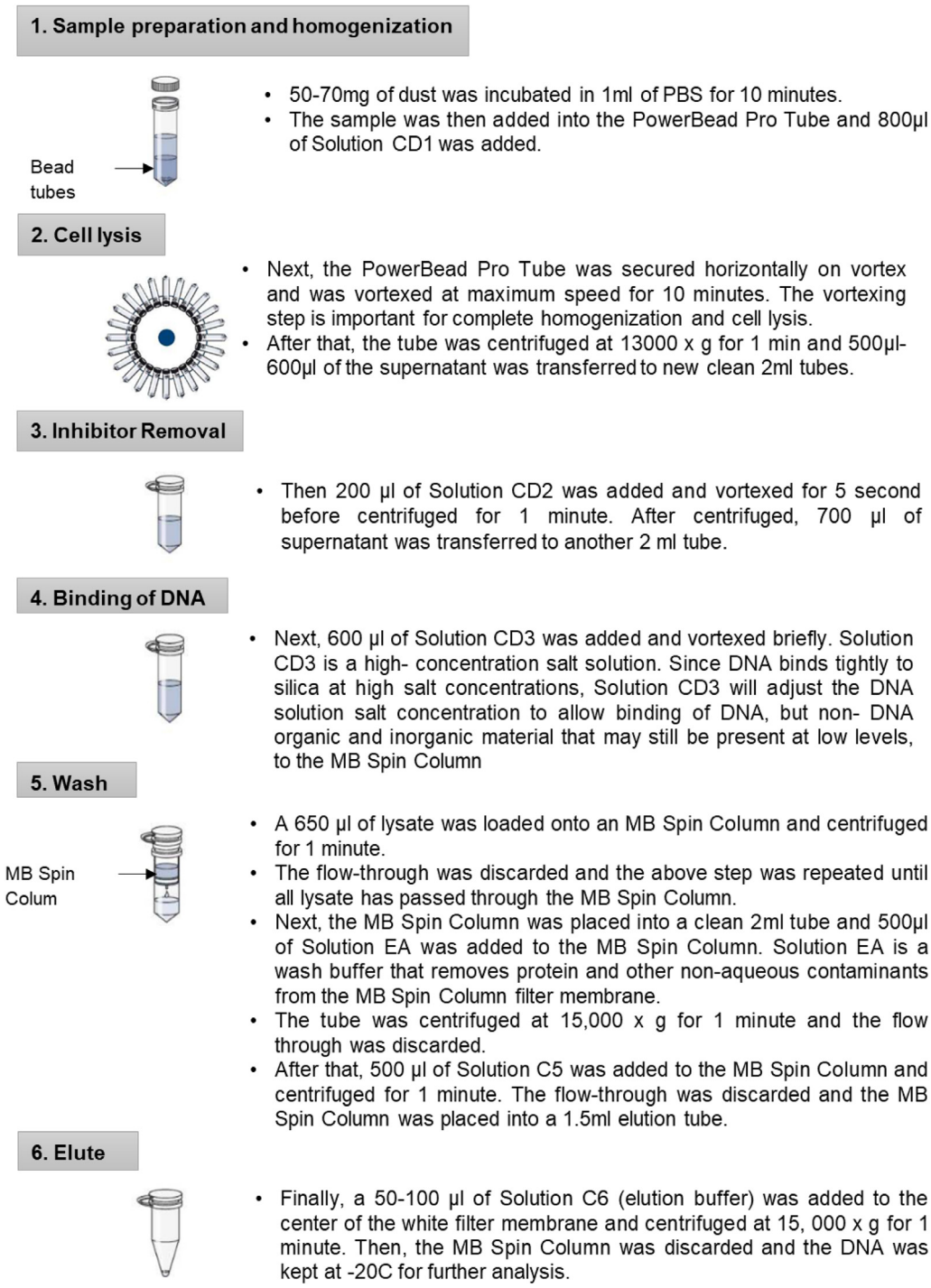

Fig. 1. Procedures for DNA isolation.

