Indoor Air Microbiome in Crowded Public Spaces: A Study of Classrooms and Public Transport Hubs in Owerri, Imo State, Nigeria

Chinaza Gloria Diala [1*], Chisom Rejoice Okenwa [2], Shittu Umar Hammed [3], Similoluwa Mercy Ibishagba [4], Victory Jesuolueminosen Uduebholo [5], Conlethann Chiemerie Ohaekwe [6], Olanrewaju Ahmed Binuyo [7]

[1] Department of Microbiology, Imo State University Owerri, Nigeria
[2] Department of Community Health and Primary Health Care, MBBS, Lagos State University College of Medicine, Lagos, Nigeria
[3] Bayero University Kano, Nigeria
[4] Department of Microbiology, University of Ilorin, Nigeria
[5] Research Assistant, Covenant University Bioinformatics Research Lab, Nigeria
[6] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Nigeria
[7] Department of Animal Science, North Carolina Agricultural and Technical State University, USA

* Corresponding Author: **Chinaza Gloria Diala**

Article Info

ISSN (online): 2582-8940
Volume: 06
Issue: 04
October-December 2025
Received: 12-08-2025
Accepted: 13-09-2025
Published: 06-10-2025
Page No: 11-17

Abstract

Indoor air comprises a mixture of many microorganisms, like bacteria, fungi and viruses, and this impacts human health unknowingly to them. This study focused on two crowded environments in Owerri, Imo State: school classrooms and public transport hub. Our aim was to collect and study airborne microorganisms, and the amount that were present in these environments. To test the microbial aspect of this prediction, we exposed Nutrient Agar and Sabouraud Dextrose Agar plates to the indoor air. Microbes were collected at different times of the day, and factors such as crowd density, ventilation and cleaning schedule affect what we found.

Our result showed that classrooms with open windows had a higher presence of environmental bacteria such as, *Bacillus*, *Pseudomonas*, ranging from 996 – 6889 Cfu/m^3 and fungi like *Mucor*, *Aspergillus* and *Penicillium* ranging from 1448 – 3422 Cfu/m^3. On the other hand, human-associated bacteria such as *Staphylococcus* and *Cutibacterium* found on human skin and respiratory droplets, were isolated from the sample from crowded public buses with a range of 897 – 3868 Cfu/m^3.

Microbes found in indoor air appeared to be largely affected by ventilation, though regular cleaning seemed to reduce surface contamination. Spaces with good airflow had more varied, balanced microbial communities, while poorly ventilated spaces showed higher levels of potentially opportunistic microbes.

This study showed that proper ventilation, and reduced overcrowding makes indoor environments healthy. Proper understanding of these differences will help in making good decisions when building and maintaining public spaces, with clear implications for indoor public health management.

DOI: https://doi.org/10.54660/IJMBHR.2025.6.4.11-17

Keywords: Airborne Microorganisms, Indoor Air, Ventilation, Crowd Density, Public Transport Hub, Classrooms, Public Health

Introduction

The major cause of poor ventilation is Overcrowding and this increase airborne microorganisms found in indoor air. This is as a result of poor ventilation, and humans who spend most of their time in public environments such as classrooms, workplaces, and in public buses also contributed to it, as proven by research [1, 2]. Less ventilated and crowded spaces create room for microorganisms such as, bacteria, fungi, and viruses to grow, survive and multiply. This make it easier for them to be transmitted

through contact especially in places like buses and schools [3, 4].

The major carriers of microorganisms in indoor environments are humans as their saliva, breath and cells from the skin contain microorganisms which are released into the air every time [5]. Majority of the content of indoor air is bacteria from humans, and this were mostly found in classrooms or public transport that is crowded. Our research indicates that microbes such as *Staphylococcus*, *Micrococcus*, and *Cutibacterium* are found on human skin and are often detected in indoor air [6].

In indoor environments, when sneezing or coughing happens without proper coverage of the mouth and nostrils, and there's the presence of bacteria, fungi, or other microorganisms, poor ventilation or circulation through air conditioning allows these microorganisms to spread and stay in the air longer [7, 8]. In public places, where there's a constant need for communication, speaking alone can also contribute to the spread of airborne microorganisms.

