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Microbiological pollutants in air and antibiotic resistance profile of some bacterial isolates

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Abstract

Microbiological quality assessment is one of the most important investigations to determine the pollution of indoor and outdoor air. To evaluate the microbial load in air, samples were collected from 3 different outdoor and 3 different indoor sites within Jahangirnagar University campus. In outdoor air, bacterial and fungal counts varied from 117 - 7284 CFU/m^3 and 88 - 5287 CFU/m^3, respectively. On the other hand, in indoor air bacterial and fungal counts varied from 440 - 6226 CFU/m^3 and 88 - 5874 CFU/m^3, respectively. Furthermore, to reveal the antibiotic resistance profile, *Staphylococcus aureus* isolates were subjected to antibiogram study against 14 antibiotics. Among the isolates, 87.5% exhibited resistance to ceftazidime; 50% to penicillin G; 31.25 % to cefotaxime; 25 % to ceftriaxone, cefuroxime, cloxacillin; and 18.75% to amoxicillin. None of the isolates showed resistance to amikacin, ciprofloxacin, erythromycin, gentamicin, imipenem, nitrofurantoin and vancomycin. The presence of antibiotic-resistant bacteria in air may cause serious health hazard to the people living in this area.

Key words: Air pollution, bacteria, fungi, antibiotic resistance.

INTRODUCTION

Human being, on an average, inhales 14 m^3 air per day (Brochu *et al*., 2006). Presence of high concentration of microorganisms in the inhaled air thus can adversely affect health and activities of the people. Pathogenic living cells present in the air or the chemical substances secreted by the airborne microbes can cause severe human infections and diseases (Stryjakowska-Sekulska *et al*., 2007). Information on microbial concentration in the indoor and outdoor air is necessary not only to estimate the health hazard associated with the inhaled air but also to formulate the strategy to minimize microbial air pollution.

Microbiological contamination of air is mostly caused by bacteria and fungi. They can exist in air as an individual entity or create aggregates of biological structures. However, the survival of microbial cells in air depends on their ability to resist different types of stress *viz*., ultraviolet radiation, desiccation, starvation etc. Some microbial cells produce pigments or mucous halo to protect them form harmful effect of ultraviolet radiation. Spore formation is one of most widely used strategies adopted by many microbes to survive in unfavorable conditions and dissemination of offspring. Airborne microscopic contaminants of biological origin, known as bioaerosol, are easily translocated by winds and air currents from one ecosystem to another, making them an important vehicle for the

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spread of hazardous microorganisms (Eduard *et al*., 2012). Bioaerosols occur as droplets or solid particles and derive from a multitude of natural and artificial sources, such as surface waters, dry soils and agricultural activities (Brandal *et al*., 2014).

Jahangirnagar university (23°52'44.62"N latitude and 90°16'8.57"E longitude), situated about 32 km north-west from the capital city Dhaka, spread over an area of 697.56 acres and about 16 meter high from the mean sea level. There are many woodland, grassland and small lakes in the campus which is a habitat for wildlife and migratory birds. The Jahangirnagar University (JU) campus is surrounded by livestock farms, training centers, agricultural fields and Dhaka-Aricha highway. Due to the presence of large number of herbs, shrubs and trees, the JU campus is generally considered as sanitarium. However, systematic study on the microbiological quality of indoor and outdoor air of this University and the antibiotic resistance profile of the airborne bacteria is almost absent. Thus, this study was undertaken to reveal the actual picture of the air quality of JU campus.

MATERIALS AND METHODS

Sampling strategy: Air samples were collected from 3 different indoor (ID1: Old Arts Building, ID2: Central Library, and ID3: Faculty of Biological Sciences Building) and 3 different outdoor sites (OD1: Bishmile Area, OD2: Outside of Al-Beruni Hall, and OD3: Central Playground) of JU campus (Fig. 1).

Collection of samples: The settle plate method was used to collect airborne culturable microbes (Hayleeyesus and Manaye, 2014). Petri plates containing nutrient agar and potato dextrose agar were used to collect bacteria and fungi, respectively. The plates were exposed to air about 1 m above the land or floor for about 30 minutes in the day time.

