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CHARACTERISTICS AND ANTIBIOGRAM OF AIRBORNE BACTERIAL COMMUNITIES

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Abstract

Composition and concentration of the bacterial communities in air of using a culture-dependent plate exposure technique and air sampling with filter were analysed. An average value of 2.8×10^3 bacterial CFU/m^3 was found in different seasons. A total of 8 bacterial genera were identified among 58 species. The most predominant bacteria were *Bacillus* (73%), *Planococcus* (9%), *Staphylococcus* (6%), *Micrococcus* (4%), *Deinococcus* (2%), Arthrobacter (2%), *Microbacterium* (2%), and *Exiguobacterium* (2%). The distribution of predominant airborne bacteria varied greatly between different sampling sites and seasons and was limited by environmental parameters, as well as human activities.

Introduction

Many environments contain diverse communities of microorganisms (Aziz 2018). The atmosphere is the main vehicle for the transportation and dispersion of microorganisms (Nugari *et al.* 1993). Constituents of the bioaerosol in the atmosphere may originate from both the natural and anthropogenic sources, including vegetation and soil (Lighthart 1998), vegetables (Lindemann 1985), animal feeding (Wilson *et al.* 2002), and traffic (Wu *et al.* 2007). Airborne microorganisms are crucial components of the atmosphere, with bacterial cells exceeding 1×10^4 m^{-3} (Burrows *et al.* 2009), and they can threaten human health by disseminating allergens and pathogens (Griffin 2007). Many workers have investigated structural variation in the airborne bacterial community during haze events (Zhen *et al.* 2017, Du *et al.* 2018, Sun *et al.* 2018, Yan *et al.* 2018). Investigations have focused more and more on the ecological, environmental, and climatological effects of microbiology in air (Tong 2000, Rosenfeld *et al.* 2019). The air is often considered as an important carrier medium for bacterial pathogens, such as *Streptococcus pneumoniae, S. pyogenes*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* (Richards *et al*. 1999). To manage outdoor pathogens, it is important to understand and identify their source, survival, dispersal, and relation to the environment, in addition of these more information on the detection, monitoring, and transportation of bacterial pathogens in the atmosphere need to be considered also (Kuske 2006, Lai *et al.* 2009). Indoor and outdoor airborne bacterial community types changed independently of haze events, and the special airborne bacterial community type was closely related to the incidence of pneumonia in the heavy hazy season (Guo *et al.* 2020).

Dusts are the most common carriers of microorganisms that could be explored in the environment depending on the air movement and concentration of dust particles. Ambient air pollution contributed substantially to the global burden of disease in 2015, which increased over the past 25 years, due to population ageing, changes in non-communicable disease rates, and increasing air pollution in low- and middle-income countries. Modest reductions in burden will

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occur in the most polluted countries unless $PM_{2·5}$ values are decreased substantially, but there is potential for substantial health benefits from exposure reduction (Cohen *et al*. 2017). According to UN statistics, level of dust in the air in Dhaka is one of the highest of the world. Common people are the worst victims as they must go out for work and many of them work outdoors. Airborne microorganisms in the Dhaka city are extremely important because of the economic and health problems. Thus the present study was conducted for the characterization and antibiogram of bacterial diversity in air spaces of Dhaka city.

Materials and Methods

Dhaka a mega city and one of the major cities of South Asia is located in central Bangladesh at 23°42′0″N 90°22′30″E, on the eastern banks of the Buriganga river, Dhaka, along with its Metropolitan area, with a population of 20.28 million in 2019 and making it the largest city in Bangladesh (BBS 2019). With a density of 47,400 people per square kilometer, Dhaka remained the most densely populated city on earth for the third consecutive year and it has ranked the second least livable city in the world, according to the Economist Intelligence Unit's annual global survey (Amin 2018).

Samples were collected from seven different sampling sites of the Dhaka metropolis, *viz*., Hazrat Shahjalal International Airport, Gulshan-2 circle, Farmgate bus stand, Dhaka Medical College and Hospital (DMCH), Curzon Hall University of Dhaka, Railway Station, Kamlapur, and Sadarghat Launch Terminal. Global Positioning System (GPS) tracks satellites orbiting the earth to calculate precise location. This is done often through means of a handheld, portable Apple iphone 10S (USA), which monitors the satellites to determine exact latitude and longitude.