Ventilation is a major factor to consider in relation to indoor microbes. School classrooms that are well ventilated tend to have more outdoor microbes than those with less ventilation. These microbes usually include bacteria, fungi, and viruses from soil, plants, and animals. On the other hand, classrooms with poor ventilation mostly contain human-associated microbes. Some studies also suggest that low microbial diversity indoors might affect the health of the people inside [9]. The level and types of microorganisms found in air vary in different places based on factors such as crowd density, ventilation, and cleaning schedules. Classrooms with open windows have more airborne microbes, while public buses usually carry microbes linked to human skin, hair, nails, and even respiratory droplets [10].

Although crowd density, ventilation, and cleanliness are known to affect the presence of indoor microbes, few studies have examined all these factors together in resource-limited settings like Imo State [11].

Indoor microorganisms have not received as much attention as other forms of pollution, like dust or smoke from car engines. Microbes should be put into consideration having public health and the quality of air in mind, although they are invisible to human eyes, they are very active in the environment and affect human health especially in constantly used public spaces, while pollutants are often visible to the human eyes and are measured. Microbes from the human body and its environment are mostly found in school air, and are highly affected by ventilation. Classrooms where teachers and students spend most of their time in enclosed space, including public buses, should contain quality air as this is important to avoid airborne diseases, which will affect their health without their knowledge [12].

DNA-based methods are always scarce and not constantly in use in many parts of the world, it was used by earlier research despite it being expensive and requiring advanced tools, and more experience to operate them [12]. Currently culture-based methods are used because of its practical and affordable nature in monitoring the presence of microorganisms both in humans and the environment, especially in underserved settings, where there are limited or no resources. Though DNA sequencing provides more information [13, 14, 15].

These culture methods are simple, accessible, and yet they can still reveal useful information about the types of microbes found in different indoor spaces. They are helpful for general studies or for communities with fewer resources, as they show patterns in microbial growth based on environmental factors like ventilation and human activity.

Overall, understanding indoor air microbiome in public spaces is critical for public health, particularly as urban populations grow and concerns about airborne disease transmission increase. Simple, cost-effective monitoring methods can help fill knowledge gaps and guide interventions [16, 17].

The aim of this study is to collect and study the types of airborne microbes found in public places such as classrooms and public transport in Imo State. The goal is to understand the relationship between indoor conditions and microbial presence using simple and effective methods.

Materials and Methods

Sampling Location

This research was carried out in the central part of Owerri, Imo State, involving two schools with varying classroom ventilation and two public transport vehicles. Some have large open windows that allow natural airflow, while others depend on artificial cooling such as air conditioning in closed environments. During the busy hours of the day, mainly in the morning and evening when passenger numbers are high, city buses that serve as public transport often carry large numbers of passengers.

Fig 1: Map of Imo State showing the sample location.

Air Sampling

To collect airborne microbes, we used sterile Nutrient Agar and Sabouraud Dextrose Agar plates, a reliable and low-cost method for culturing bacteria and fungi from the air. In school classrooms, plates were placed on desks, windowsills, and corners of the room where airflow patterns were likely to vary. They were exposed to indoor air early in the afternoon for one hour during school hours, when classrooms were actively in use. In buses, plates were positioned near window edges, under seating, and in the middle aisle. These were placed during peak passenger periods and kept exposed for one hour during ongoing operations.

Sterile gloves were worn while handling and transporting all the plates in sealed containers to avoid contamination during setup and retrieval. After exposure, plates were incubated at 30°C for 48 hours in a clean lab setting.

The colony counts obtained from the agar plates were converted to colony-forming units per cubic meter (CFU/m³) using the settle plate method, which estimates the volume of air sampled during passive exposure over one hour.

Identification of pathogens

After the incubation was complete, microbial colonies were counted manually and subjected to biochemical tests such as indole, catalase, methyl red, oxidase, sugar fermentation, coagulase, Voges Proskuer and were differentiated based on their size, shape, texture, and pigment with reference to [18, 19]. Fungal colonies were also noted based on visual features, including hyphae and spore formation.

