MULTI-DRUGS RESISTANT BACTERIA ASSOCIATED PARTICULATE MATTER IN THE AMBIENT ATMOSPHERE OF DHAKA, BANGLADESH

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Abstract

Nowadays, air pollution is confined to the level of air pollutants and bio-aerosols since it might be pathogenic or induce allergic reactions. The research was carried out to discover the bacteria associated with suspended particulate matter in indoor and outdoor air, as well as to investigate their antibiotic susceptibility. The results revealed that the extent of outdoor air pollutants (i.e., $PM_{2.5}$, PM_{10}, HCHO, and TVOC) was significantly higher ($p< 0.01$) than the extent of indoor air pollutants. Culturing the sample filter paper in Nutrient Agar (NA) media at two different temperatures, 25°C and 37°C, allowed us to measure the quantity of bacteria in the air. The concentration of bacteria was 622±22 $CFUm^{-3}$ at 26±2°C and 11±2 $CFUm^{-3}$ at 20±2°C in both outdoor and indoor air, respectively. Positive *Bacillus, Micrococcus luteus, Pseudomonas stutzeri* and *Brevundimonas diminuta* bacteria were identified. All of the identified bacteria were found to be pathogenic. Moreover, some of the identified bacteria showed resistance to some commercially available antibiotics, such as Cefixime, Ceftazimidine, Nalidixic acid, Ampicillin, Ciprofloxacin, Gentamycin, etc. The positive correlation between fine particles and the bacterial concentration (R^2= 0.75 for indoor and R^2= 0.68 for outdoor air) revealed that the bacteria were highly associated with fine particles than coarse particles. Furthermore, the number and growth of bacteria were affected by the meteorological parameters (i.e., temperature and relative humidity). The rise in relative humidity favoured the increase in bacterial concentration. Therefore, the risk of being affected by bio-aerosol is higher in the wet season than that of the dry season.

***Key words*:** Bio-aerosol; Airborne bacteria; Particulate matter; Antibiotic; Air pollutants.

INTRODUCTION

Atmospheric fine particulate matter ($PM_{2.5}$) is the most serious environmental risk factor for premature death in the globe (Rao *et al*. 2021). The 92% of the global population is exposed to $PM_{2.5}$ concentrations which is above the world health organization air quality guidelines (WHO AQG) annual average limit of 10 µg/m^3 (WHO 2016). The presence of humans and animals in the environment may cause the formation of airborne particles. If bacteria are present in the air together with particulate matter, the pollution becomes worse (Kalogerakis *et al.* 2005). Bio-aerosols are a subgroup of atmospheric particles that are directly released into the atmosphere from the biosphere. Matthias *et al*. (1995), Glikson *et al*. (1995) reported that bio-aerosols contribute around 24% of the total atmospheric particles and 5-10% of the total suspended particulate matter. Mandal *et al*. (2011) estimated that bioaerosols were responsible for approximately 5 to 34% of indoor particulate matter air pollution. Bio-aerosols are composed of both living and non-living components such as bacteria, viruses, fungus, and pollens. However, bio-aerosol is now posing a serious health threat to the world. Srikanth *et al*. (2008) predicted that the occurrence of bioaerosols could cause certain human infections, such as pneumonia, influenza, measles, asthma, allergies, and gastrointestinal illness. Several studies suggest that certain airborne bacteria are antibiotic resistant (Yoneyama *et al.* 2006, Zhu *et al.* 2020). Additionally, if the bacteria are resistant to antibiotics, the risk to health immunity grows substantially. The resistance of these bacteria might be inherited or acquired from the other pathogenic bacteria. The inherited resistance might be due to the low membrane permeability (Yoneyama *et al*. 2006). The resistance might be acquired by the genetic exchange between

