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Occurrence of *Amphibacillus* and *Bacillus* Endospores in the Atmospheric Suspended Particulate Matter of Lahore City

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Abstract.- Suspended particulate matter (SPM) of atmosphere of Lahore was collected from four different locations. Portions of the glass microfiber filter were stored for about two years at room temperature in uncontaminated conditions. The filter sheets were then processed for enumeration of viable bacterial endospores. All the samples indicated the presence of bacterial endospores with the highest number for the samples collected from Thokar Niaz Baig, a location characterized by very high traffic activity. Physicochemical characteristics of bacterial isolates indicated the endospores of *Amphibacillus* and *Bacillus* genera. Apart from the physicochemical nature of SPM, the bacterial and especially endospore content of them posed microbiological atmospheric pollution that may lead to contaminate many fomites in addition to cause food poisoning and disseminate infectious diseases.

Keywords: Endospores in air, aerial microflora, aeromicrobiology, atmospheric particulate matter and bacteria.

INTRODUCTION

Atmospheric pollution is an inevitable consequence of modern life. Industrial, traffic and other urban activities necessitate the presence of substances in the atmosphere in such quantities and of such duration, liable to cause harm to life or damage to human made materials and structures or change in the weather and climate. The air pollution results from the release of gases, aerosols and many microorganisms, at a rate that exceeds the harmless accommodative capacity of the atmosphere. Many microbes are suspended in air, which may then fall in to food, water and milk. Consequences of microbially contaminated foods range from food poisoning to serious adverse effects on human health. In big cities, life is characterized by heavy traffic that may result in to atmospheric pollution (Wittoroff *et al*., 1994; Sacre *et al*., 1995). Dust and soot particles are the major components of atmosphere of such cities (Schwartz *et al.,* 1993; Anderson *et al*., 1996). This form of pollution is concerned with acute human health problems, ranging from skin irritation to cardiac and respiratory diseases (Dockery and Pop, 1994; Burnett *et al.,* 1995; Schwartz, 1996; Hwang and Chan, 2002). The air borne chemicals also contain heavy metals (Tepper *et al.,* 1994; Dusseldorp *et al*., 1995).

The suspended particulate matter, soot particles and aerosols are associated with microbes. These on falling to daily use products contaminate them. Many procedures are used to kill them but some microbes especially bacteria of genera *Bacillus* and *Clostridium* are very resistant, due to a unique property of forming endospores under unfavorable conditions. *Bacillus* species are among main spoilage organisms due to their versatile metabolism and heat resistant spores (Deak and Timar, 1988). Microbes that have been generated in hospitals use various routes to infect the patient body. *Clostridium* may be distributed in hospital environment via the insufficient or defective air conditioning system with or without humidification. Dust settled on furniture is a very important microbial reservoir. Several potential nosocomial pathogens have been recovered from an area adjutant to surgical field (Schal, 1991).

Owing to the prevailing environmental conditions and highly resistant nature of bacterial endospores, the present study was aimed at isolating endospore forming microbes from atmospheric suspended particulate matter (SPM) sampled about two years back and stored properly. These

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information express the contamination potential of the atmospheric SPM for water, food, milk and long term persistence of the bacterial endospores.

MATERIALS AND METHODS

Four samples from different areas of Lahore i.e. Thokar Niaz Baig, Quaid-e-Azam Campus, Model Town and Choburji were collected with the assistance of the Environmental Protection Agency (EPA) using air sampling facility (Table I).

Table I.- Attributes of the samples and colony forming units (C.F.U) of suspended particulate matter (SPM) on standard count agar.

Sample No.	Area of study	Colony forming units / SPM entrapped on 25 cm² area
1	Thokar Niaz Baig	9.2×10^6
2	Quaid-e-Azam campus	9.3×10^4
3	Model Town	1.0×10^4
4	Chouburji	4.6×10^5

Whatman glass microfiber filter (20.3×25.5 cm) were kept in polythene bags and exposed over night to formalin gas in a closed chamber. They were removed out of glass chamber and polythene envelopes were closed. These pre-sterile filters were fitted in Hi volume air sampler after arrival at the sampling point and allowed to suck in the air for 15 minutes. The air filters were removed from the sampling facility, put in polythene bags and the flaps of the envelopes were closed. Parts (2.9×2.2 cm) of these filters were cut under sterile conditions and used in an earlier study (Qazi *et al.,* 2002). While the remaining portions were stored in the sterilized polythene bags at room temperature to be used in the present study.

