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Antibiotic resistance of bioaerosols in particulate matter from indoor environments of the hospitals in Dhaka Bangladesh

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The emergence and spread of antibiotic resistance in microorganisms pose significant challenges to public health, especially in hospitals. This study investigated the existence or occurrence of bacterial bioaerosol and their antibiotic resistance patterns in particulate matter (PM) collected from hospitals in the greater Dhaka region, Bangladesh. The real-time particulate matter concentrations ($PM_{1.0}$, $PM_{2.5}$, and PM_{10}) were measured in four hospitals and two ambient locations. Air sampling was conducted using a filter-based method with a low-volume air sampler, while AEROCET-531 S (USA) was employed to monitor particulate matter concentrations. Bacterial bioaerosol concentration was determined using a culture-based method, and eleven bacterial species, including nine individual species, i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *P. stutzeri*, *Bacillus cereus*, *Acinetobacter schindleri*, *Proteus vulgaris*, *B. subtilis*, *Escherichia coli*, and *B. aerius*, were isolated. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method with 21 antibiotics. Bacterial isolates were detected using partial sequencing of the 16 S rRNA gene. Bioaerosol concentration ranged from 194.65 ± 22.48 CFU/m^3 to 948.39 ± 84.14 CFU/m^3, showing significant correlations with $PM_{1.0}$ and $PM_{2.5}$ concentrations ($R^2 = 0.80$ and 0.85, respectively). All bacterial isolates collected from the hospitals exhibited resistance against four or more antibiotics, indicating multidrug resistance (MDR). Notably, the bacterial isolates displayed the highest resistance rate against ampicillin (90.90%), azithromycin (81.81%), erythromycin (81.81%), cefixime (81.81%), and cotrimoxazole (54.54%), among the tested antibiotics. Except *B. aerius*, all other bacterial isolates were associated with hospital-acquired infections (HAIs). These findings highlight the high rates of antibiotic resistance, underscoring the pressing requirement for infection control measures and continuous surveillance strategies in hospital settings. These findings emphasize the necessity for global hospital infection control strategies focusing airborne multidrug-resistant microorganisms.

Keywords Bioaerosols, Particulate matter, Antibiotic resistance, Hospital-acquired infections, Nosocomial infections, Air Quality

Quantitative studies of airborne particulate matter (PM) have focused a great deal of attention on bioaerosols in recent years, with an increasing recognition of their massive significance and impact on health, climate, and environmental pollution concerns[1]. Bioaerosols contain living as well as non-living elements, such as pollens, bacteria, fungi, and viruses[2]. Therefore, they can consist of both pathogenic and non-pathogenic microbes, whether dead or alive[3]. According to Humbal et al. (2019)[4], these bioaerosols comprise solids and semi-solids containing biotic and abiotic components whose size ranges from 0.001 nm to 100 µm. Sneezing, coughing, talking, washing, flushing the toilet, etc., can all cause biological particulate matter to become airborne[5].

In hospitals, the scenario regarding bioaerosols is more complex compared to other commercial and residential buildings. The airborne form of bacteria can cause infections in patients and hospital staff, with heightened vulnerability observed in operating theaters, intensive care units (ICUs), and delivery rooms[6]. Hospitals commonly use and expose pathogens to antibiotics, resulting in high levels of antibiotic resistance[7].

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Antibiotic-resistant bacteria (ARB) create significant challenges in treating infectious diseases[8]. A variety of chemicals produced by various items, such as antibacterial agents, sterilizers, laboratory materials, various medical operations, and therapeutic management, as well as medical wastes, might be significant indoor sources of pollution in hospitals[9]. The air pollutants dictated by zonal origins and long-range transmission (prevailing wind direction) may pertain to indoor air quality (IAQ), but localized air pollution sources, such as hospital parking lots' emissions, road traffic etc. may also contribute[10]. Furthermore, outdoor and interior construction, humidifiers, contaminated carpets, and cooling towers can all contribute to hospital pollution[11].

In particular, $PM_{2.5}$ and PM_{10}, which are significant sources of airborne microorganisms, have been demonstrated to correlate with the concentration of bacterial colonies[12]. Air movements can carry these airborne bacteria upward, as they are tiny enough to survive for a long time in the environment[13]. Bacteria may be eliminated from the air by procedures like dry deposition and/or wet deposition (being removed by precipitation, i.e. rain or snow)[14]. Depending on the amount of haze and the severity of the pollution, the influence of temperature on bioaerosols might differ. For instance, in the winter, hub bacteria were more prevalent in $PM_{2.5}$ on days with high pollution levels than on days with lower levels[15]. However, during foggy days in the fall and early winter in Beijing, the airborne bacterial load and community structure were mostly influenced by relative humidity and particulate matter concentrations[16]. These findings emphasize the complex connections among bioaerosols, temperature, humidity, and particulate matter in different environmental conditions. Bioaerosols have been linked to chronic health difficulties, respiratory disorders, and infectious infections[17]. Furthermore, there is a remarkable correlation between the Air Quality Index (AQI) and the quantity of airborne bacteria and fungi. The quantity of airborne fungus and the number of airborne bacteria steadily rise when the AQI hits 200[18]. The interactions between PM and AQI, and subsequently, the types of bacteria present, depend significantly on the chemical and microbiological compositions[19]. The composition and variety of bioaerosols have been reported to significantly change at high or extreme pollution levels[15].

Exposure to biological agents, for example, fungi, bacteria, parasites, and viruses, causes infectious diseases, which can spread through indirect contact, such as coughing or sneezing, or direct contact, such as biting, touching, or licking[20]. *Mycobacterium tuberculosis*, which infected individuals expel into the environment when they cough, sneeze, or talk, causes tuberculosis (TB) through air contamination[21]. *Bacillus anthracis* causes anthrax, which individuals can contract through ingestion, inhalation, or skin contact with infected animals[22]. Recognizing the potential health risks posed by bioaerosol in occupational settings is crucial, and industries where bioaerosol exposure is a concern should take appropriate measures to mitigate exposure and protect worker health. Bioaerosol components may threaten hospital facilities' indoor air quality (IAQ). The microorganisms found in bioaerosols have the potential to affect patients and healthcare workers by increasing the prevalence of occupational illnesses and hospital-associated infections. The objectives of this research work are to estimate the concentrations of bacterial bioaerosols and particulate matter from different hospitals and two ambient locations in the greater Dhaka region. In addition, the identification of the bacterial isolates obtained from the sampling sites was identified to get information about nosocomial infection and other health impacts in the hospital environments and to evaluate the antibiotic resistance of the identified bacterial isolates.

Results

Particulate matter (PM) concentrations

The average and standard deviation of particulate matter concentrations ($PM_{1.0}$, $PM_{2.5}$, and PM_{10}) for all sampling locations are given in Supplementary Table S1 and Fig. 1. Differences in average $PM_{1.0}$, $PM_{2.5}$ and PM_{10} concentrations were found to be statistically significant ($p < 0.05$) across all study locations.