pathogenic bacteria and environmental bacteria, resulting in pathogenic environmental bacteria. Bacteria can resist antibiotics in two distinct ways, i) preventing the antibiotic from reaching the target at a sufficiently high concentration (Walsh *et al.* 2000), and ii) by modifying or evading the antibiotic's target (Martinez *et al.* 2014). Humbal *et al*. (2019) predicted that temperature and relative humidity are the most important meteorological parameters that enhance bacteria's feasibility. During the twenty-first century, Antibiotic Resistant Bacteria have emerged as a severe global threat to human health and have become a critical global issue (Zhu *et al*. 2020). Antibiotic resistance is widely dispersed from human-made sources, such as sewage, sludge, hospitals etc. (Tang *et al*. 2015). Jing *et al*. (2018) stated that the urban air is getting polluted by ARG (antibiotic-resistant genes). Different cities are being risked with unpredictable health risks associated with airborne ARG exposure. According to Wehener (2002), antibiotic usage in animals contributes to the development of antibiotic resistance in human pathogens. Yoneyama *et al*. (2006) suggested that the broad use of antibiotics has raised a serious public health problem due to multidrug resistance of bacterial pathogens, which gradually grow resistant to every new drug introduced in the clinic. Zhu *et al*. (2020) showed that snowfall commendably spreads ARGs from point sources over the Earth's surface. According to Bragoszewska *et al.* (2018), the strains of *Micrococcus luteus* D. and *Micrococcus equipercicus* exhibited the most antibiotic resistance among their interesting bacteria. Ribeiro *et al*. (2009) predicted that the soil, dust, and plants contain the most common spore bearer *Bacillus cereus*. According to Arnesen *et al*. (2008), this species might be presented in the food production environment by its adhesive nature in the endospore. Based on this, Bottone (2010) suggested that the bacteria spread to all kinds of food due to their characteristics. According to Andersson *et al*. (2018)'s prediction, bacterial transmission from environment to human host or from host to host can be occurred by associating with a genome that results from the successful transmission of antibiotic resistance to the human host.

Bangladesh suffers from severe air pollution, which has resulted in the deaths of around 137 thousand people. Dhaka became the topmost polluted city during the last couple of years due to the worst air quality. It is quite unusual to find research studies on this topic. To our knowledge, no research has been conducted in Bangladesh to identify bio-aerosols and ARGs. Only a small number of people across the world have conducted research on the identification of bacteria in the environment, as well as their antibiotic susceptibility. To address this problem, a study was conducted to estimate the amount of airborne bacteria present in Dhaka's ambient atmosphere. The main objectives of this work were to quantify and identify bacteria in both the indoor and outdoor environments and investigate their antibiotic susceptibility.

METERIAL AND METHODS

Sampling Site

The sampling sites were divided into two groups to assess the extent of bacteria and suspended particulate matter, as seen in Fig. 1. The spot one was an indoor atmosphere and the other was an outdoor environment. The ground floor of the Mokarram Hussain Khundker Bhaban, University of Dhaka, was chosen as an indoor environment. It is the corridor between classrooms and laboratories, commonly used by teachers and students. On the other hand, the gate of the Curzon Hall area near the Doyel Chattar was selected as an outdoor environment. It is a low-traffic area but crowded by different kinds of mobile food corners. Moreover, this area is surrounded by numerous plants.

Sample Collection

Particulate matter was collected, and atmospheric parameters (temperature and relative humidity) were monitored from both indoor and outdoor conditions during the winter season (January-

February 2020) to investigate air quality. The winter season was chosen because its environment is dry and relatively more polluted than the summer and rainy seasons.

The air sampling (filter based particulate matter) was carried out by following a filtration process in which the air is passed through a filter medium separating the particulate matter. The Low Volume Air Sampler collected suspended particulate matter for 8 hours using UV (254 nm) sterilized Quartz filter paper (Gelman, Membrane Filters, Type TISSU Quartz 2500QAT-UP, 47 mm diameter). Real time particulate matter ($PM_{2.5}$ and PM_{10}) concentrations were measured using the IGERESS Air quality monitoring device (Model: WP6930S, VSON Technology Co., Ltd, Guangdong, China). It has a laser-based sensor that uses the light scattering method to detect and count particles of different size fractions. The Formaldehyde Detector (Air Quality Monitor, TVOC, HCHO, Temp/Humid (B078ZS8RVL)) was also used simultaneously to monitor the meteorological parameters (viz. temperature, relative humidity, gaseous substances, etc.) at three different times of the day, (10.00am, 2.00pm, and 6.00pm) while collecting the samples.