Two different techniques *viz.*, exposure plate technique and membrane filtration technique were used for the enumeration and isolation of microorganisms from free air spaces. Air sampler (BK-G2.5, Ambient air sampler, Bulgaria) was used to collect the sample from different sampling sites and air was passed through the filter for two minutes. Prior to sampling, the instruments were placed on the ground level with their membrane filter paper holder about 1.5 meters above the ground level. The air passed through the filter sampler (Z269360, USA) was set to 0.029 m^3 per minute average on the Petri dishes containing two different media *viz.*, nutrient agar medium and plate count agar which were used for the enumeration and isolation of aerobic heterotrophic bacteria corresponding to filter collection of airborne particles (Atlas 2010). Exposure plate technique was used for the enumeration and isolation of bacteria (Sharp and Lyles 1969). All the culture plates were marked with sample name and incubated at 37°C for 48 hrs. Bacterial colonies were counted by a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were transferred onto nutrient agar slants. During this investigation, a total 46 out of 350 isolates from nutrient agar medium were randomly selected and purified for detailed identification.

After incubation, the number of colonies forming units (CFUs) on each culture plate was counted. For each sample, the average CFU was calculated from the duplicate plates, and the standard deviation was found. The average CFU values and the standard deviations found were converted to concentrations per m^3 air, by multiplying with the dilution factor and dividing by the amount of collected air (Fang *et al.* 2007).

$$CFU/m^3 \text{ air} = (\text{Average CFU} \times 200)/(120 \times 0.3\ m^3 \text{ air})$$

CFUs on each plate were enumerated, and bacterial concentrations were expressed as CFU per cubic meter of air (CFU/m^3). Thirty-eight bacterial colonies from the samples were identified using conventional methods by Bergey's manual of systematic bacteriology II (Sneath *et al.* 1986)

and Bergey's manual of determinative bacteriology (Holt *et al.* 1994), and eight isolates were identified by 16S rRNA gene sequencing.

All the bacterial isolates were cultured in LB agar to grow single colony and one colony for each was resuspended in 50 µl sterile miliQ water and subject to heat lysis by heating at 100°C water bath for 5 mins. The lysed cell suspension was centrifuged for 1 min at 13000 rpm in microcentrifuge and then the supernatants were used as the source of template DNA for PCR amplification of 16S rRNA gene. It was stored at – 20°C for further use.

The primer of following sequences CC [F] 5′- CCAGACTCCTACGGGAGGCAGC and CD [R] 3′-CTTGTGCGGGCCCCCGTCAATTC were used for the partial amplification of 16S rRNA gene and for automated sequencing (Moghaddam *et al.* 2018). Added 25 µl volume master mixture in the oil free thermal cycler (UNO II, Biometra) contain PCR buffer, dNTPs, Tag DNA polymerase, sterile miliQ water, primers, and template DNA. The amplifications were repeated 30 times for initial denaturation 5.0 mins at 95°C, denaturation 1.0 min at 94^{o}C, primer annealing 30.0 seconds at 55^{o}C, polymerization 1.0 min at 72°C, final extension 10.0 min at 72^{o}C and the final product was stored at 4^{o}C for further process. The PCR product was run in 1.0% agarose gel electrophoresis under UV light after ethidium bromide staining (Borst 2005). DNA bands were observed on UV-transilluminator and photographed by a Gel Documentation system (Microdoc DI-HD, MUV2I-254/365, Cleaver Scientific, UK). The amplified bands were gel purified using Gel purification kit (Invitrogen) and sequenced from Macrogen, South Korea.