The concentration of $PM_{1.0}$ was found to be much higher in all the hospital sites. The greatest values of $PM_{1.0}$ and $PM_{2.5}$ were found at sampling point I5 of Dhaka Medical College Hospital (DMCH) (80.46 ± 11.32 μg/m^3, 220.60 ± 16.52 μg/m^3), while the highest PM_{10} concentration was 1452.21 ± 189.78 μg/m^3 for sampling point I6 of DMCH. The PM_{10} concentrations were highest near 1:00 p.m. at BSMMUH and KBMH (Supplementary Fig. S1) and it might be due to the increasing activities of the people coming to the hospitals and also due to the temperature elevation[28].

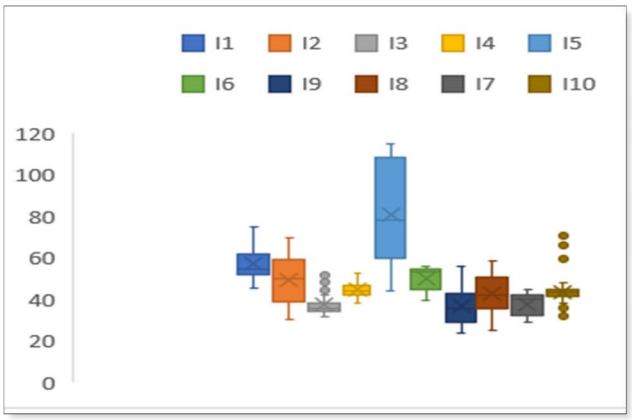
Bioaerosol concentration at the sampling locations

The concentration of bacterial bioaerosols at various sample locations of the hospitals was considerably varied based on the data acquired (Supplementary Table S1). The hospitals considered include a mix of public and private institutions with different patient volumes, infrastructure, and air handling systems, providing a broad view of hospital indoor air conditions in greater Dhaka. We explored various environmental parameters affecting bioaerosol load and resistance. Following an 8-hour sampling period, the location I5 of DMCH exhibited the highest concentration of culturable total aerobic bacterial colonies in PM (948.39 ± 84.14 CFU/m^3). The total concentration range of bacterial colonies across the hospital sites varied from 194.65 ± 22.48 CFU/m^3 to 948.39 ± 84.14 CFU/m^3.

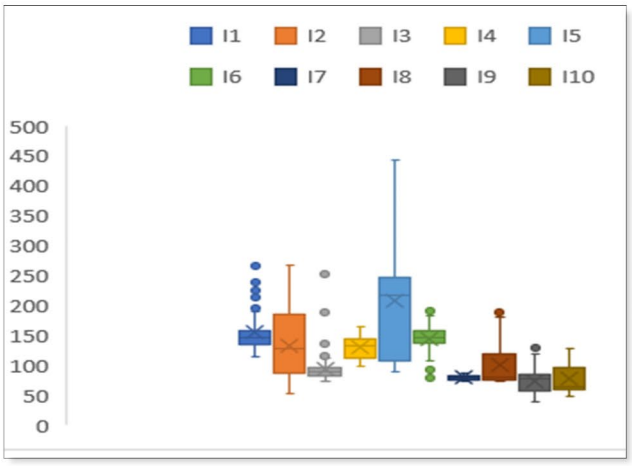
The lowest mean concentrations of culturable bacterial bioaerosol were 59.37 ± 16.51 and 48.65 ± 18.47 CFU/m^3 found at I9 and I10 or the control sites, respectively. Figure 2 shows the bioaerosol concentration with standard deviation at different sampling sites. Bacterial bioaerosol concentration was significantly lower at sampling location I3 of KBMH, which has an air conditioning system.

Correlation between bioaerosol concentration and particulate matter (PM)

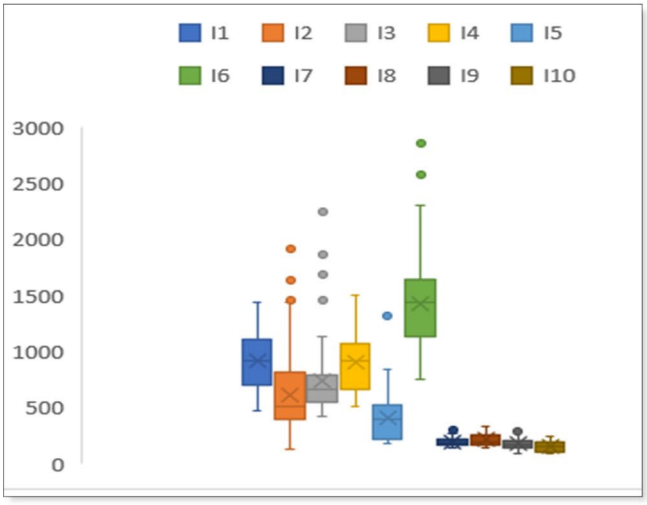
A significant relationship ($p < 0.05$) between bacterial concentration and particulate matter has been shown in Fig. 3, in which an increase in bacterial concentration was observed with the increasing concentration of particulate matter. The regression parameters in Fig-3(c) show the modest connection ($R^2 = 0.27$) between the



$PM_{1.0}$



$PM_{2.5}$



PM_{10}

Fig. 1. Particulate matter (PM) and bioaerosol concentrations at different sampling sites (all units are in μg/m^3 except bioaerosol concentration which is in CFU/m^3). Here, I1 & I2 = Bangabandhu Sheikh Mujib Medical University Hospital (BSMMUH), I3 & I4 = Khwaja Badrudduja Modern Hospital (KBMH), I5 & I6 = Dhaka Medical College Hospital (DMCH), I7 & I8 = Monno Medical College Hospital (MMCH), I9 = Green Model Town (GMT), I10 = Mukarram Hussain Khundker Bhaban (MHKB).