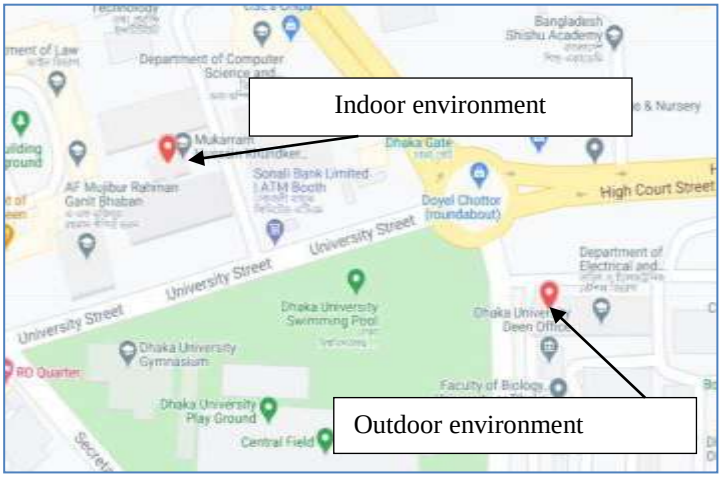


Fig. 1. Map of the sampling locations at both indoor and outdoor environment, University of Dhaka, Bangladesh.

Conditioning of Filter Paper

The blank quartz filter paper was sterilized by ultraviolet irradiation process for 8 hours. The irradiated filter paper was moisturized with autoclaved water and instantly placed in the filter paper holder of the Low Volume Sampler. After the sampling, the filter paper had been preserved at 4°C temperature till further analysis.

Analysis method

The sample in the filter paper was extracted in nutrient broth. The filter papers were cut into small pieces with a pre-sterilized anti-cutter and added to the nutrient broth. The loaded sample was then stirred for 30-40 minutes to ensure that it was completely dissolved in the nutrient broth. Then 100 µl of the extracted sample placed on nutrient agar (Hi media, India) media plates with a micropipette were spread with a sterilized bent glass rod. The plates were then incubated at 25°C and 37°C temperatures both for 24 hours. Then the total colony forming units (CFU) were counted.

The cell morphology of bacterial culture was initially identified through microscopic observation using the Gram-staining method (Rita *et al*. 2009). The cultured bacteria were identified using the VITEK 2 (BioMerieux) (Thomas *et al.* 2003). The selected bacterial isolates were then subjected to antibiotic susceptibility tests by the Kirby-Bauer disk diffusion method (James *et al*. 1973). For this,

100µl of the above culture was spread on Muller-Hinton agar (Difco, USA) plate. Antibiotic discs were then placed and incubated overnight at 37ºC. After that, the inhibition zone was measured in millimetres and compared with the standard chart to determine their sensitivity (James *et al*. 1973). Antibiotic discs (Oxoid, England) used for this experiment were Ampicillin, Cefixime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Doxycycline, Gentamicin, Imipenem, Nalidixic acid, Penicillin G, Tetracycline, and Vancomycin. The resistance and susceptibility of antibiotics were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (James *et al*. 2007).

Statistical Analysis

Single factor ANOVA (p value) was used to evaluate whether the difference in abundance of bacteria in different air pollution level is statistically significant or not using Microsoft office (excel) 2013. Regression analysis (R^2) was performed to test the association between different atmospheric parameters using Microsoft office (excel) 2013 (Zhu *et al*. 2020). The correlation between the two items was considered statistically strong with $R^2 > 0.8$ and a p value < 0.01 according to Li *et al*. (2015).

RESULTS AND DISCUSSION

Predominant Extent of Outdoor Particulate Matter

The concentration of particulate matter ($PM_{2.5}$, PM_{10}) in outdoor conditions was greater than in indoor environments as shown in fig. 2. This might be as a result of anthropogenic activities and transportation pollutants near the outdoor sample site (Maraziotis *et al*. 2008). The use of mobile food stalls might also result in an increase in particulate matter emissions. However, on day 3, both $PM_{2.5}$ and PM_{10} concentrations were higher in the indoor atmosphere than in the outdoor environment during the midday time. On the day of the sampling, building extension work was underway, which may explain the higher concentration of particulate matter in the indoor environment.