Enumeration of microbes: The exposed plates after the required time were covered with the lid and incubated at 37 °C for 24-48 h for the growth of bacteria and at around 25 °C for at least 5 days for the growth of fungi, respectively. The resultant colonies were counted and converted into colony forming unit per cubic meter of air (CFU/m^3) using Omeliansky formula (Hayleeyesus and Manaye, 2014):

$N = 5a \times 10^4 (bt)^{-1}$

Where:

N = CFU /m^3 of air

a = number of colonies per Petri dish

b = dish surface (cm^2)

t = exposure time (min)

Isolation and identification of *Staphylococcus aureus*: For isolation of *S. aureus*, colonies grew on nutrient agar plates were streaked onto mannitol salt agar (MSA). The plates were incubated at 37 °C for 24 h for the growth of bacteria. The appearance of golden yellow colony with a yellow zone surrounding the colony was considered to be

presumptive *S. aureus*. The suspected colonies of *S. aureus* were identified by morphological and standard biochemical tests (Brooks *et al.,* 2007; Cappuccino and Sherman 1996; Holt *et al.,* 1994). Based on the test results, 16 isolates were finally identified as *S. aureus*.

Antibiotic susceptibility test: Susceptibility of the isolated *S. aureus* to antibiotics was determined *in vitro* by employing disc diffusion method (Bauer *et al*., 1966) and recommendations of Clinical and Laboratory Standard Institute (CLSI, 2006). Available antibiotic disc (Oxoid, UK) of amoxicillin (10 µg), amikacin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), cloxacillin (5 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg), penicillin G (10 units) and vancomycin (30 µg) were used for the test. A portion of *S. aureus* colony grown on nutrient agar medium was inoculated in nutrient broth and incubated at 37 °C to obtain a young culture.

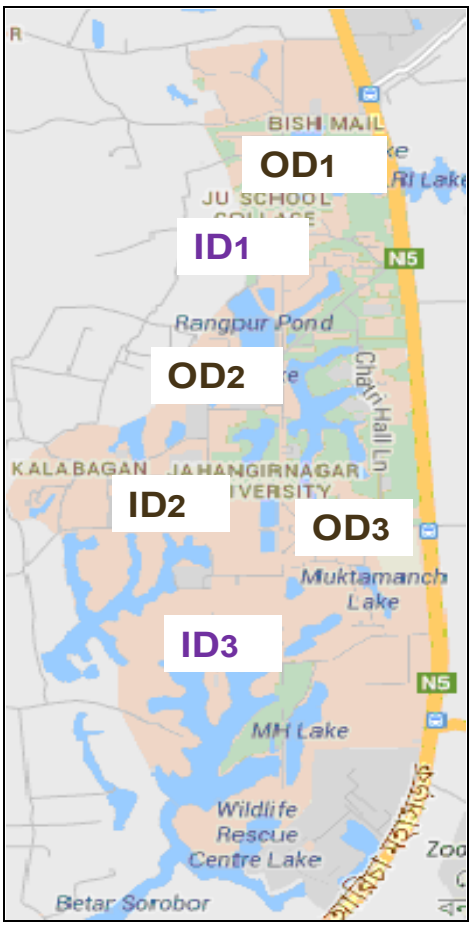

Fig. 1. Map showing outdoor (OD) and indoor (ID) sample collection sites

A cotton swab was dipped in the suspension and the excess fluid was removed by pushing and rotating the cotton swab inside the wall of the tube just above the fluid level. Then the swab was streaked over the surface of Mueller-Hinton agar to obtain uniform inoculums. Antibiotic impregnated discs were then aseptically placed on the surface of the Mueller-Hinton agar medium with the help of sterile forceps. Each disc was gently pressed down onto the medium to ensure complete contact with agar surface. The plates were inverted and incubated at 37 °C. After 18 h incubation, the plates were examined and the diameter of the zones of inhibition was measured to the nearest whole millimeter. The *S. aureus* isolates were classified as sensitive and resistant to a particular antibiotic based on the diameter of zone of inhibition.