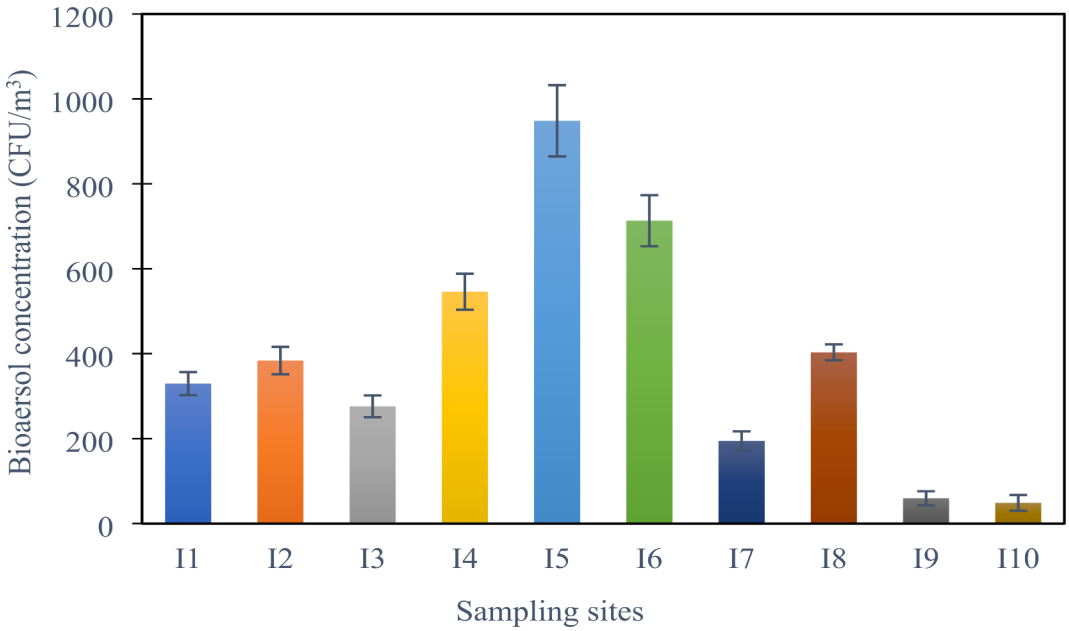


Fig. 2. Bioaerosol concentration (CFU/m^3) at different sampling sites. Here, I1 & I2 = Bangabandhu Sheikh Mujib Medical University Hospital (BSMMUH), I3 & I4 = Khwaja Badrudduja Modern Hospital (KBMH), I5 & I6 = Dhaka Medical College Hospital (DMCH), I7 & I8 = Monno Medical College Hospital (MMCH), I9 = Green Model Town (GMT), I10 = Mukarram Hussain Khundker Bhaban (MHKB).

PM_{10} concentration and the concentration of bacterial bioaerosol, though the value of the concentration of microbes and PM_{10} is significant ($p < 0.05$).

Based on the information provided in Fig. 3 (a) and (b), it can be deduced that there is a strong connection between bacterial bioaerosols and fine particles, especially $PM_{1.0}$ and $PM_{2.5}$. The high R^2 values for $PM_{1.0}$ and $PM_{2.5}$ (0.80 and 0.85) suggest a significant correlation between bacterial bioaerosols and these fine particulate matter fractions.

Correlation between bioaerosol concentration and meteorological parameters

The meteorological parameters (i.e. temperature, relative humidity) were measured simultaneously to study the influence of these on the number and growth of the bacterial bioaerosol (Supplementary Table S2).

A substantial ($p < 0.05$) correlation was found by the ANOVA-single component test between temperature and the number and growth of bacteria. Temperature change and $R^2 = 0.68$ showed a positive correlation for indoor microorganism variation (Fig. 4a). Relative humidity (RH) functions similarly to temperature and has a significant impact on airborne microbial concentration, diversity, and composition, among other factors. The R^2 value was 0.51 (Fig. 4b), demonstrating a positive association between the concentration of bacterial bioaerosol and relative humidity.

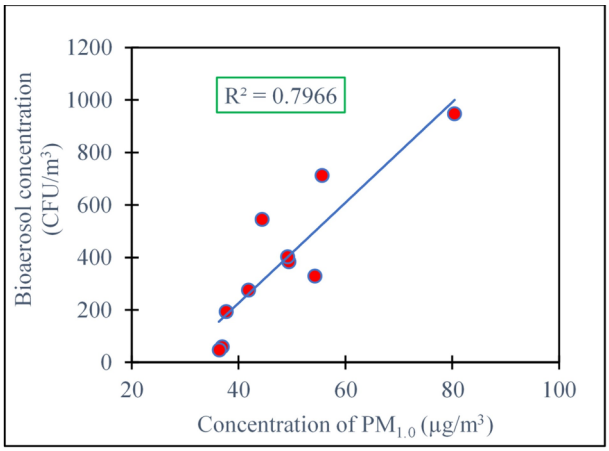
Gram positive and negative bacteria in bioaerosol samples

The colony features and structural morphology of these isolates were recorded (Supplementary Table S3**).** Eleven different bacterial colonies were isolated based on their apparent colony characteristics. Eight (isolates 2, 3, 5, 6, 7, 8, 9) were gram-negative (73% of the total isolated samples), and the other four (isolates 1, 4, 10, 11) were gram-positive (27% of the total isolated samples).

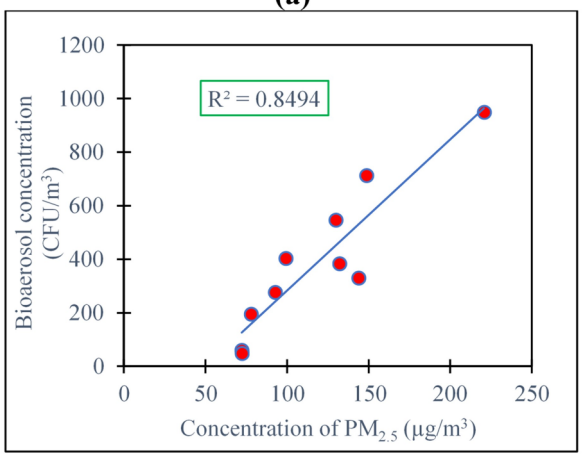
Antibiotic susceptibility profile of isolates

Twenty-one different antibiotics were employed in the antibiotic susceptibility test. With the exception of isolate-11, every examined bacterial isolate shown resistance to the majority of drugs. Isolate-11 was sensitive to all the antibiotics, and it was obtained from the bioaerosol sample of one of the controlled sites (I10) (Table 1; Fig. 5). All bacterial isolates were sensitive to only 3 antibiotics (gentamicin, tigecycline, and vancomycin). Only one isolate was found to be resistant against imipenem, meropenem, and colistin (isolate-9 against imipenem, meropenem and isolate-6 against colistin). The isolates' multi-drug resistance (MDR) was demonstrated by these findings.

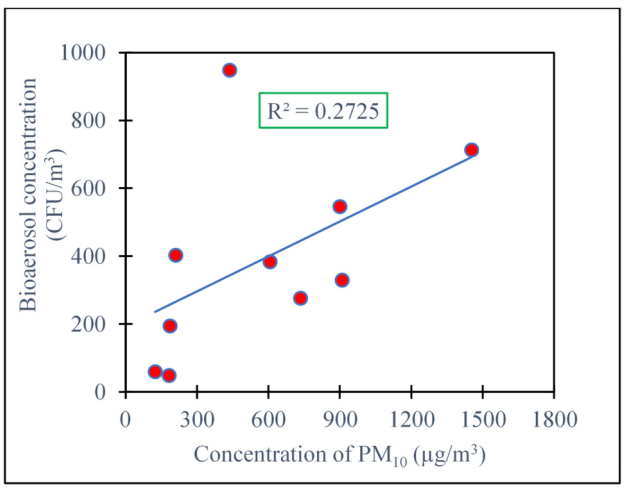
The highest antibiotic resistance was observed in the case of isolate-9, which was about 71.43% of the total antibiotics used, and the lowest was observed in isolate-10 at 19.05% (Table 2**)**. Isolate-11 showed no resistance against the antibiotics used. Isolates of bioaerosol samples collected from BSMMUH (I1, I2) and DMCH (I5, I6) showed higher resistance than other hospitals.