Gram staining was performed on the selected colonies to determine the type of bacteria cultivated. A light microscope was used to observe the stained slides and classify the bacteria as Gram-positive or Gram-negative. Fungal colonies were also identified through visuals including hyphae and spore formation, and microscopic observation, mainly looking at common molds and yeasts [20].

In addition to microbiological sampling, observational data were collected at each site. This included noting whether windows were open or closed the estimated number of people in the room or bus at the time of sampling, and any ongoing activities that might affect microbial dispersal (e.g., talking, moving, or cleaning). Cleaning routines were documented through interviews with cleaning staff and facility managers, who provided information on how frequently each space was cleaned and what cleaning products were used.

Environmental factors like room size, temperature, and humidity were also roughly recorded using portable devices to help understand their potential influence. All procedures were carried out with permission from school administrators and local transport authorities. At no point were individuals directly involved in the study, and sampling was done without causing disruption to daily routines or operations.

Results

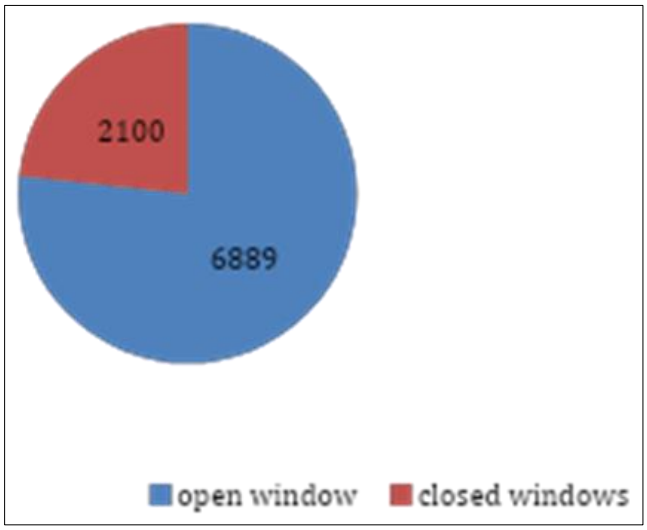
Our sampling revealed clear differences in microbial presence and diversity between classrooms and buses, largely shaped by how well the spaces were ventilated and how many people were present during collection.

Among the 10 classrooms that were studied, 7 with open windows consistently showed a higher number of microbial colonies compared to the remaining 3 classrooms with closed windows. These colonies were not just numerous, but are more diverse. They included Bacteria typically found in soil, dust, and outdoor air, such as *Bacillus*, *Staphylococcus*, *Micrococcus*, *LactoBacillus*, *Escherichia coli*, *Pseudomonas*, *Chromobacterium* and Serratia were isolated from the sample. The number of bacteria isolated from open-window classrooms ranges from 996 – 6889Cfu/m^3. Windowsills had more environmental colonies than central desks, suggesting that natural airflow was bringing in outdoor microbes.

However, classrooms with closed windows had lower overall colony counts ranging from 324 – 2100 Cfu/m^3, but a narrower range of species. Most of the microbes isolated from this classroom were associated with human presence, including *Staphylococcus* and *Micrococcus* species, bacteria that are mostly found in skin and respiratory droplets.

Table 1: Bacterial Genera and CFU Ranges in Classrooms (Open vs. Closed Windows) (n=10)

Ventilation type	No of classrooms	Bacterial genera isolated	Cfu range (Cfu/m^3)
Open windows	7	*Bacillus, Staphylococcus, Micrococcus, LactoBacillus, E. coli, Pseudomonas, Chromobacterium, Serratia.*	996 – 6889
Closed windows	3	*Staphylococcus* and *Micrococcus*	324 – 2100
Total	10		