The isolates were identified based on alignment of partial sequence of 16S rRNA gene with the existing sequences available in the database. The sequence generated from automated sequencing of PCR amplified DNA was analyzed through NCBI-BLAST (http://blast.ncbi. nlm.nih.gov/blast.cgi) and rRNA BLAST (http://bioinformatics.psb.ugent.be/cgi-bin/rRNA/ blast-form.cgi) programs to find out possible similar organisms in the databases. A phylogenetic tree of the isolates was generated using neighbor joining (NJ) distance-based algorithm of phylogenetic analysis (Saitou and Nei 1987). Sequences obtained from BLASTN (nucleotide blast) were in FASTA format and relation between each sequence could be known by multiple sequence alignment using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). The data were analyzed to determine the descriptive statistics *viz.*, statistical mean and standard deviation (Sd) with SPSS v.16.0 for Windows (SPSS, SAS Institute Inc. Cary, USA).

The selected isolates were grown in different flasks containing media with various initial pH (4.5, 5.5, 6.5, 7.5 and 8.5) at 37°C and at various cultivation temperature (5, 10, 30, 40, 50, 55, and 65°C) maintaining initial pH of the medium at 7.0 to check the optimum pH and temperature for growth, respectively.

Antibacterial sensitivity test with gentamycin (CN 120), streptomycin (S 10), penicillin G (P 10), polymyxin B (PB 300), neomycin (N 30), vancomycin (VA 30) and rifampicin (RD 5) against the selected isolate was carried out for their ability to grow in the presence of different antibiotics at concentration selected for diagnostic value. The filter paper disks placed on the surface of Muller Hinton agar plates inoculated with 0.1 ml of bacterial suspension (Atlas 1997). Inoculated plates incubated at 37ºC for 24 hrs. Development of a clear zone around the disk indicated sensitivity, while antibiotic disk without clear zone indicated resistance to the antibiotic.

Results and Discussion

`Sampling sites location measured by the GPS technique and are presented in Table 1. Considering the cultural, morphological and biochemical characteristics of the bacterial isolates, provisional identification was made for 38 isolates (Table 2). All 38 isolates were Gram-positive, of which 30 were under the genus *Bacillus* and the others were under the genus *Planoccoccus*,

Staphylococcus and *Micrococcus* (Table 3). Under the genus *Bacillus*, the provisionally identified species were *B. subtilis* (6), *B. alvei* (6), *B. brevis* (5), *B. firmus* (3), *B. stearothermophilus* (3), *B. alcalophilus* (2), *B. pumilus* (2), *B. cereus*, *B. macerans* and *B. panthothenticus* (Table 3).

Table 1. GPS reading of sampling sites.

Sampling sites	North	East
Hazrat Shahjalal International Airport	23°05′53″	90°24′18″
Curzon Hall	23°43′39″	90°24′48″
Dhaka Medical College and Hospital (DMCH)	23°43′35″	90°23′54″
Farmgate bus stand	23°45′28″	90°23′28″
Gulshan circle-2	23°47′42″	90°24′51″
Kamalapur Railway Station	23°43′57″	90°24′52″
Sadarghat Lunch Terminal	23°42′21″	90°24′23″

The rest 8 isolates were identified as *Bacillus megaterium* Y18-10, *Micrococcus luteus*, *Arthrobacter nicotianae* YNA111, *Microbacterium esteraromaticum*, *Bacillus thuringiensis* RUNI, *Exiguobacterium arabatum* 27CpOI17 and *Bacillus cereus* MSSRFS78, *Bacillus thuringiensis* RUNI based on sequence analysis (Table 4).

A phylogenetic tree of the 8 isolates was generated using neighbor joining (NJ) distance-based algorithm (Fig. 1). It was detected that there was only one cluster comprising of two sub-clusters. These sub-clusters grouped *Bacillus cereus* along with *Micrococcus luteus*, *Arthrobacter nicotianae* and *Microbacterium esteraromaticum* in one cluster and *Exiguobacterium arabatum* in other. On the other hand, there were also two small sub clusters completely comprised of *Bacillus thuringiensis*. This observation suggested the evolutionary divergence of these bacteria indicated their evolutionary trend.