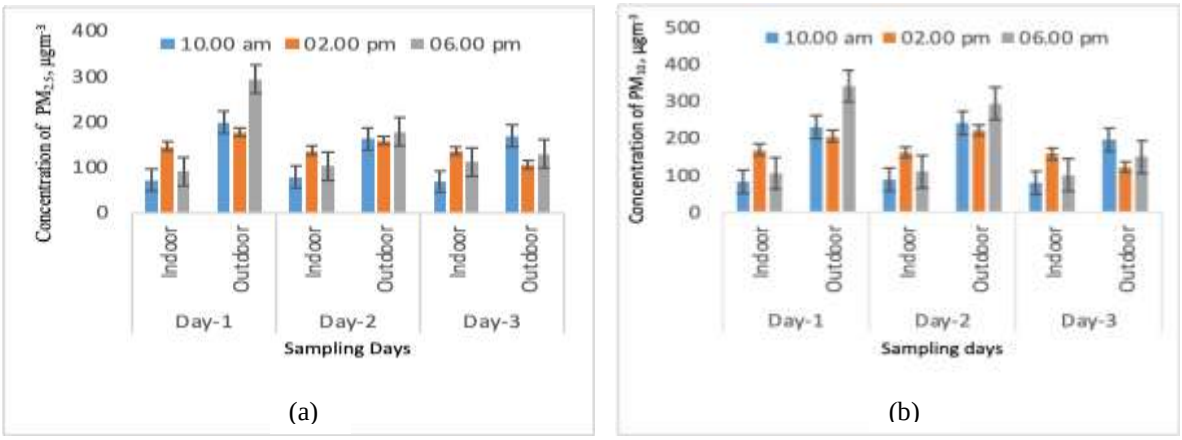
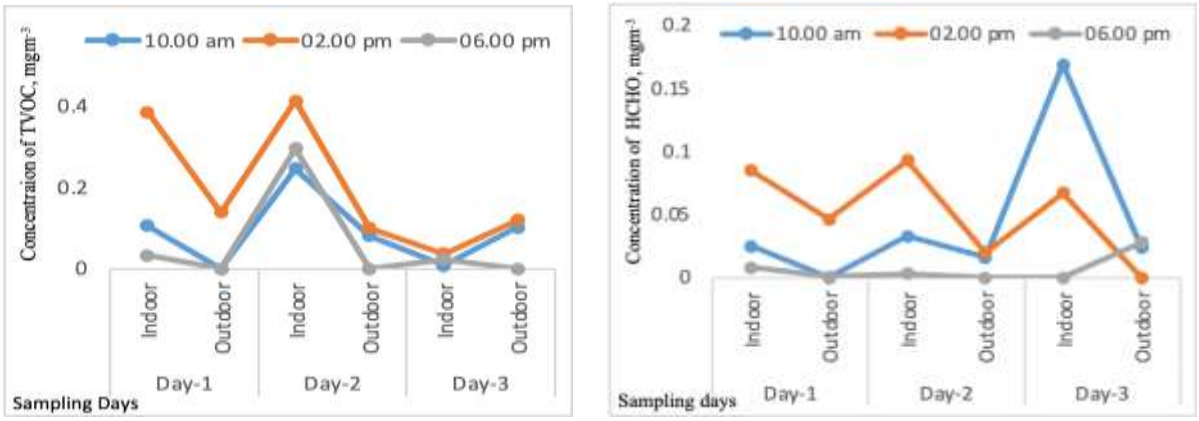


Fig. 2. Comparison of the concentration of (a) $PM_{2.5}$, and (b) PM_{10} in both indoor and outdoor environments.

Comparison between Indoor and Outdoor Gaseous Pollutants

The concentration of gaseous contaminants, such as TVOC and HCHO, was higher in the indoor atmosphere than in the outdoor environment (Fig. 3). The presence of animals, humans, and their activities, such as natural gas combustion, pressed-wood products, and paintings results in a high concentration of HCHO in the indoor environment. The biomass burning on 19th January in the indoor environment may elucidate the higher concentration of HCHO in the morning on the day 3.

The greater extent of TVOC might be due to the higher anthropogenic sources, such as paints, aerosol sprays, cleansers and disinfectants in the indoor environment, which generates higher gaseous pollutants than that of the outdoor environment.



(a) (b)

Fig. 3. Comparison between the indoor and outdoor gaseous pollutants (a) HCHO (mgm^{-3}) and (b) TVOC (mgm^{-3}).

The outdoor environment contains a higher concentration of bacteria than the indoor environment (Table 1). The number of colonies forming units per meter cube ($CFUm^{-3}$) of cultivable bacteria was determined and is shown in Table 1. The average $CFUm^{-3}$ of bacteria in the indoor and outdoor environment was 11.33±0.50 and 600±22.00 $CFUm^{-3}$, respectively. The total load of bacteria in the indoor environment was substantially lower than in the outdoor environment, as shown by this finding. The isolated bacteria were then identified. Positive *Bacillus*, *Micrococcus luteus*, *Brevundimonas diminuta*, and *Pseudomonas stutzeri* were found to be the most commonly isolated bacteria (Table 1).