Source: Table 1

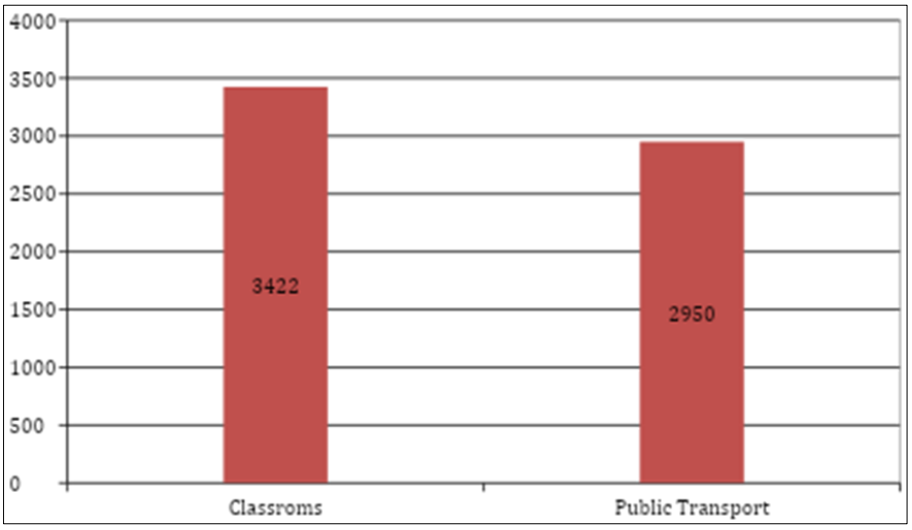
Fig 2: Showed the colony-forming unit (Cfu/m^3) isolated microbes from both classrooms with open and closed windows.

Fungal colonies such as *Mucor*, *MucorAspergillus*, *Microsporum*, *penicillium*, *Cladosporium* and *Rhizopus* were also isolated from these open, well-ventilated classrooms. The number of fungi isolated from the classroom's ranges from 1448–3422Cfu/m^3.

In public transport, fungal colonies such as *Fusarium*, *Mucor* spp, *Rhizopus* spp, *Alternaria* spp, and *Microsporum* spp were isolated from buses. The number of fungi isolated from public transport ranges from 619 - 2950Cfu/m^3.

Table 2: Fungal Genera and CFU Ranges in Classrooms and Public Transport

Location	Fungal genera isolated	Cfu range (Cfu/m^3)
Classrooms	*Mucor*, *MucorAspergillus*, *Mucor*, *MucorAspergillus*, *Microsporum*, *penicillium*, *Cladosporium* and *Rhizopus*	1448–3422Cfu/m^3
Public Transport	*Fusarium*, *Mucor* spp, *Rhizopus* spp, *Alternaria* spp, and *Microsporum* spp	619 – 2950Cfu/m^3



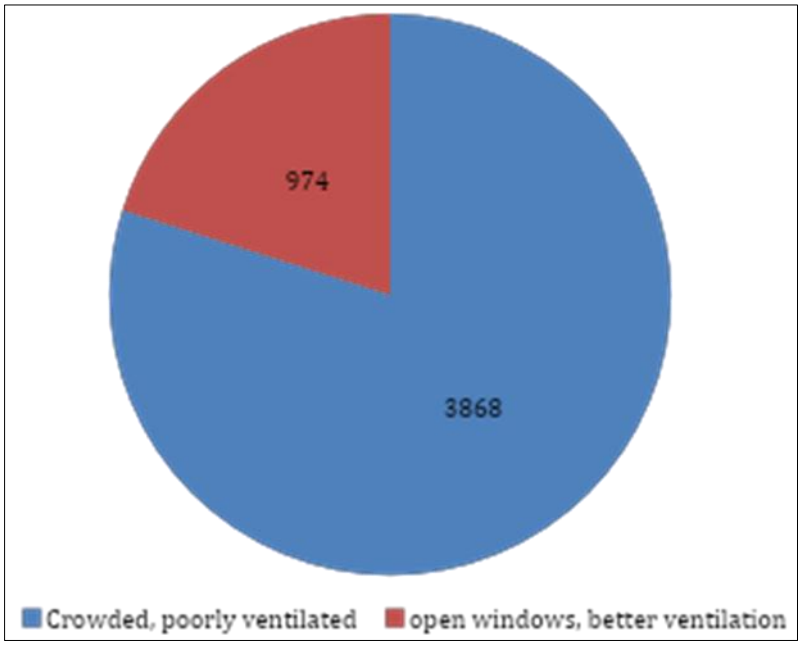
Source: Table 2

Fig 3: illustrate the colony-forming unit of fungi isolated from classrooms and public transport.