In order to enumerate the endospores of SPM, standard plate count (SPC) method was used (Benson, 1994). A piece (5x5cm) was cut from four exposed sheets and dipped in 100ml sterile water. The bottles were shaken for 20 minutes and given a heat shock for 10 minutes at 80°C. One ml of this dilution was mixed in 99ml of sterilized water and shaken for 20 minutes. Third dilution was made in the same way, out of which 100µl was spread on Petri plate containing standard plate count agar (Merck Co., Germany) and incubated for 24 hours at 37°C. The plates showing 30 to 300 bacterial colonies were selected for counting. Representative colony of each morphologically distinct group was restreaked for pure culturing on the standard plate count agar. The pure cultures thus obtained were transferred to standard plate count agar slants in test tubes. The tubes were cotton plugged and preserved in refrigerator.

Each organism was inoculated on nutrient agar slants and nutrient broth. The growth was processed for Gram's and endospore staining. The latter staining was performed by Schaeffer-Fulton method (Benson, 1994). Biochemical characteristics like carbohydrate fermentation, catalase production, oxidase production, indole production, haemolytic activity and antibiotic resistance was also checked (Benson, 1994). On the basis of these characteristics the bacteria were identified up to genus level as described by Holt *et al*. (1994).

RESULTS AND DISCUSSION

The present microbiological evaluation of Lahore city indicated that the heavy amount of SPM had retained viable endospores of bacteria of genera *Amphibacillus* and *Bacillus*. Highest colony forming units i.e. above 90 appeared for the samples collected from Thokar Niaz Baig and Chouburji while the lowest count was observed for the sample representing Model Town area (Table I). Regarding the cultural characteristics, the bacterial isolates indicated irregular, round, and filiform colonies on standard plate count agar. Colonies margins appeared as smooth, wavy, and spreading for the different isolates. The colonial growth expressed raised, umbonate, convex and rough surface (Table II). All the bacterial isolates were found gram positive rods and indicated no gas production when they were grown in the presence of starch, mannitol and xylose. For other characteristics the bacteria showed variable responses (Table III). Apart from the 53% endospore formers 47% nonendospore forming bacteria were also isolated that indicated thermoduric. 72% of the endospore formers showed α-haemolysis on blood agar, while 28% expressed β-haemolysis (Table IV).

Table II.- Colony characteristics of bacterial isolates from the atmospheric particulate matter and corresponding number of colonies on standard plate count agar.

Sample No.	Colony morphology				Colony forming units/SPM entroipped on 25 cm^2 area
	Bacterial Isolates	Configuration	Margin	Elevation	
1	1A	Irregular	Spreading	Hilly	1.0×10^6
	1B	Round	Smooth	Raised	2.0×10^6
	1C	Round	Wavy	Hilly	8.9×10^6
2	2A	Round	Wavy	Hilly	9.3×10^4
3	3A	Round	Smooth	Umbonate	4.0×10^4
	3B	Irregular	Wavy	Convex	5.0×10^4
	3C	Round	Smooth	Hilly	1.0×10^4
4	4A	Round	Smooth	Convex	2.0×10^5
	4B	L-form	Smooth	Raised	1.0×10^5
	4C	Irregular	Wavy	Hilly	6.0×10^5
	4D	Round	Smooth	Flat	5.0×10^5
	4E	Filiform	Smooth	Raised	4.0×10^5
	4F	L-form	Smooth	Convex	1.0×10^5

Table III.- Biocharacterization of the bacterial isolates recovered from atmospheric particulate matter of Lahore