The DNA library was prepared through polymerase chain reaction (PCR) amplification by targeting the 16S rRNA V3 region using the primers 341F:CCTACGGGNGGCWGCAG and 518R:ATTACCGCGGCTGCTGG [18,19]. An additional 5 bases of inline barcode were incorporated at the 5′ end of the primers to enable inline barcoding [20]. The PCR reaction mixture was prepared in sterile PCR tubes as described in Table 1 in biosafety cabinet to ensure no cross contaminations that may affect the result. The PCR amplification was the carried out using the Eppendorf 5331 MasterCycle Gradient Thermal Cycler (Eppendorf, Germany) in accordance with the PCR conditions outlined in Table 2.

Table 1
Composition for PCR reaction.

Component	Volume (μL)	Final concentration
ProMega GoTaq Green Mastermix, 2X	10	1X
Forward primer, 1 μM	5	0.25 μM
Reverse primer, 1 μM	5	0.25 μM
DNA template	1	–
Total	21	–

Table 2
PCR condition for PCR reaction.

Conditions	Temperature	Time	Cycle
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	10 s	30
Annealing	50 °C	10 s	
Extension	72 °C	10 s	
Final extension	72 °C	30 s	1

Table 3
PCR condition for index PCR.

Conditions	Temperature	Time	Cycle
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	10 s	8
Annealing	55 °C	10 s	
Extension	72 °C	10 s	
Final extension	72 °C	30 s	1

Upon completion, the PCR products were visualized using 2 % agarose and purified using SPRI Bead. Following specified PCR condition (Table 3), an additional index PCR was performed on the purified product to incorporate Illumina-specific barcode and the remaining Illumina adapter sequence.

5. *Raw data and statistical analysis, and data visualization*

The barcoded amplicons were visualized on the gel and pooled based on their band intensity followed by DNA cleanup using 0.7 X (v/v) ratio of SPRI bead. The purified, pooled and barcoded amplicons were assessed using D1000 ScreenTape system (Agilent). The expected fragment size was obtained and libraries were quantified using the Promega Glomax kit. Low yield of some libraries was accounted for by creating two separate library pools, diluted and mixed with a ration of samples between them. The concentration of the final pool was determined using Qubit Fluorometric Quantification (Thermo Fisher Scientific). Amplicon libraries and including two controls, were sequenced at 2 × 150 bp configuration on an Illumina Novaseq6000 (Illumina, San Diego, CA).

Raw paired-end reads were overlapped using fastp [21] followed by primer trimming using Cutadapt v4.4 [22]. The overlapped reads were subsequently imported into QIIME2 followed by denoising and count table construction using DADA2 [23]. Subsequently, the Amplicon Sequence Variant (ASVs) were classified with qiime2-feature-classifier that has been trained on the GTDB 16S rRNA V3 region database (r207 release). Statistical analysis of microbiome data, and visualization were performed using MicrobiomeAnalyst 2.0 [24] as described in Fig. 2.

Method validation

The QIAamp PowerFecal DNA Kit (QIAGEN, Germany) was used to extract all bacterial DNA from the dust samples. Fig. 3 showed the smearing of DNA bands of extracted bacterial DNA from three different locations on gel electrophoresis: return vent from indoor air-conditioning unit in clinic, supply and return vent from AHU room. The estimated DNA sizes for F1, F2, and F3 were 14,135 bp, 9416 bp, and 9682 bp, respectively. A blank sample (no DNA template) was run over the gel to act as quality control, and no visible DNA band was found. Next, Fig. 4 showed the distinct bands of PCR product from extracted DNA. The sizes of PCR products were approximately ~ 300 bp To avoid false positive results during amplification process, the B sample was included in the PCR reaction as other sample but contain no DNA template. The absence of bands in the B in gel electrophoresis validated the result, suggesting that DNA was successfully isolated from the dust samples.