(a)



(b)



(c)

Fig. 3. Correlation between concentration of particulate matter (**a**) $PM_{1.0}$, (**b**) $PM_{2.5}$, (**c**) PM_{10} with concentration of bioaerosol.

Identification of the bacterial isolates

The isolates obtained from the study were subjected to phylogenetic and 16 S rRNA sequence analyses, which revealed that they belong to various bacterial families, including *Staphylococcaceae*, *Pseudomonadaceae*, *Bacillaceae*, *Acetobacteraceae*, and *Enterobacteriaceae*.

Distance tree analysis demonstrated that isolate-3 was very closely associated with *P. stutzeri* (Table 3; Fig. 6), with a 100% similarity to the database's reference sequence. Isolates-1 and 10 exhibited 99% resemblance to

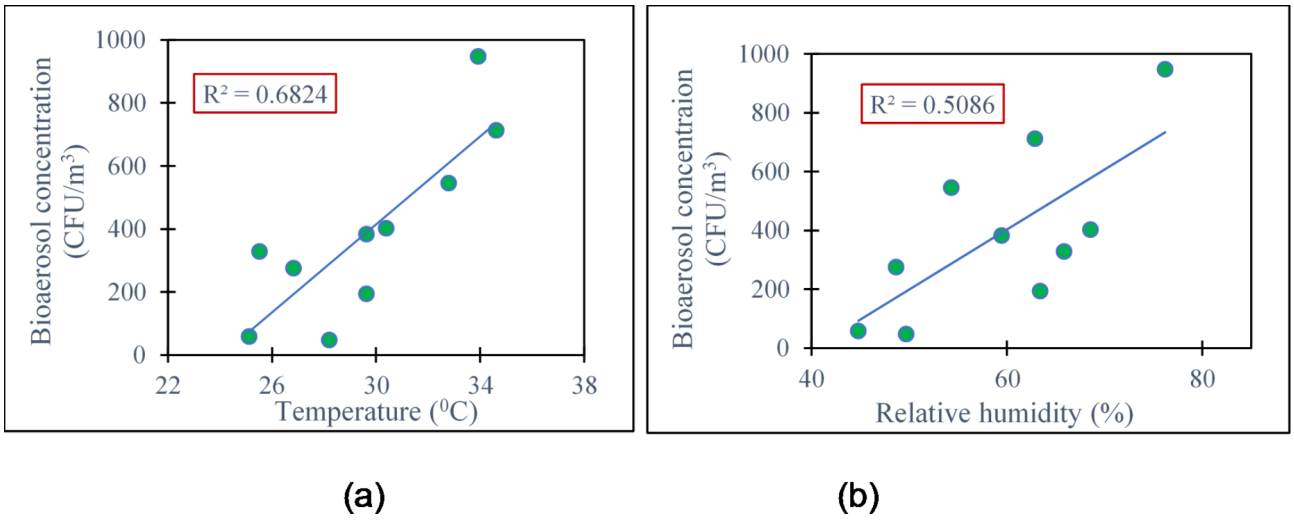


Fig. 4. Correlation between Temperature (**a**) and Relative Humidity (**b**) with bioaerosol concentration at different sampling sites.

the standard sequence, indicating a close relationship with S. aureus. On the other hand, 98% closeness to the reference sequence indicated that isolates 7 and 9 were closely related to *E. coli*. Isolate-2 and 3 were *P. aeruginosa* and *P. stutzeri* found to have 99% and 100% similarities, respectively. *B. cereus*, *B. subtilis* and *B. aerius* were some of the species (isolate-4, 8 and 11) of *bacillaceae* family, also found to have 99% likeliness to the reference sequence. Isolate-6 was found to be *P. vulgaris* and had 99% similarity.

Supplementary Fig. S2 shows the DNA sequencing chromatogram of isolate-3 which was generated by using Chromas software (Version 2.6.6). The sequences generated by automated sequencers are displayed as a graph called a chromatogram, which contains a series of peaks in four different colors. The DNA sequences of the bacterial isolates were aligned with the reference sequences. According to Supplementary Fig. S3, in the isolate-3 sequence examined by Chromas software, all 643 nucleotide base pairs were matched with the reference *P. stutzeri* with no gaps observed.

Bacterial species identified at different sampling locations

In the hospital environments, *S. aureus* and *E. coli* were the most frequently detected bacteria (Table 4). The majority of the bacterial species, which are primarily pathogens or opportunistic pathogens, were discovered in the Bangabandhu Sheikh Mujib Medical University Hospital and the Dhaka Medical College Hospital. *Bacillus spp.* was mostly found in the controlled sites which rarely create any health issues. So, hospitals contained many harmful bacterial species compared to other places.

Discussion

The values of particulate matter concentration at the controlled sites were significantly lower than those at the hospital locations, likely due to the absence of a source of particulate matter pollution[29]. All the hospital sites had considerably higher PM1.0 concentrations, a significant concern given that smaller particulate matter easily deposits in the human lower respiratory tract, leading to serious public health issues[26,27]. There were also significant variances in the mean values of different hospitals. National Ambient Air Quality Standards (NAAQS) for Bangladesh are 65 μg/m^3 for $PM_{2.5}$ (24-hour average) and 150 μg/m^3 for PM_{10} (24-hour average). So, the concentration of particulate matter at different hospitals exceeded the NAAQS value and also the WHO value, which is 15 μg/m^3 for $PM_{2.5}$ and 45 μg/m^3 for PM_{10} (24-hour average)[30].

The higher concentrations of bioaerosol found in this study might have been attributed to a number of factors, including a high patient and visitor volume, inadequate air conditioning, and prolonged window closures. Artificial air cooling has been proven in several studies to have the potential to lower indoor bacterial counts[31]. The highest values in I5 and I6 may also be associated with the building's age, non-standard flooring, consumable materials, wall seams, a high percentage of outdated beds in the wards, natural ventilation, and a high patient density in the wards[32]. The number of patient beds is one of the primary factors promoting the generation and release of airborne bioaerosols, according to several studies[33].

Bacteria, particularly pathogenic bacteria, positively correlate with PM's physical and chemical makeup[34]. A moderate correlation ($R^2 = 0.27$) was found between PM_{10} levels and bacterial bioaerosol concentrations, with both showing statistically significant values ($p < 0.05$). This might be because of the high concentration of fungi that greatly contributes to the PM_{10} concentration[28]. Bacterial bioaerosols and fine particulate matter fractions appear to be significantly correlated, as indicated by the high R^2 values for $PM_{1.0}$ and $PM_{2.5}$ (0.80 and 0.85). It appears that the elements in the particulate matter provide ideal circumstances for the development of microorganisms.

