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Bacteriological Study of Chittagong City Area

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Abstract: To study environmental bacteria, samples were collected from different indoor and out door sites of Chittagong city over one year from Jan 2002 to December 2002. The frequency of over all load in different media was recorded as 25.87, 44.20 and 29.91% in the rainy season, winter and summer, respectively. The prevalence of bacteria found 82.52% and fungal flora 17.48% in out door sites and in case of indoor sites, bacterial flora was 76.93% and fungal flora was found 23.07%. The most frequently isolated organisms *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* were found all over the sites and seasons of the year. The percentage of *Staphylococcus* spp was dominant among all 110 staphylococcus media in tea stall (13.69%) and Kodomtoli (14.37%) in case of indoors and out doors, respectively all the year round.

Key words: Air borne bacteria, antibiotic sensitivity, seasonal variation

INTRODUCTION

The finding of *Bacillus subtilis* in samples of ambient air and air-conditioner was frequent also many others^[1]. Most of the air borne bacteria originate from natural sources include soils, lakes, oceans, animals and men^[2,3]. Sources such as sewage treatment^[4,5], animal rendering^[6] fermentation processes^[7] and agricultural activities also contribute to the bacterial population of the atmosphere. The number of bacteria in the air at any given time depend on such factors as the amount of movement, sunshine, humidity, location and the amount of suspended dust or spray. Numbers vary from less than one per cubic feet at a mountaintop to thousands in very dust air^[8]. Numbers of bacteria in air are increased by air currents caused by movement of people, by ventilation and by breezes. Dry air contains more organisms than does similar air in moist condition. Rainfall remove bacteria from the air, so that a hard steady rain fall may particularly free the air from organism or a little number of bacteria present in that time. Bacteria exit virtually everywhere, so the number of bacteria is always present in the bio aerosol.

In this study, the quantitative distribution of air borne bacteria and their seasonal variation under prevailing climatic condition, antibiotic sensitivity of the organisms have been determined in different sites of Chittagong City.

MATERIALS AND METHODS

Sampling sites: Samples were collected from different sites of Chittagong city for the isolation of environmental

bacteria. Out door samples were collected from New market, Shishu park, Kodomtoli, Banani and Agrabad residential areas, Fays lake and Chittagong stadium. Indoor samples were collected from operating theater, delivery rooms, incentive care, tea stall (Andar Killah), hotel and restaurant (Chak Bazar), jetty area (Sea Port) and neonatal wards (Chittagong Medical College).

Sampling procedure: Air borne bacteria were collected by open plate procedure. Plates of uniform size (7.9 cm diameter) containing nutrient agar media (NA), 110 *Staphylococcus* medium, Tryptic soy agar and blood agar medium were exposed for 30 min in each sampling sites. Exposure of two plates for each media, that is eight plates were exposed for each site. The plates were incubated at 37°C for 48 h.

Colony counting and Identification: For enumeration of different types of total bacteria in air, NA media and tryptic soy agar media were used, 110 *Staphylococcus* media was used for isolation of total *Staphylococcus* spp. and blood agar medium were used to grow wide range of pathogenic specially *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Necesseria* spp. which are difficult to grow other media. After observing colony and slide observation coagulase test was performed for *Staphylococcus aureus* This study tried to identify hemolytic conditions especially for *Streptococcus* spp. Crystal violate blood agar were used to find out *Streptococcus pyogenes*, chocolate agar were used to select *Neisseria* spp., *Strpetococcus pneumoniae* and *Haemophilus influenzae*. From all blood agar grown

bacteria we obtained those, which are cocci (Screening was done by Light Microscope, 10X, Nikkon Co) selected air borne bacteria and their morphological and biochemical characters were studied. Provisional identification was made with the help of Bergy's Manual of determinative bacteriology.

For the study of seasonal variation the meteorological data were collected from Govt. Meteorological Information Center, Chittagong. The whole year was divided into three seasons (rainy, winter and summer) considering the temperature, relative humidity and rainfall.