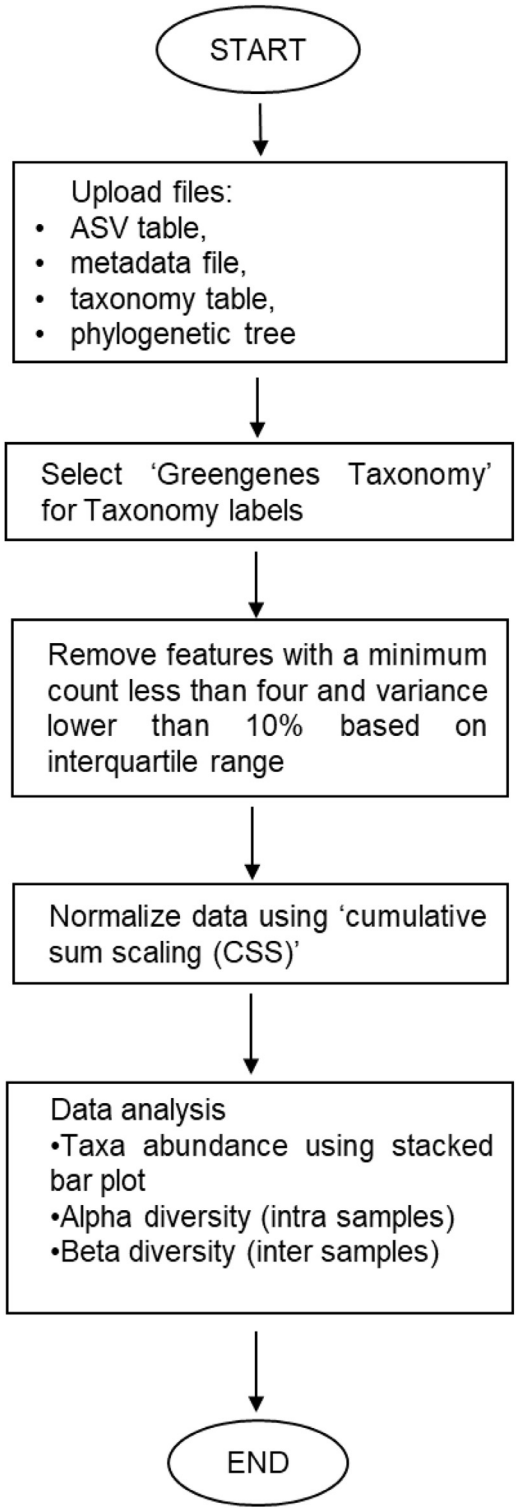

Fig. 2. Workflow for data analysis using MicrobiomeAnalyst 2.0.

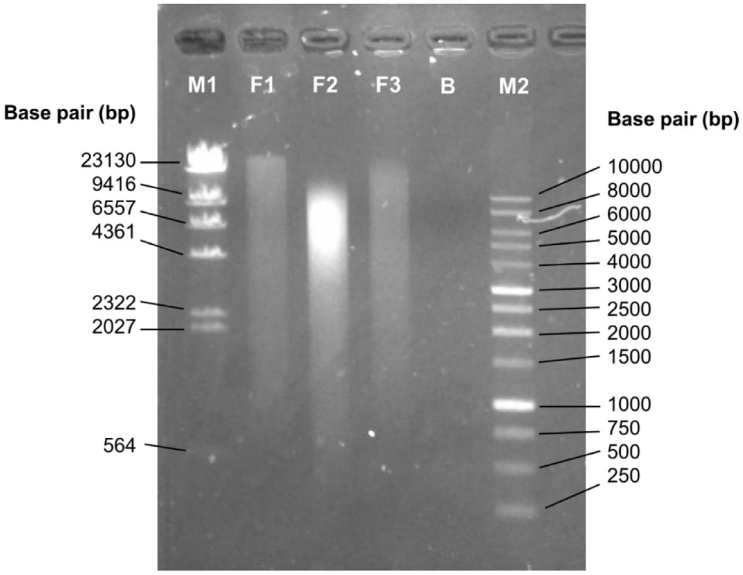

Fig. 3. Gel electrophoresis of extracted DNA from dust samples collected from supply and return vent from AHU room and indoor HVAC. M1: Lambda DNA/HindIII marker, Lane 1–3: extracted DNA, B: blank sample with no DNA template, M2: 1 kb DNA ladder.