Table 1. Identification and Quantification of bacteria in the indoor and outdoor environment.

Indoor				Outdoor			
Sampling site	**Sampling day**	**Bacteria present in the sample**		**Sampling site**	**Sampling day**	**Bacteria present in the sample**	
		CFU/m^3	**Identified species**			**CFU/m^3**	**Identified species**
Ground floor of Mukarram bhaban	Day-1 (05/01/20)	11 ±0.50	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*	A traffic area in the Curzon hall gate	Day-1 (19/01/20)	600 ±22.00	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*
	Day-2 (12/01/20)	11 ±0.50	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*		Day-2 (26/01/20)	622 ±22.00	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*
	Day-3 (20/01/20)	12 ±0.50	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*		Day-3 (02/01/20)	578 ±22.00	Positive *Bacillus* *Micrococcus luteus* *Brevundimonas diminuta* *Pseudomonas stutzeri*

According to these findings, the outdoor environment has a higher concentration of bacteria than the indoor environment. There were so many mobile food corners, mobile shops, and crowded with

people in the outdoor environment's sampling site, resulting in higher emission of bacteria. The weekly variance, on the other hand, revealed that the environmental atmosphere holds about the same number of bacteria. Many studies have shown that Positive *Bacillus*, *Micrococcus luteus*, *Pseudomonas stutzeri, and Brevundimonas diminuta* are potentially pathogenic (Xiang *et al.* 2005, Gilardi *et al.* 2016, Qudiesat *et al*. 2009, Bottone *et al.* 2010).

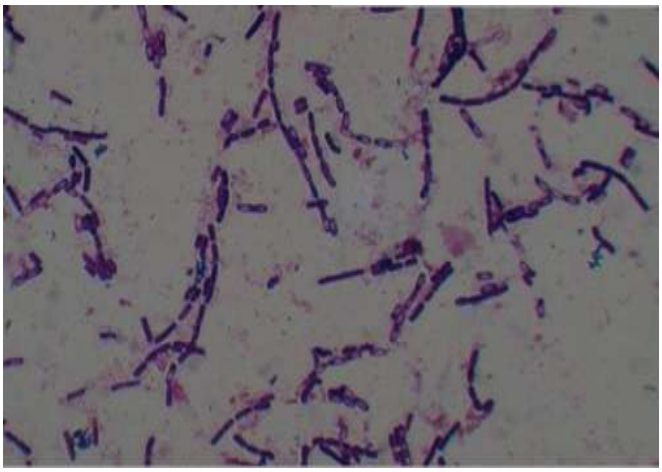


Fig. 4. Gram Staining of Positive bacilli. The image was taken under a light microscope (X 100).

The ANOVA analysis reveals that the concentrations of bacteria in both indoor and outdoor environments are significantly correlated ($p< 0.01$). Because of the higher exposure, the outdoor bacterial abundance is observed to be higher than that of the indoor environment. This may be because of anthropogenic activities, such as talking, sneezing, and coughing are more prevalent in outdoor environments (Chen *et al*. 2009). Additionally, the crowding of people and mobile food vendors outside the Curzon Hall gate may be contributing to the high concentration of outdoor bacteria. Furthermore, a higher concentration of air pollutants in the outdoor atmosphere favours the growth of bacteria.