Microbial diversity was obviously lower in public transport, but the total number of colonies increased significantly during crowded conditions. Buses operating at busy hours of the day, with passengers in close proximity and limited ventilation, showed a rise in human-associated bacteria which ranged from 897 – 3868 Cfu/m^3. Gram-positive cocci dominated, and most of the colonies isolated resembled skin-related genera. These included shiny, yellowish colonies that are characteristic of *Staphylococcus*, Corynebacterium and *Cutibacterium* spp. Buses with open windows and better ventilation showed greater microbial diversity that ranged from 312 - 974 CFU/m^3, Bacteria such as *Bacillus*, *Pseudomonas*, and Actinobacteria spp were isolated. These environmental bacteria which are introduced by outdoor air reduce the relative abundance of human-associated microbes.

Table 3: Bacterial Genera and CFU Ranges in Public Transport (Crowded vs. Ventilated Buses)

Condition	Bacterial genera isolated	Cfu range (Cfu/m^3)
Crowded, poorly ventilated	*Staphylococcus, Corynebacterium and Cutibacterium spp*	897 – 3868Cfu/m^3
Open windows, better ventilation	*Bacillus, Pseudomonas, Actinobacteria spp*	312 – 974 CFU/m^3



Source: Table 3

Fig 4: Showed the CFU range of bacterial isolated from both poor and better ventilated environments.

The impact of passenger density is noticeable in buses. An increase in the number of passengers corresponded with a rise in airborne microbial load, indicating a direct relationship between human presence and microbial load in enclosed public transport. On the other hand, buses with fewer passengers or open windows showed fewer colonies and more microbial diversity, although human-associated species still dominate.

Cleaning schedules had a more noticeable effect on surfaces than on air samples. Classrooms and buses with regular cleaning had visibly fewer surface residues and minimal microbial growth when swabbed separately, but this didn't significantly lower the number of colonies caught on airborne exposure plates. In several cases, we observed that even shortly after cleaning, microbes quickly reappeared in the air, likely due to human activity and airflow patterns.

Among all the factors observed, ventilation stood out as the most influential. Open windows in classrooms increase air exchange, bringing in more environmental microbes and reducing the dominance of any single group. Closed environments, especially crowded buses, promoted conditions where human-associated microbes could accumulate and thrive.

Discussion

Our findings confirm that ventilation plays a crucial role in shaping indoor air microbial communities. The richer microbial diversity found in classrooms with open windows highlights the importance of fresh air in introducing environmental microbes that might contribute to a balanced indoor microbiome. This aligns with research showing that indoor airborne bacterial communities in naturally ventilated rooms closely resemble outdoor communities and are more diverse than those in mechanically ventilated spaces [21].

Skin and respiratory droplets, usually found in buses, could lead to higher levels of microbes in crowded and poorly ventilated spaces, which could be a threat to human lives. These droplets, often released through breathing, talking, or coughing, accumulate more easily where air circulation is limited and people are packed closely together [22]. This is common during outbreaks of respiratory or other airborne diseases, when enclosed public spaces become hotspots for transmission [23]. Modeling of urban buses confirms that opening windows can reduce aerosol concentration by about half, and that stagnant air in crowded conditions significantly elevates infection risk [24, 25].

While cleaning routines help reduce microbial contamination on surfaces, they have limited impact on airborne microbes. Airborne particles can easily be inhale due to its ability to last longer for a period of time in an environment, and this makes cleaning of the surface incomplete for maintaining indoor hygiene [26]. According to studies on bioaerosol which showed that airborne bioaerosol can still be found on the surface even after cleaning, this indicates that proper ventilation is the best in controlling aerosolized microorganisms [27]. This showed that improving ventilation and managing crowd in an environment may be more effective strategies for maintaining healthier indoor air in public spaces. By reducing the concentration of airborne microbes and improving air exchange, such measures directly address the root causes of indoor microbial buildup.