RESULTS AND DISCUSSION

In outdoor air, bacterial count varied from 3965 - 7284 CFU/m^3, 646 - 4112 CFU/m^3, 117 - 2408 CFU/m^3 in March, April and May 2014, respectively. Whereas, fungal count in outdoor air varied from 2056 - 5287 CFU/m^3, 88 - 4141 CFU/m^3, 822 - 1821 CFU/m^3 in March, April and May 2014, respectively (Table 1). Bacterial count in indoor environments varied from 2643 - 6226 CFU/m^3, 1087 - 1586 CFU/m^3, 440 - 1380 CFU/m^3 in March, April and May 2014, respectively. Whereas, in indoor air fungal count varied from 881 - 4024 CFU/m^3, 88 - 646 CFU/m^3, 617 - 5874 CFU/m^3 in March, April and May 2014, respectively (Table 2). The daily activities of people, wastes generated from laboratories and medical centre, wastes from temporary restaurants, movement of transport vehicles and droppings from wildlife are thought to be the principal factors contributing to the buildup and spread of airborne microbial flora in Jahangirnagar University campus. High level of microbial pollution of air was also reported in different countries of the world (Azimi *et al*., 2013; Ekhaise and Ogboghodo, 2011; Hayleeyesus and Manaye, 2014; Stryjakowska-Sekulska *et al*., 2007). In this study, among the bacterial genera, *Bacillus*, *Micrococcus* and *Staphylococcus* were most abundant and among the fungal genera, *Aspergillus* and *Penicillium* were most abundant. Brandal *et al*. (2014) reported that *Bacillus* and *Staphylococcus* are the most frequent in airborne microflora whereas Bonetta *et al*. (2010) found *Staphylococcus* and *Micrococcus* as the most common bacterial genera in indoor air.

The Commission of the European Communities in 1993 formulated air quality standards for non-industrial premises (CEC, 1993). According to that standard, degree of pollution was classified into 5 categories: very low (bacteria < 50 CFU/m^3, fungi < 25 CFU/m^3 of air); low (bacteria 50-100 CFU/m^3, fungi 25-100 CFU/m^3 of air); intermediate (bacteria 100-500 CFU/m^3, fungi 100-500 CFU/m^3 of air); high (bacteria 500-2000 CFU/m^3, fungi 500-2000 CFU/m^3 of air); and very high (bacteria > 2000 CFU/m^3, fungi > 2000 CFU/m^3 of air). Although this standard was recommended for indoor air, we used the same for both indoor and outdoor air to get an overall picture of the pollution status in JU campus.

Table 1. Microbiological quality of outdoor air during the study period

Month (in 2014)	Sampling site	Replication	Bacterial count (CFU/m^3)	Fungal count (CFU/m^3)
March	OD1	1	5343	3935
		2	5874	5287
		3	7284	2643
	OD2	1	3965	2203
		2	5110	2320
		3	6315	2261
	OD3	1	4993	2056
		2	6285	2790
		3	5727	2996
April	OD1	1	646	2408
		2	4024	2373
		3	4112	4141
	OD2	1	852	411
		2	1057	88
		3	1410	499
	OD3	1	1087	264
		2	1322	441
		3	1175	352
May	OD1	1	117	1674
		2	822	1234
		3	793	1439
	OD2	1	1468	1821
		2	264	1116
		3	1380	822
	OD3	1	2408	940
		2	2174	910
		3	676	822

In outdoor air, highest average bacterial count was at OD1 (6167 CFU/m^3) in March and lowest at OD1 (577 CFU/m^3) in May 2014. The average fungal count was also highest at OD1 (3955 CFU/m^3) in March and lowest at OD1 (333 CFU/m^3) in April 2014 (Table 3). In indoor air, highest average bacterial count was at ID3 (5786 CFU /m^3) in March and lowest at ID2 (764 CFU/m^3) in May 2014. The average fungal count was highest at ID3 (2731 CFU/m^3) in May and lowest at ID3 (303 CFU/m^3) in April 2014 (Table 4). Based on the sanitary standards for non-industrial premises (CEC, 1993), very high microbial pollution was observed at all the sampling sites in March 2014 (Table 3 and 4). Air pollution status gradually improved in all the 3 outdoor sampling sites in May 2014 (Table 3). Microbial air pollution status also improved in 2 indoor sampling sites at the same time (Table 4).