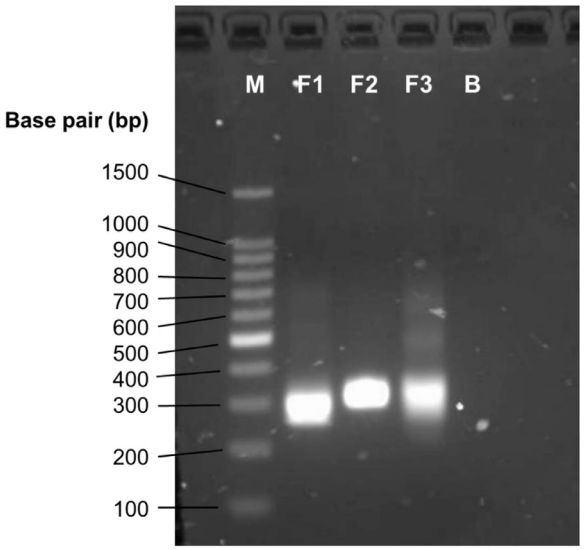

Fig. 4. Gel electrophoresis of PCR products from dust samples. M: 100 bp DNA ladder, Lane 1–3: PCR products, and B: blank sample with no DNA template.

The amplicon sequencing resulted in 1732,345 reads, and a total of 49 bacteria species were recovered from the dust collected in the hospital building. Fig. 5 illustrated the top 20 bacterial species identified in indoor air, AHU supply, and AHU return at species level. *Pseudomonas spp*. predominated the supply vent with 51.1 %, while *Rickettsia spp*. (49.9 %) and unclassified *Sphingomonadaceae* (23.3 %) predominated the return vent of AHU. The beta diversity analysis using PERMANOVA indicated insignificant differences in bacterial composition at the species level across three sets of dust samples with F-value: 1.064, R^2: 0.053 and p-value: 0.362 (Fig. 6). Next, the alpha diversity analysis using the Shannon and Observed indices revealed no significant difference in species richness and evenness within each location in collected dust samples (p-value: 0.5757, [ANOVA] F-value: 0.5604) (Fig. 7). These statistical analysis results can often be biased when using the culture-dependent method since not all bacteria are cultivable and the bacterial count is generally dominated by certain types of bacteria only. Thus, it does not represent the true bacterial population. Meanwhile, a culture-independent method allows us to profile the complete microbial community, allowing us to accurately assess the microbial diversity observed within and between the dataset or groups.

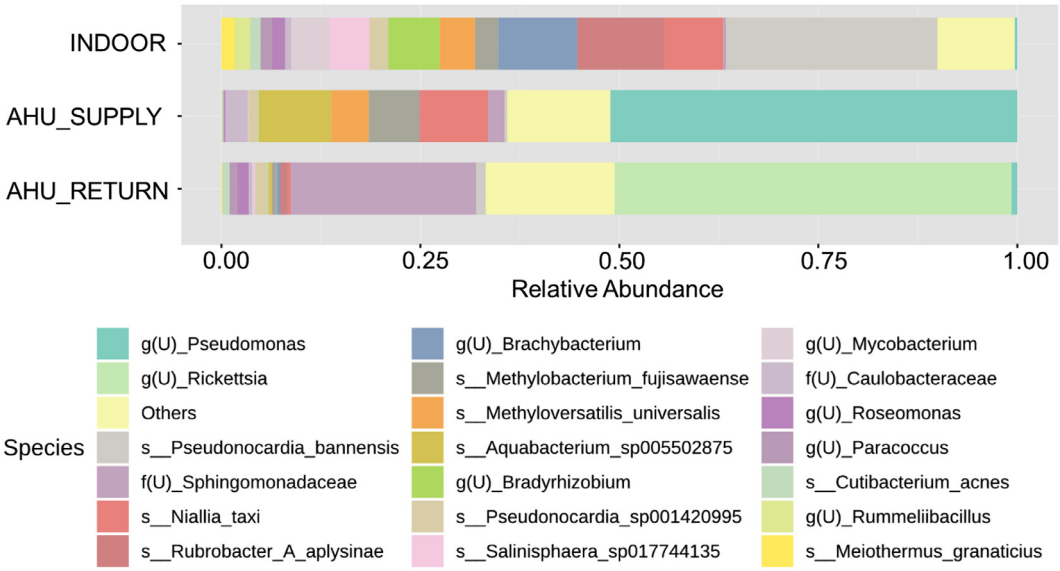

Fig. 5. Top 20 bacterial composition of indoor air, AHU supply and AHU return at species level.