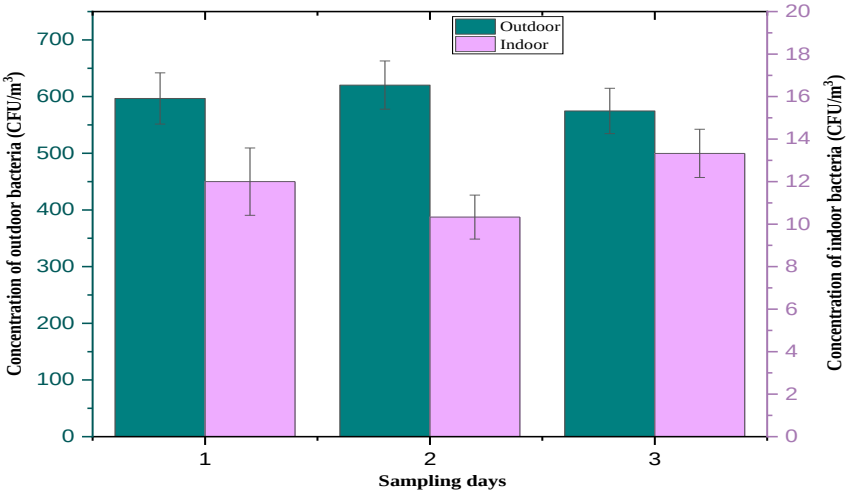


Fig. 5. Concentration of bacteria (CFU/m^3) at both indoor and outdoor sampling locations in Dhaka, Bangladesh.

Resistance of Isolated Bacteria against Multiple Antibiotics

Antibiotic susceptibility test showed that all of the bacteria tested, except for positive Bacillus isolate-2, were resistant to some of the antibiotics. Positive Bacillus isolate-2 was found to be antibiotic-sensitive against all antibiotics tested. Among the isolates, isolate-1 was found resistant against 2 antibiotics (Cefixime and Ceftazidime), isolate-3 against 5 antibiotics (Cefixime, Ceftazidime, Ceftriaxone Penicillin G and Tetracycline), and isolate-4 against 3 antibiotics (Cefixime, Ceftazidime and Nalidixic Acid), isolate-5 against 3 antibiotics (Cefixime, Ceftazidime and Ceftriaxone), Table 2 and Fig. 6. These findings indicated that the isolates were MDR (Multiple Drug Resistant), which is concerning from a public health standpoint.

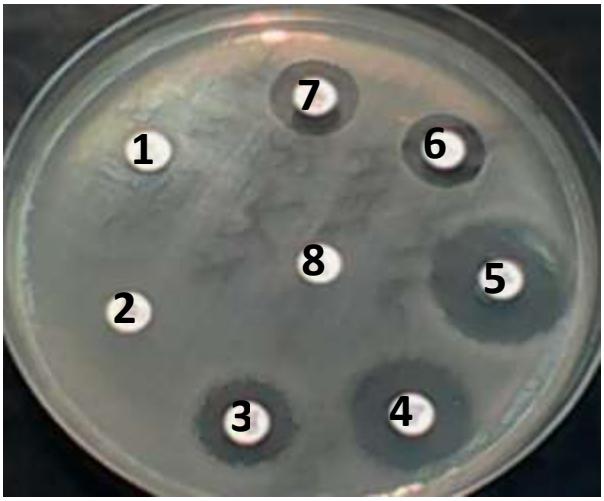


Fig. 6. Representative figure of antibiotic sensitivity of positive *Bacillus* isolate-4. showing, 1. Cefixime, 2. Ceftazidime, 3. Gentamycin, Ceftriaxone, 4. Ciprofloxacin, 5. Imipenem, 6. Ampicillin, 7. Vancomycin and 8. Nalidixic acid.

According to the results, there may be a large number of contaminants in the air that we were unable to detect or that are not cultivable. Additionally, the whole procedure was carried out during the dry season, when the bacterial viability was limited. This may be one of the causes for the inability to identify additional pathogens. However, during the wet season, bacterial viability is increased due to the increased humidity, which favours the number and growth of bacteria. As a result, the health risks are greater during the rainy season than during the dry season.

Table 2. Antibiotic Susceptibility Test of Identified Bacteria

Isolates	Name of the Antibiotics											
	Ampicillin	Cefixime	Ceftazidime	Ceftriaxone	Ciprofloxacin	Doxycycline	Gentamycin	Imipenem	Nalidixic Acid	Penicillin G	Tetracycline	Vancomycin
1	S	R	R	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S
3	R	R	R	R	S	S	S	S	S	R	R	S
4	S	R	R	S	S	S	S	S	R	S	S	S
5	S	R	R	R	S	S	S	S	S	S	S	S

R- resistant; **S-** sensitive

Correlation between Bacteria and Meteorological Parameters

The influence of metrological parameters (viz. temperature, relative humidity) on the bacterial number and growth were also studied. Due to temperature variations, the development of culturable bacteria was significantly higher in the spring/winter season than in the summer season (Humbal *et al*. 2019). The bacterial concentration positively correlates with the temperature supported by the previous study (Humbal *et al*. 2019).