This study showed that ventilation and number of people in a space affect the concentration of microbes in indoor environments. Ensuring that classrooms and buses have open windows and are not overcrowded will help in limiting the spread of airborne microbes which affects human health. Putting this measure into practice is important in settings where people spend longer periods of time. For example, increasing classroom ventilation rates to 12 –16 liters per second per student increasing classroom ventilation rates has been linked to 80% decrease in the risk of contracting airborne infection compared to depending on natural ventilation.

Conclusion

From our research culture-based techniques showed to be effective and accurate in providing quality results on microorganisms found in indoor air environments. Open windows increase the presence of good microorganisms from the environment.

The number of microorganisms found in the air and human body increases as a result of a crowded environment in places like buses and classrooms. Cleaning has little effect on the number of microorganisms found in the air. This indicates how important ventilation and crowd control is.

Healthy indoor air can be achieved by opening windows and reducing crowds in schools and public buses.

These results support policies and building plans that focus more on proper ventilation and don't allow overcrowding, as it will help in reducing airborne diseases transmission caused by these microbial loads in indoor environments.

Strong policies such as, buildings having wide open windows and minimum number of passengers in a bus especially during busy hours, should be implemented by Schools and transport authorities in Owerri, Imo State.

Raising awareness concerning indoor airborne microorganisms and its health effect will help in reducing the spread of airborne diseases, by actions such as, opening windows and reducing the number of people in a setting.

Combining DNA-based and Culture-based approaches should be carried out by future researchers to improve the understanding of microbial diversity and its seasonal patterns, though simple methods still give out quality results and practical guidance for intervention.

References

1. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, Peccia J. Human occupancy as a source of indoor airborne bacteria. PLoS ONE. 2012;7(4):e34867. doi:10.1371/journal.pone.0034867
2. Stanley HO, Bamidele IF. Microbial assessment of indoor air quality of selected institutions in Rivers State, Nigeria. Int J Pathogen Res. 2022;10(2):22-28. doi:10.9734/ijpr/2022/v10i230244
3. Al-Shaarani S, Pecoraro L. A review of pathogenic airborne fungi and bacteria in indoor environments and their health implications. Front Microbiol. 2024;15:1428415. doi:10.3389/fmicb.2024.1428415
4. World Health Organization. The world health report 2002: reducing risks, promoting healthy life. Geneva: World Health Organization; 2002. Available from: http://www.who.int/publication/i/item/9241562072
5. Zampolli J, De Giani A, Rossi M, Finazzi M, Di Gennaro P. Who inhabits the built environment? A microbiological point of view on the principal bacteria colonizing our urban areas. Front Microbiol. 2024;15:1380953. doi:10.3389/fmicb.2024.1380953
6. Leung MH, Wilkins D, Lee PK. Insights into the pan-microbiome: skin microbial communities of Chinese individuals differ from other racial groups. Sci Rep. 2015;5:11845. doi:10.1038/srep11845
7. Firatoglu ZA. The effect of natural ventilation on airborne transmission of the COVID-19 virus spread by sneezing in the classroom. Sci Total Environ. 2023;896:165113. doi:10.1016/j.scitotenv.2023.165113
8. Korany HZ, Almhafdy A, AlSaleem SS, Cao S-J. Numerical modelling of ventilation strategies for mitigating cough particles transmission and infection risk in hospital isolation rooms. Indoor Built Environ. 2024;33(5). doi:10.1177/1420326X241226467
9. Matthews K, Cavagnaro T, Weinstein P, Stanhope J. Health by design: optimising our urban environmental microbiomes for human health. Environ Res. 2024. doi:10.1016/j.envres.2024.119226
10. Kembel SW, Meadow JF, O'Connor TK, Mhuireach G, Northcutt D, Kline J, Green JL. Architectural design influences the diversity and structure of the built environment microbiome. ISME J. 2014;8(7):1469-1479. doi:10.1038/ismej.2014.210
11. Kim D, Yoon H, Lee J. Assessment of airborne bacteria in the indoor of public-use facilities concentrated on influencing factors and opportunistic pathogenic bacteria. Air Qual Atmos Health. 2024;17(3):1540. doi:10.1007/s11869-024-01540-3
12. Adams RI, Miletto M, Taylor JW, Bruns TD. The diversity and distribution of fungi on residential surfaces. PLoS ONE. 2013;8(11):e78866. doi:10.1371/journal.pone.0078866
13. Kalogerakis N, Paschali D, Lekaditis V, Pantidou A, Eleftheriadis K, Lazaridis M. Indoor air quality-bioaerosol measurements in domestic and office premises. J Aerosol Sci. 2005;36(5):751-761. doi:10.1016/j.jaerosci.2005.02.004
14. Cox J, Mbareche H, Lindsley WG, Duchaine C. Field sampling of indoor bioaerosols. Aerosol Sci Technol. 2019;54(5):572-584. doi:10.1080/02786826.2019.1688759
15. Upula SA, Oka IA, Ije UE. Microbiological assessment of indoor air quality in selected patient wards at a tertiary hospital in Nigeria. Ann Res Rev Biol. 2023;38(6):19-29. doi:10.9734/arrb/2023/v38i630589
16. Jain R, Chakraborty S. Quantifying indoor fungal aerosol levels in slums: implications for public health. Discov Public Health. 2025;22:295.