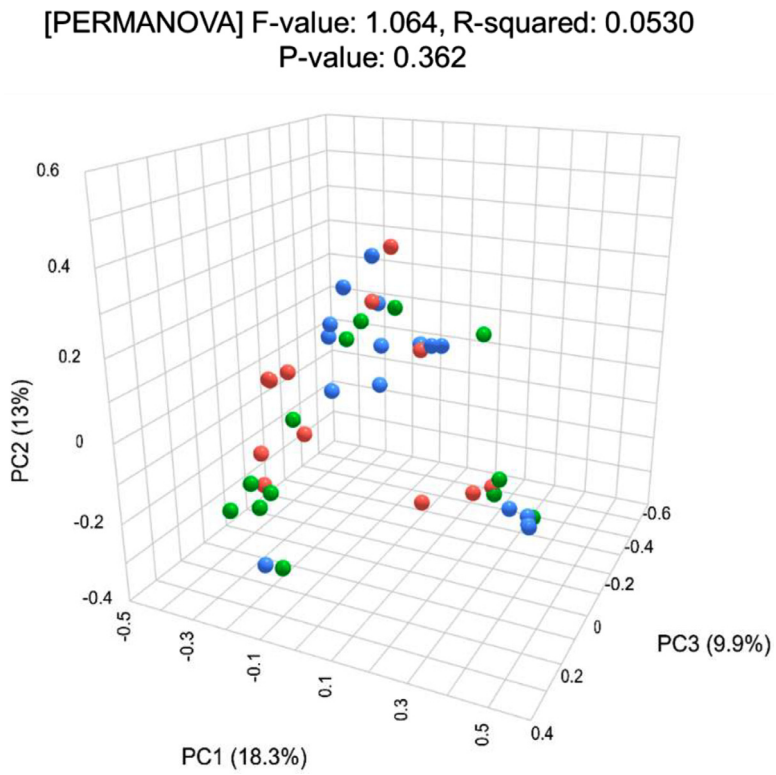

Fig. 6. The beta diversity PCoA 3D plots were constructed using Jaccard and Bray-Curtis dissimilarity index showing no significant difference between groups with a p-value> 0.05.

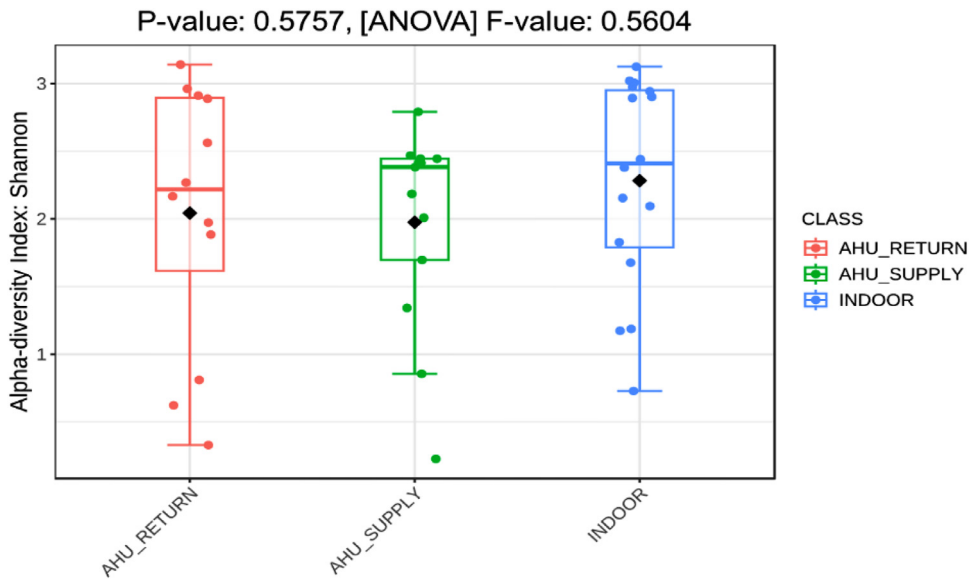

Fig. 7. The alpha diversity analysis using the Shannon diversity index showed no significant differences in bacterial composition at the species level within each location (p-value> 0.05).