The most prevalent bacterial genera are presented in Fig. 2. Significantly higher variation of the genera *Staphylococcus*, *Bacillus*, *Micrococcus*, *Microbacterium* and *Arthrobacter* were detected in Hazrat Shahjalal International Airport (30% of *Staphylococcus*, 20% of *Bacillus*, 20% of *Planococcus* and 10% of each genera of *Microbacterium* and *Arthrobacter*) than those in the other six sites (Tables 3, 4). The proportion of *Bacillus* in the Curzon Hall, Dhaka Medical College Hospital and Farmgate bus stand was 66.67, 75 and 60%, respectively, while it was 100% in the Gulshan-2 circle, Kamalapur Railway Station and Sadarghat Launch Terminal, respectively (Tables 3, 4). In the Farmgate bus stand *Planococcus* was 40% and *Exiguobacterium* was 25% in the Dhaka Medical College Hospital. High light intensity, extreme temperature variations, low amount of organic matter and a scarcity of available water making is a non-hospitable environment for microorganisms and generally unsuitable habitat for their growth. Nevertheless, substantial numbers of microbes are found in the lower regions of the atmosphere (Van Houdt *et al.* 2009). Among the bacteria detected in the atmosphere, the majority is Gram-positive, and many are spore-formers *viz.*, *Bacillus* spp. and *Microbacterium* spp. were particularly dominant in the air during a dust event (Kellogg and Griffin 2006). High microbial diversity (25 genera, 56 species), with a dominant community including *Staphylococcus*, *Bacillus*, *Acinetobacter* and *Kocuria*, was found by the cultivation-based method (Wu *et al.* 2018).

Selected bacterial isolates were allowed to grow within a varied range of temperature 5 - 65°C; of which 40°C was most preferred by all isolates, while A5, S4, S5 and S8 showed highest growth at 50°C and isolates A2, C3 and R4 can grow even at 60°C (Fig. 3). It was found that almost all isolates grew well at pH ranging from 4.5 to 8.5, but pH 6.5 was preferred by most of the isolates (Fig. 4). Out of 46 isolates, 17 viz., A4, A6, A7, A9, A10, D2, D3, D4, F1, F2,

Table 2. Biochemical characteristics of the bacterial isolates.

Isolate no.	Gram reaction	Catalase	Oxidase	Deep glucose agar	Casein hydrolysis	Starch hydrolysis	Tyrosine degradation	Liquefaction of gelatin	VP	MR	Deamination of phenyl alanine	Nitrate reduction	Citrate utilization	Production						D-glucose							
														Utilization of propionate	Indole production	Lipase	Protease	Lecithinase	Acid	Gas	L-Arabinose	D-Xylose	D-Mannitol			
A1	+	+	–	MA	+	+	–	+	+	–	–	+	–	–	–	–	+	+	+	–	–	–	+	–			
A2	+	+	–	MA	+	–	+	+	+	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–			
A3	+	+	–	MA	–	+	–	+	+	–	–	+	–	–	–	–	–	+	+	–	–	+	+	–			
A4	+	+	–	FA	+	+	+	+	+	–	–	+	+	+	–	–	–	+	+	–	–	–	–	–			
A5	+	+	+	MA	+	+	–	+	+	–	–	+	+	–	–	–	–	+	+	–	–	+	+	+			
A6	+	+	+	MA	–	–	–	+	+	–	–	+	–	–	–	–	–	–	+	–	–	+	+	–			
A7	+	+	–	FA	–	+	–	+	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–	–			
A8	+	+	–	A	–	–	+	+	+	–	–	+	–	–	–	–	–	–	+	–	–	–	+	–			
A9	+	+	+	FA	+	–	+	+	+	–	–	–	–	–	–	–	+	+	+	–	+	+	–	–			
A10	+	+	–	FA	–	–	–	+	+	–	–	+	–	–	–	–	+	–	+	–	+	+	+	–			
C3	+	+	+	FA	+	+	–	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–			
C4	+	+	+	MA	+	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–			
C5	+	+	+	FA	+	+	+	+	+	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–			
D1	+	+	+	FA	+	+	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–			
D2	+	+	+	FA	+	+	–	+	+	–	–	+	+	–	–	–	–	+	+	+	–	+	+	+			
D3	+	+	+	FA	+	+	+	+	+	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–			
D4	+	+	+	A	+	+	–	+	+	–	–	+	+	–	–	–	–	+	+	+	–	+	+	+			
F1	+	+	+	FA	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–			
F2	+	+	+	MA	+	+	+	+	+	+	–	+	–	–	–	+	–	+	+	+	–	–	–	+			
F3	+	+	+	MA	+	+	–	+	–	+	–	+	+	–	–	–	+	+	–	–	–	–	–	+			
F4	+	+	+	A	+	+	–	+	+	–	–	+	–	–	–	–	–	–	+	–	–	+	+	–			