RESULTS AND DISCUSSION

The maximum (51.21%) incidence of *Staphylococcus* spp. was recorded on 110 *Staphylococcus* media in winter season. On nutrient agar medium bacterial colonies were found maximum (35.34%) in winter. In tryptic soy agar media also found maximum load (49.94%). Low temperature, low humidity and minimal rainfall were found associated with high incidence of bacterial flora (Table 1). On the other hand, the lowest bacterial counts were observed on each media in rainy season. Nutrient agar (31.92%), tryptic soy agar (20.50%) and 110 *Staphylococcus* agar (23.06%) It indicates that, moderate temperature, moderate humidity and overly rainfall were associated with lowest incidence of bacterial flora. These findings are in agreement with those of Wright *et al.*^[9] and Webb^[10] who observed that the prevalence of airborne bacteria was immediately related with prevalent climatic conditions including wind speed and direction, temperature and relative humidity.

The air total bacterial count was maximum in second season of the year^[11] which supports present study.

Different types of airborne bacteria on different media and from sites (indoor and out door) showed seasonal variation, counts of total bacterial colonies and their abundances shown in Table 2. Out door samples showed highly polluted spectrum than indoor. It was evident that, 110 *Staphylococcus* medium was a suitable medium where maximum trapping of pathogenic *Staphylococci* were possible. This result associated with the findings^[12]. The presence of *Staphylococcus* spp. found in the Neonatal wards in winter season is abundant (Table 1) and found many of them are *S. aureus*. The members of the genus *Staphylococcus* were found higher in City Street in winter season than those of indoor air. So it is evident from the study that bacteria showed its highest incidence in winter. The number of bacteria was greater in number in dirty and untidy rooms than that of clear rooms. A damp and humid atmosphere contains fewer organisms than dry one owing to the fact that the organisms are carried down by the droplets of moisture^[3] and similar with the present results.

For the identification of the *Bacillus* spp. some biochemical tests were carried out on the basis of morphological and physiological characters and to identify *Staphylococcus aureus* and *Streptococcus* groups we used some culture tests (Table 2).

Air borne bacteria of different sites showed highest percentage in the out door sites and relatively lower percentage were found in the in door sites. (Table 1) This result corroborate with the findings^[14]. But in case of *Bacillus* spp. indoor sites possesses more organisms than found in out door sites. Because the climatic condition of

Table 1: Seasonal variation of environmental bacteria load collected from different site (CFU)

Site	Nutrient agar media			Tryptic soy agar			110 Staphylococcus media		
	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer
Indoor									
Operating theater	282	176	656	80	535	166	72	175	93
Delivery rooms	158	43	62	28	29	10	46	131	76
Incentive care	70	791	164	118	213	32	110	146	90
Tea stall (Andar Killa)	196	330	259	57	105	38	131	602	326
Hotel and Restaurant (Chack Bazar)	359	324	447	67	207	142	99	180	270
Jetty area	258	217	416	108	296	64	123	187	191
Neo natal wards	496	676	796	420	279	1796	84	643	60
Total	2315	2557	2800	1298	1664	2248	665	2064	1106
Out door									
New Market	790	380	670	199	594	162	134	272	190
Shisu park	159	92	130	41	222	66	179	169	180
Kodomtoli	298	527	198	186	1614	397	315	614	279
Banani and Agrabad Residential area	326	88	156	42	226	62	126	199	99
Fays Lake	318	838	601	215	1134	359	335	703	227
Ctg. Stadium	266	469	31	176	229	68	184	282	80
Total	2157	2394	1786	1035	4019	1114	1273	2239	1055
Grant Total	4472	4951	4586	2333	5683	3362	1938	4303	2161

Meteorological record (Average): Rainy season (June to August), Temp: 29.2°, Humid: 84%, Rain Fall: 310 mm
 Winter season (Dec to Feb), Temp: 26.82°, Humid: 68%, Rain Fall: 3 mm
 Summer Season (March to May), Temp: 27.3°, Humid: 72%, Rain Fall: 130 mm

Table 2: Biochemical tests for identification

Species (n)	Catalase	Growth at 5% saline water	Glucose		Starch hydrolysis	Mannitol fermentation	Casein hydrolysis	Nitrate reduction	Blood agar growth
			Acid	Gas					
<i>Bacillus megaterium</i> (21)	+	+	+	-	+	-	+	+	-
<i>B.cereus</i> (64)	+	+	+	-	+	-	+	+	+
<i>B.subtilis</i> (39)	+	+	+	-	+	+	+	+	-
<i>B. macerans</i> (11)	+	-	+	+	+	+	-	+	-
<i>B. licheniformis</i> (34)		+	+	-	+	+	+	+	-
<i>B. leterosporous</i> (18)	+	-	+	-	-	-	+	+	-
<i>Staphylococcus aureus</i> (24)	+	-							+
<i>Streptococcus</i> sp.	-	-	+	-	-	+	-	+	+
<i>Streptococcus pyogenes</i> (7)	+	+	-	-	-	+	-	-	+
<i>Streptococcus pneumoniae</i> (6)	+	-	+	+	-	-	-	-	+
Unknown (36)									