Airborne microbiome	Percentage (%)	Pathogenicity
Pseudomonas spp.	17.39	Human pathogens
Rickettsia spp	16.65	Gram-negative bacteria found in ticks, mites, fleas etc
Psedonorcardia spp.	11.10	Pathogenic towards fungus
Niallia spp.	5.41	Opportunistic pathogen
Methylobacterium spp.	4.26	Non-pathogenic to human
Rubrobacter spp.	4.01	Non-pathogenic to human
Brachybacterium spp.	3.49	Potential pathogens for bloodstream infection
Methyloversatilis spp.	3.08	Plant pathogens
Aquabacterium spp.	3.07	Non-pathogenic
Massilia spp.	2.49	Human pathogens
Bradyrhizobium spp.	2.26	Non-pathogenic
Mycobacterium spp.	1.68	Human pathogens
Salinisphaera spp.	1.74	Rarely pathogenic
Roseomonas spp.	1.10	Opportunistic pathogens
Paracoccus spp.	0.82	Opportunistic human pathogens associated with blood infections
Staphylococcus spp.	0.67	Human pathogens

This work confirms the high importance for bacterial pathogens detection due to their various uses, as reported before [1,2,7,25–31].

Conclusion

In conclusion, culture-independent techniques and amplicon sequencing based on V3 provide better resolution on profiling of pathogenic and non-pathogenic airborne microbiome.

Ethics statements

Not relevant as this study did not involve human or animal samples.

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.

CRediT authorship contribution statement

Nor Husna Mat-Hussin: Data curation, Writing – original draft, Visualization, Investigation, Writing – review & editing. **Shing Wei Siew:** Software, Validation, Writing – review & editing. **Mohd Norhafsam Maghpor:** Writing – review & editing. **Han Ming Gan:** Software, Validation, Writing – review & editing. **Hajar Fauzan Ahmad:** Conceptualization, Methodology, Software, Visualization, Investigation, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Universiti Malaysia Pahang Al-Sultan Abdullah for Made-in-UMP Grant [PDU223001–1] and Post-graduat Research Grant [PGRS220382]. And the National Institute of Occupational Safety and Health (NIOSH) Malaysia [RDU220703]. We thanked Dr Ummu Afeera Zainulabid who assist in sample collection from Sultan Ahmad Shah Medical centre (SASMEC).