(Contd.)

Contd.

F5	+	+	–	FA	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–	+	–
G1	+	+	+	MA	+	+	–	+	–	–	–	+	+	–	–	+	+	–	+	–	+	+	+
G2	+	+	–	A	+	+	+	+	–	–	+	+	–	–	–	–	+	–	+	–	+	–	–
G3	+	+	+	MA	+	+	+	+	+	–	–	+	–	–	–	+	+	–	+	–	–	–	–
G4	+	+	+	A	+	+	+	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
G5	+	+	+	MA	+	+	+	+	–	–	–	+	–	–	–	+	+	–	+	–	–	–	–
R1	+	+	+	MA	+	+	+	+	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–
R2	+	+	+	MA	+	+	+	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	–
R3	+	+	+	A	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	–	+	+	+
R4	+	+	+	MA	+	+	+	+	+	+	–	+	–	–	–	+	+	–	+	–	–	–	–
R5	+	+	+	FA	+	+	+	+	–	+	–	+	–	–	–	+	–	–	+	–	–	–	–
R6	+	+	–	MA		+	+	+	–	+	–	+	+	–	–	–	+	–	–	–	+	+	–
R7	+	+	+	MA	+	+	–	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
R8	+	+	+	MA	+	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
R9	+	+	+	MA	+	+	–	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	–
R10	+	+	+	A	+	+	–	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
S1	+	+	+	A	+	+	+	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
S2	+	+	+	A	+	+	+	+	+	+	–	+	–	–	–	+	+	–	–	–	–	–	–
S3	+	+	+	MA	+	+	+	+	+	+	–	+	–	–	–	+	+	–	–	–	–	+	–
S4	+	+	+	MA	+	+	–	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
S5	+	+	+	MA	+	+	–	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	–
S7	+	+	+	MA	+	+	+	+	+	+	–	+	–	–	–	+	+	–	+	–	–	–	–
S8	+	+	+	MA	+	+	–	+	–	–	–	+	+	–	–	–	+	–	+	–	+	+	+
S9	+	+	+	MA	+	+	+	+	+	+	–	+	–	–	–	+	+	–	–	–	–	–	–
S10	+	+	–	MA	+	+	–	+	–	+	–	+	–	–	–	–	+	+	+	–	–	–	–

+ indicates the positive result and – indicates the negative result, A = Aerobic, FA = Facultative anaerobic, MA = Microaerophilic.

G2, R2, S1, S2, S4, S7 and S9 grew well at pH 7.5 than pH 6.5, while A9, F1, R3 and S2 were found to be alkalophilic in nature and grew well at pH 8.5 and G1 and R7 were found to be slightly acidophilic and grew well at pH 5.5 (Fig. 4).

Table 3. Provisional identification of the selected heterotrophic isolates.