Table 2. Microbiological quality of indoor air during the study period

Month (in 2014)	Sampling site	Replication	Bacterial count (CFU/m^3)	Fungal count (CFU/m^3)
March	ID1	1	2937	2115
		2	3642	2408
		3	2643	2526
	ID2	1	3906	1116
		2	3378	4024
		3	2790	1527
	ID3	1	6226	1586
		2	5198	1469
		3	5933	881
April	ID1	1	1087	529
		2	1322	264
		3	1176	646
	ID2	1	1175	587
		2	1351	529
		3	1586	617
	ID3	1	1145	352
		2	1322	88
		3	1410	470
May	ID1	1	617	764
		2	852	617
		3	1116	1527
	ID2	1	470	646
		2	1380	764
		3	440	793
	ID3	1	587	1439
		2	1145	5874
		3	617	881

Table 3. Status of outdoor air quality (n = 3)

Month (in 2014)	Sampling site	Bacterial count (CFU/m^3)	Fungal count (CFU/m^3)	Pollution degree*
March	OD1	6167	3955	Very high
	OD2	5130	2261	Very high
	OD3	5668	2614	Very high
April	OD1	2927	2974	Very high
	OD2	1106	333	High
	OD3	1195	352	High
May	OD1	577	1449	High
	OD2	1037	1253	High
	OD3	1753	891	High

*Based on sanitary standards for non-industrial premises (CEC, 1993).

Table 4. Status of indoor air quality (n = 3)

Month (in 2014)	Sampling site	Bacterial count (CFU/m^3)	Fungal count (CFU/m^3)	Pollution degree*
March	ID1	3074	2350	Very high
	ID2	3358	2222	Very high
	ID3	5786	1312	Very high
April	ID1	1195	480	High
	ID2	1371	578	High
	ID3	1292	303	High
May	ID1	862	969	High
	ID2	764	734	High
	ID3	783	2731	Very High

*Based on sanitary standards for non-industrial premises (CEC, 1993).

S. aureus, a Gram-positive bacterium which grows in grape-like cluster, is commonly found in most environments and may survive on dry surfaces for long periods (Reygaert, 2013). Furthermore, this microorganism is naturally equipped with a battery of virulence factors which help it to promote colonization, prevent opsonization, and cytolytic activity. Although, most infections with *S. aureus* are localized at the area of entry and are self-limiting, presence of many virulence factors gives this microorganism an advantage to cause acute to chronic infections, such as boils, deep tissue abscesses, enterocolitis, bacteriuria, osteomyelitis, pneumonia, carditis, meningitis, septicemia and arthritis (Jensen and Lyon, 2009). Before the beginning of antibiotic era, treatment of severe *S. aureus* infection was difficult. However, introduction of the β-lactam antibiotic penicillin into clinical use dramatically improved the situation. But, within a few years, *S. aureus* began to show resistance to penicillin. later on although β-lactamase resistant penicillins (e.g. methicillin, oxacillin) were developed and found effective, *S. aureus* strians resistant to methicillin an oxacillin were also isolated with increasing frequency (Reygaert, 2013). Large mobile genetic elements appear to encode both antibiotic resistant factors and proteins that are responsible for increase in virulence thus giving the organism the ability to adapt to the selective pressure of antibiotics (Ojo *et al.*, 2014).

Abuse of antibiotics is a common phenomenon in developing counties including Bangladesh. Due to the widespread use of antibiotics, *Staphylococcus aureus* has rapidly developed resistance to many antibiotics making treatment difficult. So, the study was further extended to determine the antibiotic resistance profile of *S. aureus* isolates. For this purpose, all the 16 *S. aureus* isolates were tested against 14 antibiotics (Table 5). Among the isolates, 87.5% exhibited resistance to ceftazidime; 50% to penicillin G; 31.25 % to cefotaxime; 25 % to ceftriaxone, cefuroxime, cloxacillin; and 18.75% to amoxicillin (Fig 2). Agbagwa and Jirigwa (2015) also reported high level (100%) of resistance by *S. aureus* isolates to ceftazidime. In the present study, all of the *S. aureus* isolates (100%) showed susceptibility to amikacin, ciprofloxacin, erythromycin, gentamicin, imipenem, nitrofurantoin and vancomycin. One hundred percent susceptibility of *S. aureus* isolates to vancomycin was also reported by Akpaka *et al.* (2006). Our result is also in agreement

with previous studies where erythromycin and gentamicin were found highly effective against *S. aureus* (Agbagwa and Jirigwa; 2015, Akpaka *et al.* 2006; Ako-Nai *et al.,* 2005).