References

[1] S.W. Siew, M.Y. Choo, I.P.G. Marshall, H. Abd Hamid, S.S. Kamal, D.S. Nielsen, et al., Gut microbiome and metabolome of sea cucumber (Stichopus ocellatus) as putative markers for monitoring the marine sediment pollution in Pahang, Malaysia, Mar. Pollut. Bull. 182 (2022) 114022.
[2] S.W. Siew, S.M. Musa, S.N. 'Azyyati, M.F. Farida Asras, H.F Ahmad, Evaluation of pre-treated healthcare wastes during COVID-19 pandemic reveals pathogenic microbiota, antibiotics residues, and antibiotic resistance genes against beta-lactams, Environ. Res. 219 (2023) 115139 Available from: https://www.sciencedirect.com/science/article/pii/S0013935122024665.
[3] A.S. Mat Yassim, M.F.F. Asras, A.M. Gazali, M.S. Marcial-Coba, U.A. Zainulabid, H.F. Ahmad, COVID-19 outbreak in Malaysia: decoding D614G mutation of SARS-CoV-2 virus isolated from an asymptomatic case in Pahang, Mater. Today Proc. (2021).
[4] U.A. Zainulabid, A.S. Mat Yassim, M. Hussain, A. Aslam, S.N. Soffian, M.S. Mohd Ibrahim, et al., Whole genome sequence analysis showing unique SARS-CoV-2 lineages of B.1.524 and AU.2 in Malaysia, PLoS One 17 (2) (2022) e0263678.
[5] U.A. Zainulabid, N. Kamarudin, A.H. Zulkifly, H.M. Gan, D.D. Tay, S.W. Siew, et al., Near-complete genome sequences of nine SARS-CoV-2 strains harboring the D614G mutation in Malaysia, Microbiol. Resour. Announc 10 (31) (2021) Aug 5. [cited 2021 Dec 8], doi:10.1128/MRA.00657-21.
[6] J.P. Gangneux, M. Sassi, P. Lemire, P. Le Cann, Metagenomic characterization of indoor dust bacterial and fungal microbiota in homes of asthma and non-asthma patients using next generation sequencing, Front. Microbiol. 11 (2020) Available from https://www.scopus.com/inward/record.uri?eid=2-s2.0-85089429490&doi=10.3389%2Ffmicb.2020.01671&partnerID=40&md5=6cea992d26869b1be3bbb06fac67af43.
[7] M.H.Y. Leung, X. Tong, P.K.H. Lee, Indoor microbiome and airborne pathogens, in: Comprehensive Biotechnology, 6, 3rd ed., Elsevier, 2019, pp. 96–106, doi:10.1016/B978-0-444-64046-8.00477-8.
[8] S. Yang, M. Hua, X. Liu, C. Du, L. Pu, P. Xiang, et al., Bacterial and fungal co-infections among COVID-19 patients in intensive care unit, Microbes Infect. 23 (4–5) (2021) 104806, doi:10.1016/j.micinf.2021.104806.
[9] X.L. Gao, M.F. Shao, Q. Wang, L.T. Wang, W.Y. Fang, F. Ouyang, et al., Airborne microbial communities in the atmospheric environment of urban hospitals in China, J. Hazard. Mater. 349 (January) (2018) 10–17.
[10] Z. Ashuro, K. Diriba, A. Afework, G. Husen Washo, A. Shiferaw Areba, G. G/meskel Kanno, et al., Assessment of microbiological quality of indoor air at different hospital sites of dilla university: a cross-sectional study, Environ. Health Insights (2022) 16.
[11] A.B. Chandika, R.S. Mkala, B. Lugoba, B.C. Kipilipili, W. Saitot, C.E. Kamkunguru, et al., Bacterial contaminants on exposed surfaces and their antibiotic sensitivity patterns at the Benjamin Mkapa hospital, Dodoma-Tanzania, Asian J. Res. Infect. Dis. 7 (1) (2021) 1–11.
[12] S. Okten, A. Asan, Airborne fungi and bacteria in indoor and outdoor environment of the pediatric unit of Edirne government hospital, Environ. Monit. Assess. 184 (3) (2012) 1739–1751.
[13] S. Ökten, B. Şen, F. Kaynak Onurdağ, M. Kolukirik, A. Asan, Is next generation sequencing an alternative to cultivation-based methods for investigating fungal diversity in indoor air samples? Aerobiologia 36 (3) (2020) 433–440 (Bologna).
[14] X. Libert, C. Chasseur, A. Packeu, F. Bureau, N.H. Roosens, S.C.J. De Keersmaecker, Exploiting the advantages of molecular tools for the monitoring of fungal indoor air contamination: first detection of exophiala jeanselmei in indoor air of air-conditioned offices, Microorganisms 7 (12) (2019) 1–14.
[15] L. Ciuffreda, H. Rodríguez-Pérez, C. Flores, Nanopore sequencing and its application to the study of microbial communities, Comput. Struct. Biotechnol. J. 19 (2021) 1497–1511, doi:10.1016/j.csbj.2021.02.020.
[16] D.D. Tay, S.W. Siew, M. Najib, B. Razali, H. Fauzan, B. Ahmad. Metadata analysis for gut microbiota between indoor and street cats of Malaysia. 1 (1) (2021) 56–65.
[17] A. Li, J. Xiong, L. Yao, L. Gou, W. Zhang, Determination of dust and microorganism accumulation in different designs of AHU system in Shaanxi history museum, Build. Environ. 104 (2016) 232–242, doi:10.1016/j.buildenv.2016.05.014.
[18] C. Quast, A. Klindworth, E. Pruesse, T. Schweer, M. Horn, F.O. Glo, Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, Nucleic Acids Res. 41 (1) (2013) 1–11.
[19] E. Gil-santos, J.J. Ruz, O. Malvar, I. Favero, A. Lemaître, P.M. Kosaka, et al., Optomechanical detection of vibration modes of a single bacterium, Nat. Nanotechnol. 15 (6) (2020) 469–474.
[20] T.C. Glenn, T.W. Pierson, N.J. Bayona-Vásquez, T.J. Kieran, S.L. Hoffberg, J.C. Thomas, et al., Adapterama II: universal amplicon sequencing on Illumina platforms (TaggiMatrix), PeerJ (10) (2019).
[21] S. Chen, Y. Zhou, Y. Chen, J. Gu, fastp: an ultra-fast all-in-one FASTQ preprocessor, Bioinformatics 34 (17) (2018) i884–i890, doi:10.1093/bioinformatics/bty560.
[22] M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet J. 17 (1) (2011) 10.
[23] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (7) (2016) 581–583, doi:10.1038/nmeth.3869.
[24] J. Chong, P. Liu, G. Zhou, J. Xia, Using microbiome analyst for comprehensive statistical, functional, and meta-analysis of microbiome data, Nat. Protoc. 15 (3) (2020) 799–821.
[25] Z.U. Afeera, S.S. Wei, M.S. Munirah, S.S. Nellisa, P. Petrick, A.H. Fauzan, Whole-genome sequence of a stenotrophomonas maltophilia isolate from tap water in an intensive care unit, Microbiol. Resour. Announc. 12 (2) (2023) e00995.
[26] D. Dean Tay, S. Wei Siew, M.N. Razali, H.F Ahmad, Metadata analysis for gut microbiota between indoor and street cats of Malaysia, Curr. Sci. Technol. 1 (1) (2021) 10.
[27] Z.U. Afeera, M.Z. Norirwanisyam, A. Janaki, K. Norhidayah, Z.A.M. Adil, A.M.A. Shamshir, et al., Near-complete whole-genome sequencing of two burkholderia pseudomallei strains harbouring novel molecular class D beta-lactamase genes, Isolated from Malaysia, Microbiol. Resour. Announc. 11 (10) (2022) e00468.