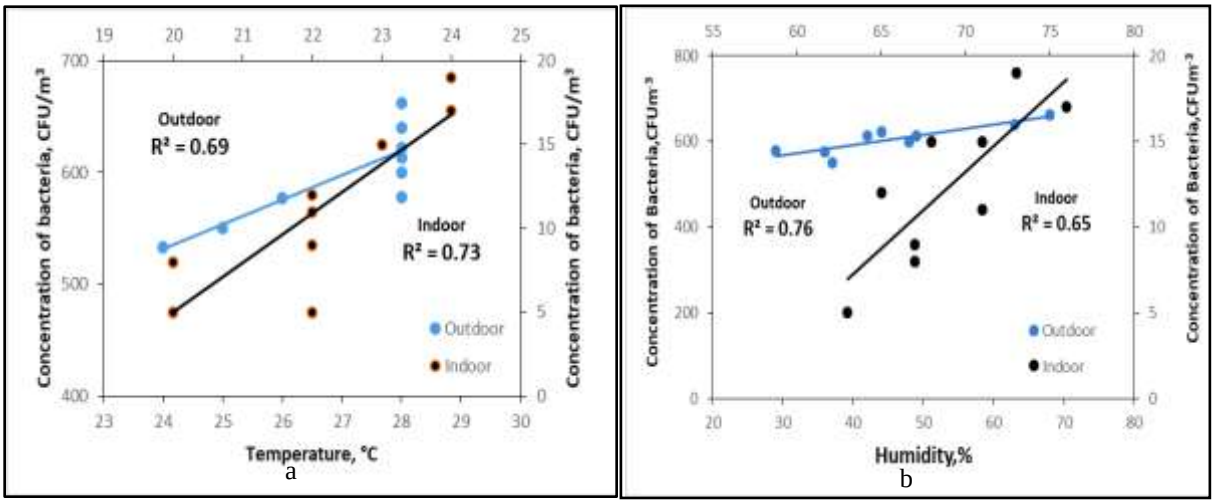


Fig. 7. Effects of meteorological parameters on the concentration of bacteria (a) effect of temperature and (b) effect of humidity.

Increase in bacterial concentration is facilitated by temperature rise depicted in Fig. 7(a). The ANOVA: single factor test indicates a significant ($p< 0.01$) effect of temperature on the number and growth of bacteria. The variation of indoor bacteria is moderately correlated ($R^2= 0.73$) with temperature than outdoor bacteria ($R^2=0.69$). Fig. 7(b) reveals that raising the relative humidity value favours bio-aerosol viability because higher relative humidity promotes the metabolic activities of bacterial particles. The growth of bacterial particles is observed to be positively correlated with relative humidity, which is consistent with previous work. The presence of water in the air promotes the growth of bacteria (Humbal *et al*. 2019). In the case of humidity, it is observed that the outdoor bacterial concentration ($R^2= 0.76$) is largely correlated with humidity than that of indoor bacteria ($R^2= 0.65$). This may be attributed to the fact that the percentage of humidity in the indoor atmosphere is higher than in the outdoor environment due to the lower temperature.

Variations of Bacteria Concentration with Particulate Matter

Fig. 8(a) depicts an important ($p<0.01$) relationship between bacterial concentration and particulate matter concentration, with a rise in bacteria concentration found with rising fine particles ($PM_{2.5}$) concentration ($R^2= 0.75$ for outdoor and $R^2= 0.68$ for indoor). Though the value of the concentration of both indoor and outdoor bacteria and PM_{10} is significant ($p< 0.01$), there is a weak correlation between them. The regression factors in the Fig. 8(b) reflect the weak correlation between both indoor ($R^2= 0.34$**)** and outdoor ($R^2= 0.27$**)** bacteria with PM_{10}. Comparing both Figs. 8(a) and 8(b)**,** it can be predicted that the bacteria are highly associated with fine particles instead of coarse particles as the associated components of the particulate matter favours bacteria's growth.