Table 5. Zone of inhibition produced by the antibiotic against *S. aureus* isolates

Isolates	Zone of inhibition (mm)													
	AML*	AK	CTX	CAZ	CRO	CXM	CIP	OB	E	CN	IPM	F	P	VA
SA1	23	31	24	18	24	33	31	9	30	35	43	28	27	19
SA2	21	27	17	14	18	25	27	20	24	29	38	23	24	16
SA3	9	29	10	8	9	7	29	0	29	26	34	20	8	16
SA4	20	28	21	13	21	25	18	19	25	28	40	23	25	17
SA5	21	25	22	14	22	30	26	15	24	27	41	25	23	20
SA6	28	28	21	15	21	18	26	21	30	25	36	19	24	16
SA7	26	27	21	14	17	23	25	19	25	24	32	21	32	16
SA8	8	26	11	9	12	9	21	7	26	21	32	18	8	16
SA9	30	32	7	8	16	16	25	21	26	34	45	21	33	20
SA10	32	35	19	9	17	15	35	26	24	35	46	21	35	21
SA11	19	28	9	7	13	10	30	21	21	33	40	22	27	20
SA12	31	35	12	7	14	9	34	0	29	30	44	22	38	19
SA13	22	24	17	13	16	21	24	18	26	24	34	21	30	16
SA14	26	28	18	13	13	21	24	17	24	28	33	25	34	17
SA15	26	30	19	9	21	25	27	21	23	30	40	21	22	16
SA16	25	27	19	12	16	23	26	20	26	27	35	22	31	16

*AML: amoxicillin; AK: amikacin; CTX: cefotaxime; CAZ: ceftazidime; CRO: ceftriaxone; CXM: cefuroxime; CIP: ciprofloxacin; OB: cloxacillin; E: erythromycin; CN: gentamicin; IPM: imipenem; F: nitrofurantoin; P: penicillin G; and VA: vancomycin.

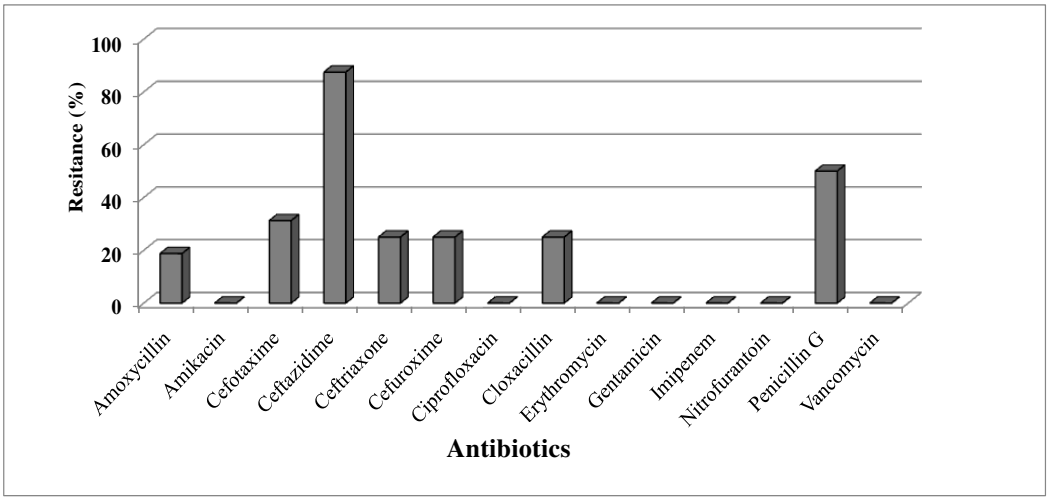

Fig. 2. Antibiotic resistance profile of the *S. aureus* isolates

The present study revealed that the indoor and outdoor air of the different sampling sites of JU campus was highly contaminated with bacteria and fungi during the study period. Furthermore, the presence of antibiotic-resistant bacteria in air has aggravated the contamination problem.

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