Blood agar positive organisms				
Hemolytic condition (α or β)	Crystal violet agar	Chocolate agar	Coagulase test	Identification
β	-	-	+	<i>Staphylococcus aureus</i>
α	-	-	-	<i>Streptococcus</i> sp
β	+	-	-	<i>Streptococcus pyogenes</i>
α	-	+	-	<i>Streptococcus pneumoniae</i>
α	-	-	-	Unknown

Table 3: Antibiotic sensitivity pattern test of the observed organisms

Antibiotics	<i>B. subtilis</i>	<i>B. cereus</i>	<i>B. coagulans</i>	<i>B. leterosporous</i>	<i>B. megaterium</i>	<i>B. licheniformis</i>	<i>B. macerans</i>	<i>B. stearothermophilus</i>	<i>S. aureus</i>	<i>St. pneumoniae</i>	<i>St. pyogenes</i>	<i>Streptococcus</i> sp.
Streptomycin	s	s	s	s	s	s	m	s	r	s	s	r
Ampicillin	m	s	s	s	s	r	s	m	s	s	r	s
Amoxicillin	m	r	s	s	s	s	s	m	s	r	m	m
Chloramphenicol	s	s	r	m	r	s	s	r	s	r	s	r
Tetracycline	s	m	s	s	m	s	r	s	s	s	r	s
Gentamicin	r	s	s	s	m	s	s	r	r	m	s	m
Sulfamethoxazole	s	r	m	m	s	s	s	r	s	s	s	s

r (resistant), s (sensitive), m (moderate) (Ref: sensitivity standard Table: Becton Dickinson Co, US. Antibiotic disk: Oxoid Co.)

Chittagong is more or less different than other places of Bangladesh as well most of the tropical countries. So, this is probably due to the presence of substrate that contains the bacteria. Air borne bacteria may also transport to the indoor sites to out door through air current pass by the open doors and through windows in room or residential areas. Crowded room (Neonatal wards and delivery rooms) possesses more organisms. Similar result was found by Frobisher^[15] who reported that crowded lunch room, busy railway station and main corridor of large city hall possess more organisms.

This study, observed air condition filters and did not get and *Bacillus subtilis* in the five samples of air condition filters which are different from the Molina *et al.*^[1] findings.

A detailed observation of *Bacillus* spp. was made in the present investigation. It was found that *Bacillus cereus* were most dominant among the species and frequently found in both indoor and out door sites in all the seasons. Similar result was found by Ehrlich^[16]. The next dominant species was *Bacillus subtilis* found in the indoor and out door sites in all seasons corroborate with the result of Wright *et al.*^[9] *Bacillus macerans* and *Bacillus coagulans* found in fewer concentrations and found mainly in indoor sites. *Bacillus leterosporus*, *Bacillus megaterium*, *Bacillus licheniformis* and

Bacillus sterothermophilus were found in various indoor and out door sites in different seasons. These findings are more or less agreed with those of Deak and Timer^[17] who reported that most aerobic airborne spore forming microorganisms were identified as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus sterothermophilus*.

This investigation were mainly conducted with and *Bacillus* spp., *Staphylococcus aureus* and some pathogenic *Streptococcus* spp. Because of storage of sufficient fund and facilities. So, the present investigation here will be of help in making future awareness program in controlling microbial pollution of environment.

In this study six *Streptococcus pneumoniae* were found and among all *Streptococcus* spp. no *Necessaria* spp. or *Hemophila influenzae* were found. Twenty four *Staphylococcus aureus* were also found in the present study.

Among the observed organisms, it was found the highest organisms are sensitive to antibiotic streptomycin, resistant to chloramphenicol. *Bacillus subtilis* are resistant to gentamycin, *Streptococcus pneumoni* showed resistant to amoxicillin and chloramphenicol. *Streptococcus pyogenes* are resistant to ampicillin and tetracycline. *S. aureus* are resistant to streptomycin and gentamycin (Table 3).

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