Isolate no.	Provisionally identified names	Isolate no.	Provisionally identified names
A3	*Planococcus* sp.	G5	*Bacillus brevis*
A4	*Staphylococcus* sp.	R1	*Bacillus firmus*
A5	*Bacillus stearothermophilus*	R2	*Bacillus brevis*
A6	*Planococcus* sp.	R3	*Bacillus pumilus*
A7	*Staphylococcus* sp.	R5	*Bacillus subtilis*
A9	*Staphylococcus* sp.	R6	*Bacillus macerans*
C4	*Micrococcus* sp.	R7	*Bacillus brevis*
C5	*Bacillus subtilis.*	R8	*Bacillus alvei*
D2	*Bacillus alcalophilus*	R9	*Bacillus subtilis*
D3	*Bacillus pumilus*	R10	*Bacillus stearothermophilus*
D4	*Bacillus cereus*	S1	*Bacillus brevis*
F1	*Planococcus* sp.	S2	*Bacillus alvei*
F2	*Bacillus firmus*	S3	*Bacillus alvei*
F3	*Bacillus brevis*	S4	*Bacillus subtilis*
F5	*Planococcus* sp.	S5	*Bacillus alcalophilus*
G1	*Bacillus stearothermophilus*	S7	*Bacillus alvei*
G2	*Bacillus firmus*	S8	*Bacillus subtilis*
G3	*Bacillus alvei*	S9	*Bacillus alvei*
G4	*Bacillus subtilis*	S10	*Bacillus pantothenticus*

Table 4. Sequence based identification of the selected bacterial isolates using bioinformatics.

Name of the isolates	Name and accession number of the bacterial isolates having highest similarity		Basis of the bioinformatics parameters		
	Accession number	Scientific name	Strain	Max. coverage score	Identities match (%)
A1	HQ333020.1	*Bacillus megaterium*	Y18-10	1002	99
A2	HE587970.1	*Micrococcus luteus*	-	573	87
A8	JQ071518.1	*Arthrobacter nicotianae*	YNA111	965	99
A10	AB646579.2	*Microbacterium esteraromaticum*	-	966	99
C3	HQ873480.1	*Bacillus thuringiensis*	RUN1	977	99
D1	JN602236.1	*Exiguobacterium arabatum*	27CpOI17	845	94
F4	HQ701803.1	*Bacillus cereus*	MSSRFS78	211	92
R4	HQ873480.1	*Bacillus thuringiensis*	RUN1	998	99

Total 20 isolates were susceptible to all antibiotics at different ranges (Table 5). Isolates A3, A6, G1 were resistant to streptomycin; A7, C3, C4, C5, D3, F2, F4, G3, R1, R2, R4, R5, R8, S2, S3, S5, S7 and S9 were resistant to penicillin G; A10 and S8 were resistant to polymyxin B; and G2 was resistant to rifampicin only. Two isolates G5 and R9 were resistant to both penicillin G and polymyxin B (Table 5).

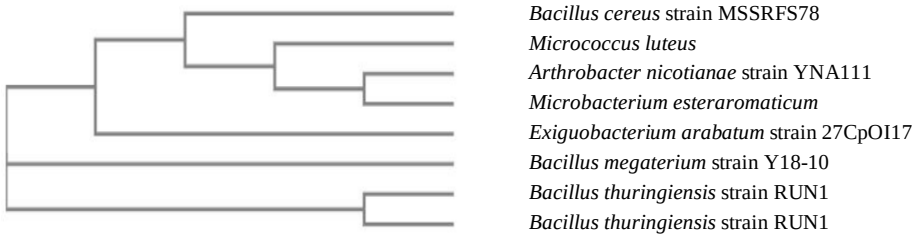

Fig. 1. Evolutionary relationships of the taxa. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987).

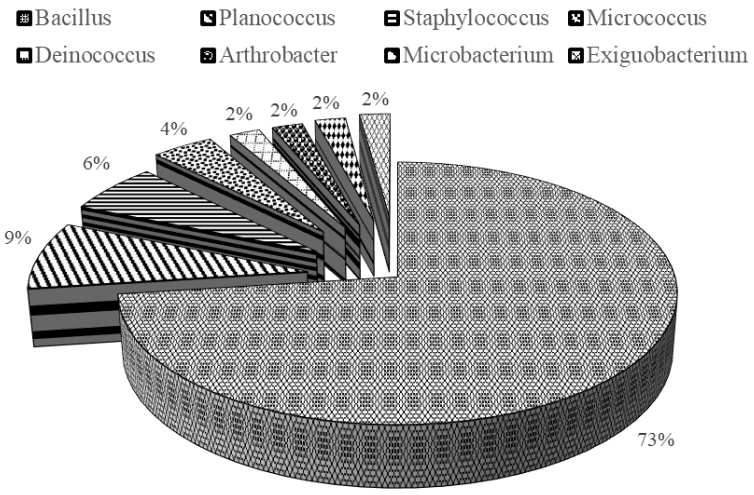

Fig. 2. Proportions of bacterial genera detected among the airborne bacterial community in the Dhaka city.