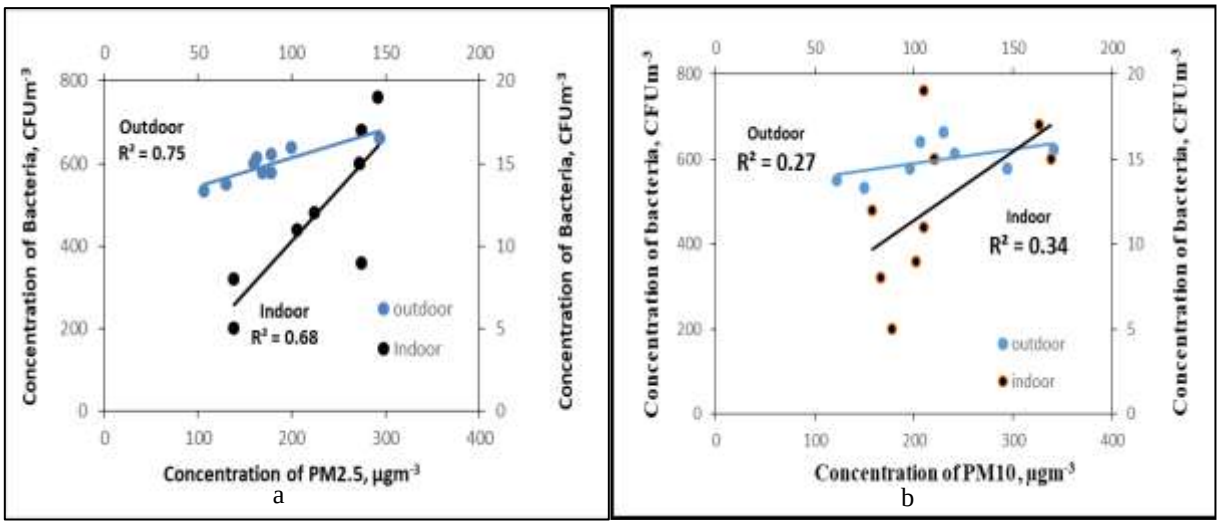


Fig. 8 Variations of bacterial concentration (a) with $PM_{2.5}$ and (b) with PM_{10}

Therefore, the more fine particles in the atmosphere, the more will be the pathogens in the air associated with the fine particles, indicating that the higher air pollution prevail higher concentration of bacteria, resulting in greater pathogenic risk to public health. The resistance of these pathogens towards antibiotics increases the headache about health hazards exponentially. Moreover, the environmental parameters control the extent of bacteria. During the high humidity or wet season, the complications become high than that of the dry season because the higher humidity helps the growth of pathogens. Therefore, to compensate for these pathogen-related health risks, humans must be aware of air emissions and take appropriate measures to regulate anthropogenic practices, which are the cause of air contaminants.

An investigation into the antibiotic resistance of bio-aerosol in the ambient environment of Dhaka city was undertaken. The identified bacteria were Positive *Bacillus*, *Micrococcus luteus*, *Pseudomonas stutzeri* and *Brevundimonas diminuta.* The first two bacteria are gram-positive bacteria that could be pathogenic, and the second two are gram-negative bacteria that could be pathogenic under certain circumstances. The results suggest that the outdoor environment has a greater concentration of bacteria as well as a higher level of air pollutants. The maximum concentration of bacteria was 622±22 $CFUm^{-3}$ at 26±2°C and 11±2 $CFUm^{-3}$ at 20±2°C in both outdoor and indoor environments, respectively. The primary reason for the increase of bacteria in the outdoor environment was human-made activities. The meteorological parameters were found to affect the number and growth of the bacteria. A positive correlation of bacterial concentration of ambient environment with both temperature (R^2= **0.73** for indoor**,** R^2= **0.69** for outdoor**)** and humidity (R^2= **0.76** for outdoor**,** R^2= **0.65** for indoor) was found, which indicates that the bacteria's concentration of ambient environment is entirely dependent on the meteorological parameters. Additionally, the extent of bacteria is highly associated with fine particles, such as $PM_{2.5}$ (R^2= **0.75** for outdoor, and R^2= **0.68** for indoor). Furthermore, the antibiotic resistance of the reported bacteria raises concerns about health risks since they were immune to certain widely active antibiotics, such as Ampicillin, Cefixime, Ceftazimidine, Ceftriaxone, Penicillin G, Tetracycline, and others. Therefore, inhaling the polluted air hinders our normal lung functions and damages our immunity

slowly but surely, as the air is associated with antibiotic-resistant bacteria. This poses a serious challenge to public health because these antibiotics could be ineffective in combating pathogens.

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