A study by Hoeksma et al. (2015)[35] investigated the effects of temperature on *E. coli*, *M. synoviae*, and *E. mundtii* by observing bacterial decay. The results demonstrated that different microbial species exhibited

Name of the Antibiotics																					
Isolates	Azithromycin	Erythromycin	Gentamicin	Chloram-phenicol	Ampicillin	Ciprofloxacin	Cotrimoxazole	Cefixime	Ceftazidime	Tige-cycline	Aztreonam	Imipenem	Cloxacillin	Cefepime	Vancomycin	Cefalexin	Ceftriaxone	Cefotaxime	Cefuroxime	Meropenem	Colistin
1	R	R	S	R	R	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S
2	R	R	S	R	R	S	S	R	R	S	R	S	S	R	S	R	R	S	R	S	S
3	R	R	S	R	R	S	S	R	S	S	S	S	R	S	S	R	R	R	R	S	S
4	R	R	S	R	R	R	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S
5	R	R	S	S	R	S	R	R	S	S	R	S	R	S	S	S	S	S	S	S	S
6	S	S	S	R	R	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R
7	R	R	S	S	R	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S
8	R	R	S	S	R	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S
9	R	R	S	S	R	R	R	R	R	S	S	R	R	R	S	R	R	R	R	R	S
10	R	R	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 1. Antibiogram of the isolates obtained from bioaerosol samples of different hospital environments. S= Sensitive, R= Resistant .

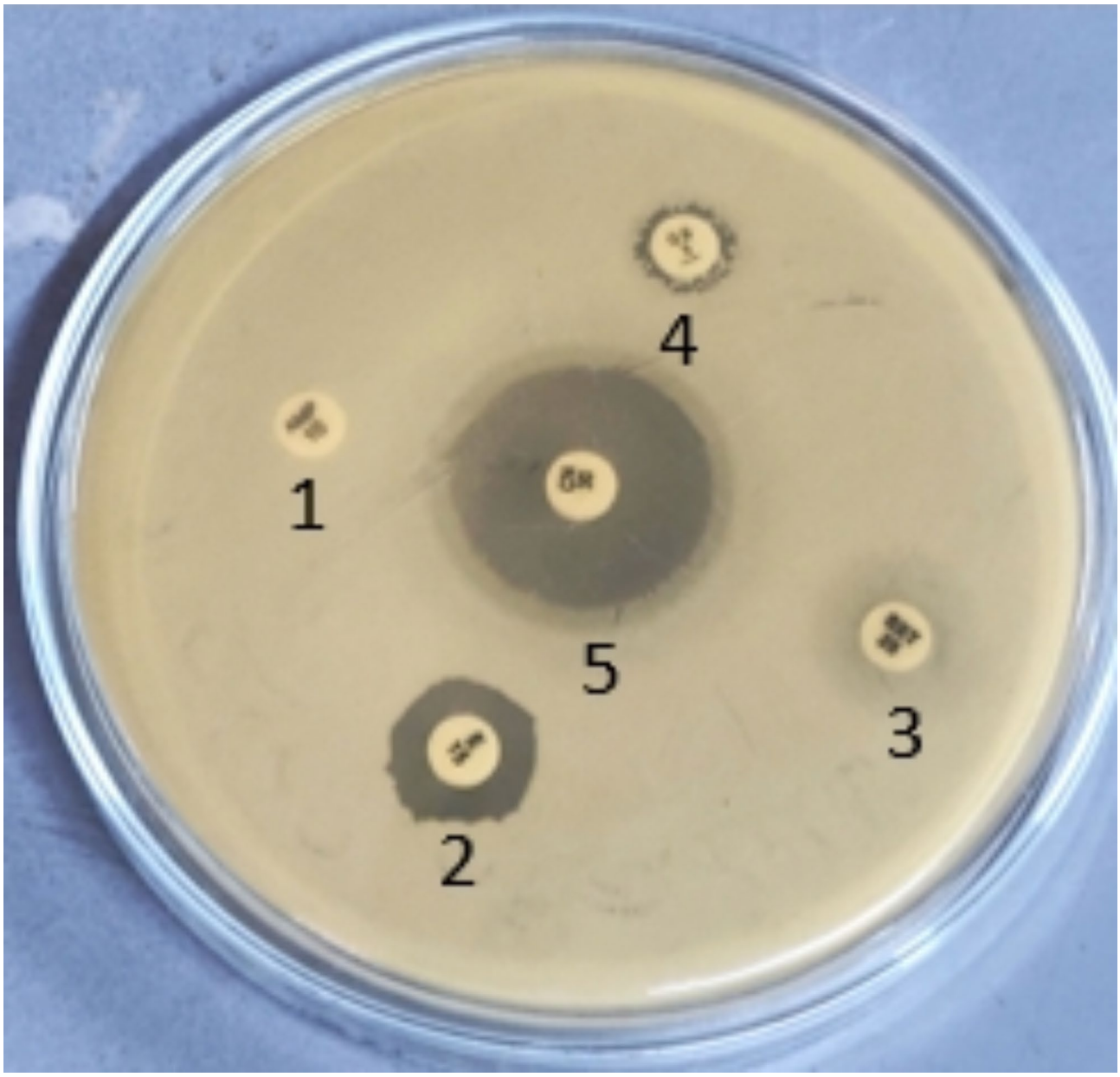


Fig. 5. A representative antibiogram of the isolate-5. 1- Ampicillin; 2-Erithromycin; 3- Cotrimoxazole; 4-Cloxacillin; 5- Gentamicin.

varying abundances at different temperature ranges, with some thriving in high temperatures, others in low temperatures, and a majority occurring at moderate air temperature values. In an indoor environment at the University of Dhaka, RS et al. (2022)[36] conducted a study that showed a positive correlation between bacterial concentration and temperature (R^2 value 0.73) and a R^2 value of 0.68 was found between the relation of bioaerosol concentration and temperature in this study which is also supported by the previous one. The growth of culturable microorganisms was more pronounced during the spring or winter compared to the summer season, primarily due to temperature fluctuations[4]. Relative humidity (RH) functions similarly to temperature and has a significant impact on airborne microbial concentration, diversity, and composition, among other factors. The combined effects of relative humidity and temperature are likely to play a crucial role in shaping the behavior of airborne microorganisms[16]. High relative humidity was advantageous for bacterial release and proliferation, but it may potentially lower bacterial viability[37]. Another study, which was performed at the University of Dhaka, found a positive correlation between indoor bacterial concentration and relative humidity, and the R^2 value was 0.68[37], which supports the result obtained here ($R^2 = 0.51$). The relative humidity and bacterial concentration were discovered to be negatively correlated[38]. According to Knudsen et al. (2017)[39], various microorganisms react to relative humidity differently, and occasionally they don't react at all.