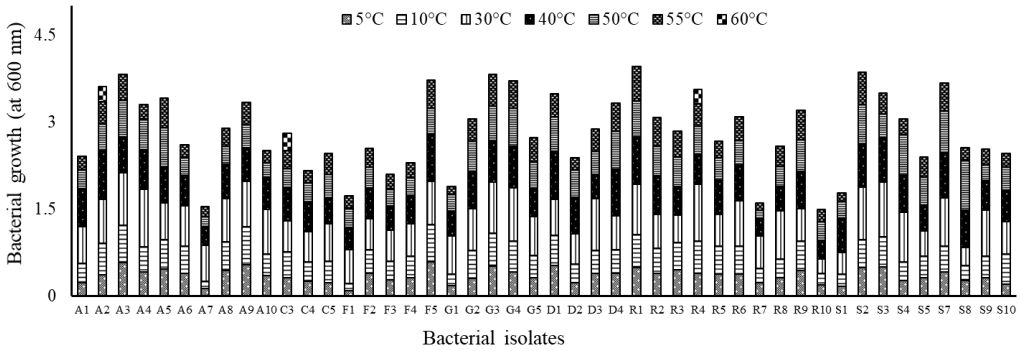

Fig. 3. Effects of temperature on bacterial growth.

Table 5. Antibiogram of the selected isolates.

Isolate no.	Inhibition zone measured in diameter (mm)						
	Name of the antibiotics						
	CN 120	S 10	P 10	PB 300	N 30	VA 30	RD 5
A1	S (9)	S (12)	S (7)	S (10)	S (10)	S (14)	S (6)
A2	S (11)	S (14)	S (22)	S (6)	S (17)	S (11)	S (27)
A3	S (6)	R	S (5)	S (6)	S (13)	S (13)	S (7)
A4	S (10)	S (14)	S (40)	S (7)	S (12)	S (14)	S (22)
A5	S (24)	S (10)	S (25)	S (6)	S (21)	S (13)	S (23)
A6	S (6)	R	S (7)	S (6)	S (11)	S (13)	S (6)
A7	S (12)	S (18)	R	S (9)	S (5)	S (8)	S (4)
A8	S (15)	S (18)	S (40)	S (9)	S (25)	S (20)	S (29)
A9	S (13)	S (15)	S (28)	S (9)	S (17)	S (11)	S (26)
A10	S (6)	S (5)	S (7)	R	S (27)	S (21)	S (4)
C3	S (11)	S (14)	R	S (3)	S (3)	S (9)	S (5)
C4	S (18)	S (14)	R	S (13)	S (22)	S (18)	S (23)
C5	S (13)	S (14)	R	S (4)	S (15)	S (9)	S (4)
D1	S (11)	S (8)	S (28)	S (5)	S (5)	S (9)	S (13)
D2	S (20)	S (14)	S (39)	S (6)	S (22)	S (13)	S (24)
D3	S (11)	S (16)	R	S (3)	S (14)	S (9)	S (6)
D4	S (14)	S (19)	S (32)	S (6)	S (16)	S (13)	S (29)
F1	S (16)	S (15)	S (14)	S (5)	S (18)	S (8)	S (13)
F2	S (16)	S (25)	R	S (2)	S (14)	S (10)	S (8)
F3	S (21)	S (13)	S (4)	S (8)	S (21)	S (20)	S (10)
F4	S (13)	S (16)	R	S (10)	S (19)	S (17)	S (11)
F5	S (4)	S (5)	S (7)	S (10)	S (8)	S (7)	S (15)
G1	S (18)	R	S (38)	S (6)	S (22)	S (14)	S (23)
G2	S (10)	S (12)	S (10)	S (19)	S (17)	S (11)	R
G3	S (13)	S (11)	R	S (4)	S (14)	S (8)	S (3)
G4	S (18)	S (8)	S (7)	S (6)	S (14)	S (16)	S (7)
G5	S (14)	S (5)	R	R	S (8)	S (3)	S (7)
R1	S (13)	S (16)	R	S (3)	S (14)	S (10)	S (3)
R2	S (19)	S (10)	R	S (6)	S (17)	S (14)	S (11)
R3	S (14)	S (12)	S (7)	S (6)	S (25)	S (14)	S (7)
R4	S (11)	S (12)	R	S (4)	S (15)	S (5)	S (3)
R5	S (13)	S (20)	R	S (3)	S (16)	S (11)	S (6)
R6	S (14)	S (16)	S (5)	S (7)	S (2)	S (13)	S (9)
R7	S (23)	S (14)	S (46)	S (7)	S (21)	S (13)	S (24)
R8	S (13)	S (15)	R	S (3)	S (15)	S (9)	S (4)
R9	S (20)	S (11)	R	R	S (16)	S (14)	S (10)
R10	S (18)	S (10)	S (7)	S (7)	S (18)	S (13)	S (9)
S1	S (25)	S (14)	S (8)	S (11)	S (25)	S (18)	S (8)
S2	S (12)	S (13)	R	S (4)	S (16)	S (11)	S (9)
S3	S (13)	S (14)	R	S (14)	S (14)	S (9)	S (3)
S4	S (15)	S (11)	S (3)	S (7)	S (21)	S (14)	S (9)
S5	S (21)	S (12)	R	S (5)	S (15)	S (13)	S (11)
S7	S (11)	S (11)	R	S (5)	S (15)	S (5)	S (4)
S8	S (20)	S (11)	S (23)	R	S (19)	S (13)	S (24)
S9	S (11)	S (11)	R	S (4)	S (16)	S (10)	S (3)
S10	S (10)	S (16)	S (10)	S (8)	S (14)	S (13)	S (15)