doi:10.1186/s12982-025-00658-8
17. Hampton-Marcell JT, Ghosh A, Gukeh MJ, Megaridis CM, *et al*. A new approach of microbiome monitoring in the built environment: feasibility analysis of condensation capture. Microbiome. 2023;11:129. doi:10.1186/s40168-023-01555-5
18. Bergey DH, Holt JG, editors. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: The Williams & Wilkins Company; 1994.
19. Cheesbrough M. District laboratory practice in tropical countries, part 2. 2nd ed. Cambridge: Cambridge University Press; 2009. doi:10.1017/CBO9780511543470
20. Tripathi N, Zubair M, Sapra A. Gram staining. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562156/
21. Leung MH, Tang X, Lee PK. Indoor microbiome and airborne pathogens. In: Moo-Young M, editor. Comprehensive biotechnology. 2nd ed. Vol. 4. Amsterdam: Elsevier; 2019. p. 96-106. doi:10.1016/B978-0-444-64046-8.00477-8
22. Yang X, Ou C, Yang H, Liu L, Song T, Kang M, Lin H, Hang J. Transmission of pathogen-laden expiratory droplets in a coach bus. J Hazard Mater. 2020;397:122609. doi:10.1016/j.jhazmat.2020.122609
23. Troko J, Myles P, Gibson J, *et al*. Is public transport a risk factor for acute respiratory infection? BMC Infect Dis. 2011;11:16. doi:10.1186/1471-2334-11-16
24. Zhang Z, Han T, Yoo KH, Capecelatro J, Boehman AL, Maki K. Disease transmission through expiratory aerosols on an urban bus. Phys Fluids. 2021;33(1):015116. doi:10.1063/5.0037452
25. Luo Q, Pan J, Hang J, Ma Q, Ou C, Luo Z, Zeng L. Effect of natural ventilation on aerosol transmission and infection risk in a minibus. Phys Fluids. 2024;36(11):115116. doi:10.1063/5.0236268
26. Mohammed MO. Surface microbial contamination and air quality before and after regular cleaning procedures. Atmosphere. 2023;14(2):352. doi:10.3390/atmos14020352
27. Jendrossek SN, Jurk LA, Remmers K, Cetin YE, Sunder W, Kriegel M, Gastmeier P. The influence of ventilation measures on the airborne risk of infection in schools: a scoping review. Int J Environ Res Public Health. 2023;20(4):3746. doi:10.3390/ijerph20043746