In a study at a hospital in Iran, it was found that the highest antibiotic resistance was in cefixime (45.8%), ceftazidime (30.2%), gentamicin (12%) and ciprofloxacin (12%)[8]. One contributing factor to the rise of antibiotic

Sampling locations	Cultured bacterial isolates	% of antibiotic resistant
Bangabandhu Sheikh Mujib Medical University Hospital (BSMMUH)	1	42.86
	2	52.38
	3	47.62
	4	42.86
Khwaja Badrudduja Modern Hospital	5	33.33
	6	23.81
Dhaka Medical College Hospital (DMCH)	7	33.33
	8	33.33
	9	71.43
Monno Medical College Hospital	10	19.05
Mukarram Hussain Khundker Bhaban	11	No resistance shown

Table 2. Percentage of antibiotics resistant by the isolated bacterial samples cultured from bioaerosol samples of different sampling locations.

Isolates	Query Recovery (%)	E Value	Identity	Species that the maximum match associated with
1	100	0.00	99%	*S. aureus*
2	100	0.00	99%	*P. aeruginosa*
3	100	0.00	100%	*P. stutzeri*
4	100	0.00	99%	*B. cereus*
5	100	0.00	99%	*A. schindleri*
6	100	0.00	99%	*P. vulgaris*
7	100	0.00	98%	*E. coli*
8	100	0.00	99%	*B. subtilis*
9	100	0.00	98%	*E. coli*
10	100	0.00	99%	*S. aureus*
11	100	0.00	99%	*B. aerius*

Table 3. Identification of bacterial isolates by blast analysis of a partial 16 S rRNA sequence that was searched in a nucleotide database.

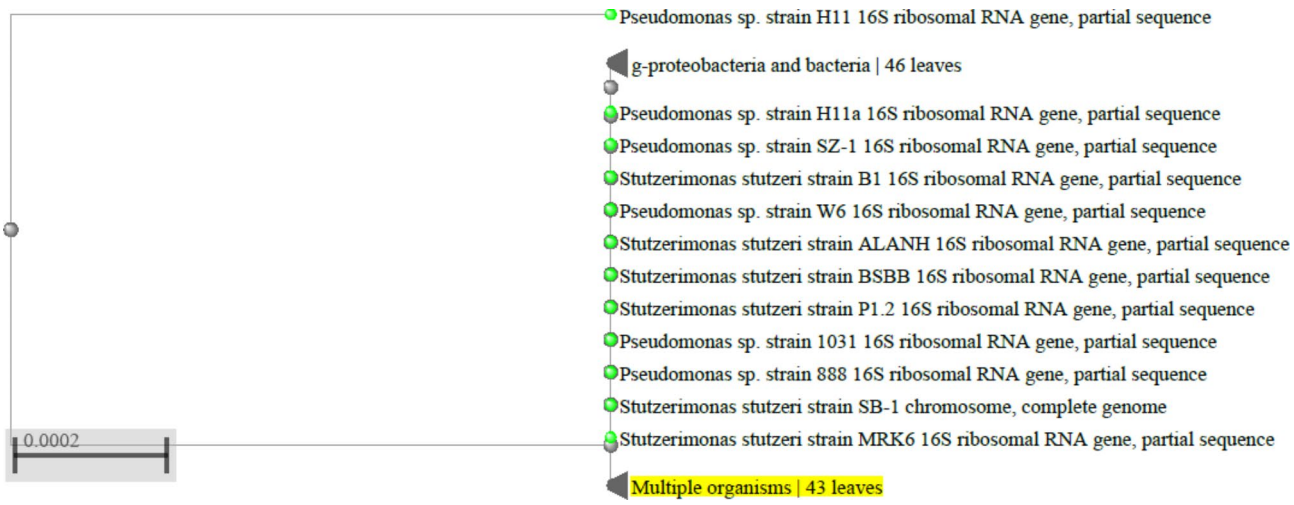


Fig. 6. A representative figure of phylogenetic tree analysis of isolate-3, which was found closely related to *P. stutzeri*.

resistance is the overuse of antibiotics, which can promote the development of antibiotic-resistant bacteria and antibiotic-resistant genes[40]. This overuse creates a selection pressure that favors the survival and proliferation of resistant strains, reducing the effectiveness of antibiotics and making it more challenging to treat bacterial infections effectively.

Sampling Sites	Bacterial isolates	Bacterial species present
Bangabandhu Sheikh Mujib Medical University Hospital	1	*S. aureus*
	2	*P. aeruginosa*
	3	*P. stutzeri*
	4	*B. cereus*
Khwaja Badrudduja Modern Hospital	5	*A. schindleri*
	6	*P. vulgaris*
Dhaka Medical College Hospital	7	*E. coli*
	8	*B. subtilis*
	9	*E. coli*
Monno Medical College Hospital	10	*S. aureus*
Mukarram Hussain Khundker Bhaban	11	*B. aerius*

Table 4. Identified bacterial isolates at different sample sites in Dhaka, Bangladesh.

P. aeruginosa, *P. stutzeri*, *(A) schindleri*, and *P. vulgaris* were also identified as potential sources of nosocomial infections in immunocompromised patients within hospital settings[44]. *(B) cereus and B. subtilis* were also found in the hospital environment, according to the research. According to reports, *E. coli* and *K. pneumoniae* are the two most prevalent nosocomial pathogens in hospitals that cause urinary tract infections (UTIs) in Europe[45]. Others have found similar patterns, with high incidence of *Staphylococcus*, *Micrococcus*, and *Bacillus* in various hospital settings[46,47]. Among the Gram-negative bacteria, Acinetobacter spp., *P. aeruginosa*, and *E. aerogenes* were detected on the plate surface, highlighting their potential association with healthcare-related infections through hospital indoor air[48]. These findings underscore the importance of monitoring and understanding the presence of different bacterial species in hospital environments to implement effective infection control measures and protect the health of patients and healthcare workers.

Coagulase-negative staphylococcus (CONS) is a common cause of nosocomial infections, particularly in neonatal and pediatric intensive care units, and is associated with significant patient mortality and morbidity[42]. Similar findings were reported by Memon et al. (2016)[43], who observed a high prevalence of *S. aureus* in various hospital wards, highlighting its role as a notorious pathogen responsible for nosocomial infections in immunocompromised patients. *S. aureus*, *P. aeruginosa*, *P. stutzeri*, *B. cereus*, *(A) schindleri*, *P. vulgaris*, *(B) subtilis*, *E. coli*, and *B. aerius* were found in this study, all of which are associated with creating nosocomial infections or hospital-acquired infections (HAI) in patients and healthcare workers, except *Bacillus aerius*[44,51]. Most of the bacterial species were found to be opportunistic pathogens. *B. cereus* and *E. coli* were identified as pathogens based on other studies. *B. aerius* was the one with no pathogenic report so far. Among the bacteria responsible for causing a wide range of clinical infections, *S. aureus* is a major human pathogen. It is known to be a leading cause of various infections, including bacteremia, infective endocarditis, osteoarticular infections, skin and soft tissue infections, pleuropulmonary infections, and device-related infections[52]. *P. aeruginosa* and *P. stutzeri* are also implicated in causing infections such as bacteremia, urinary tract infections (UTIs), and respiratory infections[53,54]. Additionally, *A. schindleri* can lead to nosocomial infections, with a predilection for aspiration pneumonia and catheter-associated bacteremia. These bacteria can pose significant health risks in hospital settings, particularly to immunocompromised patients and those with underlying medical conditions. Recent works have proposed hospitals as emission hotspots of antibiotic-resistant bacteria in urban environments[49,50] which is in accordance with this study.