S = Sensitive, R = Resistant, CN 120 = Gentamycin, S 10 = Streptomycin, P 10 = Penicillin G, PB 300 = Polymyxin B, N 30 = Neomycin, VA 30 = Vancomycin and RD 5 = Rifampicin.

The distribution of predominant airborne bacteria varied greatly between different sampling sites and seasons and was limited by environmental parameters, as well as human activities. The size distributions of bacteria during different sites also indicated the presence of different bacterial genera in the air. The predominant bacteria under the genus *Bacillus*, *Planococcus*, *Staphylococcus*, *Micrococcus*, *Deinococcus*, *Arthrobacter*, *Microbacterium* and *Exiguobacterium* were mainly distributed in the stages relating to trapping ranges.

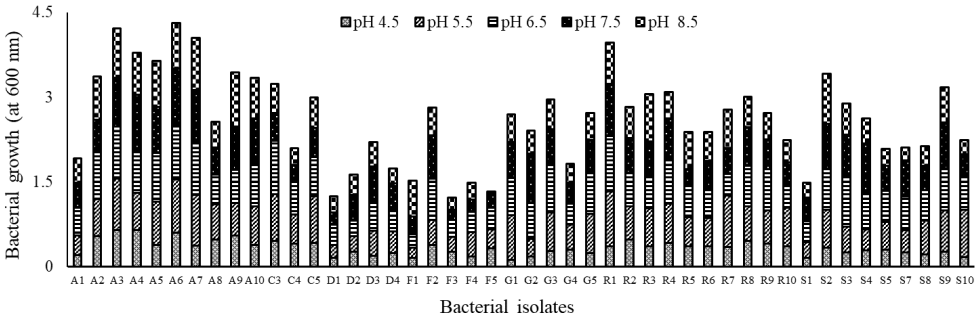

Fig. 4. Effects of pH on bacterial growth.

These results highlight the difficulty in understanding and interpreting the microbial structure of these communities, and consequently, more attention should be paid to the conservation of the city environment.

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