[28] N. Ayob, K.N. Muhammad Nawawi, M.H. Mohamad Nor, R.A. Raja Ali, H.F. Ahmad, S.F. Oon, et al., The effects of probiotics on small intestinal microbiota composition, inflammatory cytokines and intestinal permeability in patients with non-alcoholic fatty liver disease, Biomedicines 11 (2023).
[29] S.N. Soffian, M.I.H. Nasharudin, R.A. Ruzaidi, A.N.F.M. Anera, W.A.W. Hashim, M.S. Ismail, et al., Whole genome sequencing of bovine Pasteurella multocida type B isolated from haemorrhagic septicaemia during 2020 major outbreak in East Coast, Malaysia, AIP Conf. Proc. 2682 (1) (2023) 50011.
[30] M.Y. Choo, M.F. Yusof, S.S. Kamal, D.S. Nielsen, H.F. Ahmad, High-throughput amplicon sequencing of gut microbiome sea cucumber in Pahang, Malaysia, AIP Conf. Proc. 2682 (1) (2023) 50009.
[31] M.Y. Choo, S.W. Siew, N.S.A. Abdullah, M.A.N. Muhammad, H.F. Ahmad, Complete whole genome sequencing of Vibrio parahaemolyticus strain UMP001VA, isolated from sea cucumber Holothuria leucospilota from Malaysia harbouring blaCARB-33 gene, Mater. Today Proc. 75 (2023) 68–73.