The World Health Organization (WHO) has provided 100 CFU/m^3 as the maximum number for hospital guidelines for bacteria[55]. Given that each patient and staff member have a different level of immunosuppression and susceptibility to infection, the study of bioaerosol concentration and the evaluation of bacterial resistance to antibiotics is crucial for the prevention of hospital-acquired infections (HAIs) or nosocomial infections and may be impacted by ineffective management of these factors (11). Healthcare facilities and hospitals stand out among all building types for their link with pathogenic bacteria. Nosocomial infections, which impact 15% of inpatients, are particularly prone to hospitalized patients[56]. Antibiotic resistance will cause at least 700,000 deaths annually, and the rise in ARGs will cause 10 million fatalities annually by 2050[50]. A study estimated a global antibiotic consumption rate of 14.3 defined daily doses (DDD) per 1000 population per day in 2018, with a 95% uncertainty interval of 13.2 to 15.6 DDD[57]. This amounted to a total of 40.2 billion DDD consumed worldwide in 2018. This represented a significant increase of 46% from the antibiotic consumption rate of 9.8 DDD per 1000 per day in 2000, with a 95% uncertainty interval of 9.2 to 10.5 DDD. The rise in antibiotic consumption over this period raises concerns about the potential impact on antimicrobial resistance and the need for appropriate stewardship and control measures to ensure responsible and effective use of antibiotics globally. These findings emphasize the need for national and international hospital infection control guidelines to address airborne antibiotic-resistant bioaerosol threats, especially in locations with limited resources.

Methods

Characteristics of the sampling sites and hospital building

In the greater Dhaka region, the samples were collected from two public and two private hospitals (Fig. 7). The Bangabandhu Sheikh Mujib Medical University Hospital (BSMMUH), with a capacity of 1900 beds, is Bangladesh's first public and second largest hospital with numerous departments. The second hospital was

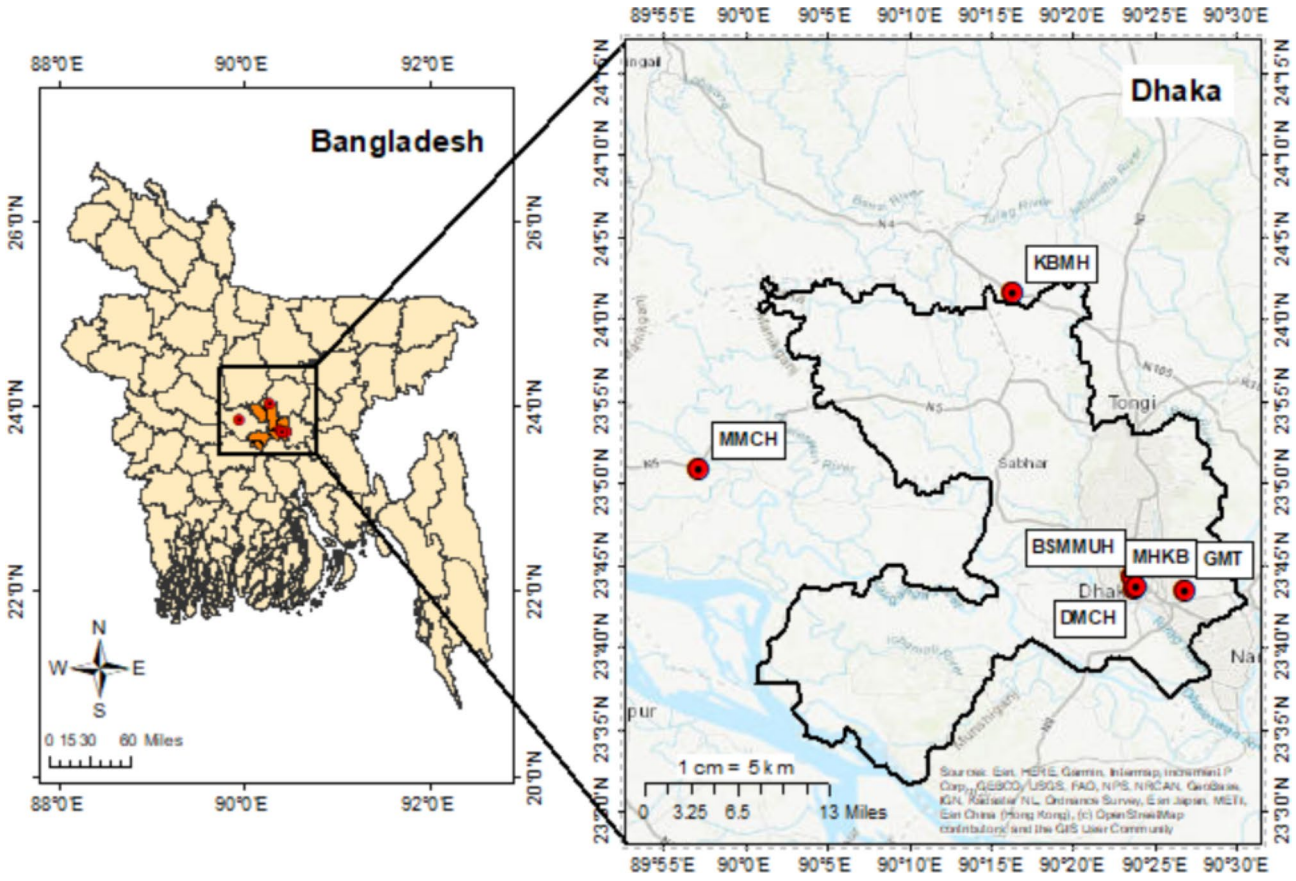


Fig. 7. Map of Bangladesh (Left); four hospitals and two sample-control sites in the greater Dhaka area, Bangladesh (Right).

Khwaja Badrudduja Modern Hospital is a compact healthcare facility comprising 20 beds primarily catering to primary care needs. The third hospital was the Dhaka Medical College Hospital (DMCH).With 2600 beds and multiple departments, it's one of the largest and most established hospitals in Bangladesh. The fourth hospital was Monno Medical College Hospital, which is located in a rural region. The hospital is a significant medical facility equipped with 500 beds and multiple departments.

As the ambient sites, Green Model Town Residential Area and Mukarram Hussain Khundker Bhaban were chosen. In the Green Model Town area, the living room space of a six-story building was chosen for the sampling point. The area is full of trees and has less traffic than other sampling places during sampling hours. At the Mukarram Hussain Bhaban, the sampling was conducted at the Atmospheric & Environmental Chemistry Research Laboratory, which was chosen to have a proper air ventilation system and to avoid the potential confounding effect of traffic congestion. Supplementary Table S4 provides an extensive overview of the features of the office structure and sampling site.

Sample collection

During the pre-monsoon season (February to June of 2023), air samples in the hospitals were collected using UV sterilized Quartz filter paper (Gelman, Membrane Filters, Type TISSU Quartz 2500QAT-UP, 47 mm diameter) with a 4.0-minute hold period between each measurement. Particulate matter was collected using a low-volume air sampler, in which the airflow rate (16.7 L per minute) was recorded by an orifice plate inserted between the filter and the vacuum pump. This design employs a filter cassette set up in a single-filter tray and a Partisol FRM® Model 2000 single-channel air sampler. The concentrations of particulate matter ($PM_{1.0}$, $PM_{2.5}$, and PM_{10}) were determined using the AEROCET-531 (USA) air quality monitoring instrument. For three days in a row, each hospital's working hours (8:00 am to 4:00 pm) were selected for the purpose of sampling. Samples of bioaerosol were taken at a height of approximately 1.5 m in order to replicate the human breathing zone's aspiration. IGERESS air quality monitoring device was used to gather temperature and relative humidity data (Model: WP6930S, VSON Technology Co., Ltd, Guangdong, China).

Conditioning of filter paper

We used an ultraviolet irradiation process for 8 h to sterilize the blank quartz filter paper, either killing off any remaining microbiological particles or rendering them inactive. The autoclaved water was used to moisten the irradiated filter paper before it was immediately put in the low-volume air sampler's filter holder. After the

completion of the sampling, a pre-sterilized anti-cutter was used to cut the filter papers into small pieces, which were then added to the 100 mL nutrient broth. The material was completely dissolved in the broth after being agitated for 30 to 40 min on a hot plate (37 °C) magnetic stirrer. Next, using a sterile bent glass rod, 25 µL of the material was spread out over the nutritional agar medium plates. The plates were then incubated at 37 °C for 24 h. Then, the total colony forming units (CFU) were counted. Following sampling, the loaded filter paper was kept at 4 °C until further examination.

Calculation of the bioaerosol concentration

The concentration of bacteria in the bioaerosol was calculated by dividing the CFU by the measured air volume (CFU/m^3)[23].

$$\text{Bioaerosol Concentration } (CFU/m^3) = \frac{Number\ of\ colonies\ \times\ Aliquot\ dilution\ factor}{Volume\ of\ total\ air\ sampling\ \ (in\ m3)} \quad (1)$$

Identification of the bacterial bioaerosol species

Obtaining pure culture

Different bacterial colonies were preliminarily determined only by observing their colony characteristics. Using sterile loops, each colony was removed, and a streak plate experiment was conducted on nutritional agar medium. Following that, the plates were incubated at 37 °C for 24 to 48 h. After getting a pure culture, gram staining, antibiotic sensitivity test, and identification of bacteria were performed.

Gram staining method

At first, a smear was made of a bacterial culture on the glass side, heat-fixed and then applied a primary stain (crystal violet) for 60 s. It was washed gently to remove the dye and added the iodine solution for 60 s. After removing iodine through washing, ethanol was added for 15 s and gently washed the slide again. Then, a counter stain was added, safranin, and the slide was kept for 60 s. After washing, the slides were air-dried and observed under a light microscope with 100x magnification, and the morphology, arrangement, and distinguishing features of the bacterial cells were observed[58].

Antibiotic resistivity of the isolates

The Kirby-Bauer disk diffusion method[24] was used to assess the antibiotic susceptibility of the chosen bacterial isolates. For this, selected bacteria were cultured in liquid nutrient broth media, and from this culture media, 100 µl was taken and spread on Muller-Hinton agar (Difco, USA) plate. After that, antibiotic discs were positioned and incubated at 37°C for the entire night. The sensitivity was then evaluated by measuring the inhibition zone in millimeters and comparing it with the reference chart[24]. Antibiotic discs (Oxoid, England) used for this experiment were tigecycline (15 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), azithromycin (15 µg), cloxacillin (1 µg), colistin (10 µg), chloramphenicol (30 µg), vancomycin (30 µg), cefepime (30 µg), cephalexin (30 µg), and meropenem (10 µg). The guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) were followed in determining the antibiotics' susceptibility and resistance[25].

Identification of bacterial isolates using 16 S rRNA sequencing

For each isolate, a portion of the 16 S rRNA gene was amplified via the Polymerase Chain Reaction (PCR) technique. Total genomic DNA was isolated using the kit (Invitrogen™ PCR Master Mix Starter, UK). Primers 27 F-AGA GTT TGA TCM TGG CTC AG and 1492 R-TAC GGY TAC CTT GTT ACG ACTT were utilized to amplify the specific region of the 16 S rRNA sequence[59]. The PCR was run for 30 cycles and the condition of PCR was annealing at 57 °C for 45 s, primer extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min. The last extension stage was carried out at 72 °C for 10 min. Gel electrophoresis (2% agarose gel) was performed to confirm PCR product amplification. The PCR product was then purified using a kit (FavorPrep™ GEL/PCR Purification Kit, Taiwan), and concentration was determined using nanodrop (Thermo Fisher Scientific, USA). The purified PCR products were then subjected to Sanger sequencing (3500 Genetic Analyzer, Thermo Fisher Scientific, USA). By using the online blast software interface, all sequences were compared to the 16 S rRNA database of bacteria and archaea. The top 10 sequences obtained from the blast findings were taken into consideration for creating phylogenetic trees for each isolate using the Maximum Likelihood procedure in MEGA version 5.25 software[41]. The most closely related sequences for each isolate were found by examining the resultant trees, and the alignment outcome was noted. The names of the species were allocated based on the best match.

Statistical analysis

All statistical analyses were performed using the MS Excel-2019 software. The variations in particulate matter concentrations were examined using one-way ANOVA (analysis of variance). Statistically significant alterations were determined using a paired t-test with a 95% confidence level (p-value = 0.05). The R^2 value was used to measure the proportion of the variance in the bioaerosol concentration with the particulate matter concentration and with the meteorological parameter. The **supplementary section** contains all the applicable ANOVA (Analysis of variance) test equations.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

B.A.K.: Experimental work, initial draft and editing. S.R. : Initial draft, review and editing. N.T. : Experimental and editing. K.B.: Experimental and editing. K.C.D.: Experimental, review and editing. M.S.I. : Conceptual, review and editing. N.A. : Conceptual, review and editing. A.S.: Conceptual and review and editing.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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