Biochemical tests	Bacterial isolates*												
	IA	IB	IC	2A	3A	3B	3C	4A	4B	4C	4D	4E	4F
Gram Staning	+	+	+	+	+	+	+	+	+	+	+	+	+
Endospore staining	+	-	-	+	+	+	-	-	+	+	-	-	+
Methyl Red test	-	+	-	+	-	-	-	+	-	+	-	-	-
Voges Prosakauer test	-	+	-	-	+	-	+	+	-	-	-	-	+
Catalase test	+	+	-	-	-	-	+	+	-	-	-	-	+
Oxidase test	-	-	-	-	-	-	-	+	+	-	-	-	+
Indole test	-	+	+	+	+	+	+	+	+	-	-	+	+
Acid production test: Starch	++	++	++	++	++	+	++	++	++	++	++	++	++
Mannitol	+++	+++	+++	+++	-	++	+++	++	+++	+++	+++	++	++
Xylose	-	+	-	+	++	++	-	-	-	+	+	++	-

Number of positive signs indicates intensity of reaction.
*2A, 3A, 3B and 4B belonged to *Amphibacillus*, while 1A and 4F to *Bacillus* genera. 1B, 1C, 3C, 4A, 4C, 4D & 4E were unidentified

Studies have correlated the amount and size of SPM with different diseases, health risks and infections. For example Romieu and Borja-Aburto (1997) proved that particles at high concentration could cause mortality. These workers have related an increase of $100 mg/m^3$ in 24 hours of average diameter of 10 microns (PM 10) to a 13% increases in total mortality. Avol *et al.* (2001) concluded that particulate matter (PM 10) had potentially important effects on lung function and growth. Similarly, Braga *et al.* (2001) have associated $10 \mu g/m^3$ increase in particulate matter, ranging from <10 to 100 µm in size, with the increase in deaths due to pneumonia. While Bhatia *et al.* (1998) have correlated diesel

Table VI.- Details of haemolytic activity and antibiotic susceptibility patterns of the bacterial isolates recovered from atmospheric SPM of Lahore city.

Bacterial isolates	Haemolytic character	Zone of growth inhibition to different antibiotics*					
		W 5 µg/disc	NV 30 µg/disc	CN 10 µg/disc	PRL 100 µg/disc	CRO 30 µg/disc	CXM 30 µg/disc
lA	α-haemolysis	2.40[a]	3.80	2.80	2.90	1.50**	R
1B	α-haemolysis	4.32**	2.55	2.25*	2.20	1.17**	R
1C	α-haemolysis	R	2.32	2.73	2.40	R	R
2A	α-haemolysis	R	2.75	2.78	2.37	R	R
3A	β-haemolysis	R	2.16	2.61	2.47	R	R
3B	α-haemolysis	3.48	2.88	3.15	2.40	R	R
3C	α-haemolysis	R	2.64**	2.99	2.76	R	R
4A	Non-haemolytic	2.38	2.91	2.77	3.93	1.91	1.79
4B	α-haemolysis	3.55	3.22	3.52	3.00	1.23	R
4C	α-haemolysis	1.60	3.68	2.60	2.80	R	1.00
4D	Non-haemolytic	9.79	2.90	4.80	2.17	2.20	1.00
4E	Non-haemolytic	R	3.67	2.75	2.40	R	R
4F	β-haemolysis	R	3.67	2.75	2.40	R	R

*Abbreviation used: W, Trimethoprim; NV, Novobiocin; CN, Gentamycin; PRL, Piperacillin; CRO, Ceftriaxon; CXM, Cefuroxim.
**= Some resistant colonies appeared in the zone of growth inhibition.
a = Diameter of zone of growth inhibition in cm.
R = Resistant to drug.

exhaust with lung cancer.

The smaller particles in the atmosphere provide more surface area for adsorption, attachment and distribution of microbes in the air. Burnett *et al.* (1997) have illustrated role of particulate size and hospitalization for cardio - respiratory diseases. Infact the SPM provide sites for the attachment of microorganisms and facilitate their spread in to the environment (Qazi *et al.,* 2002).

The present results have indicated the presence and retention of bacterial endospores in the atmosphere of Lahore, many of which were found haemolytic. It is speculated that dissemination of such microbes through SPM might have been contributing to the spread of infectious diseases and food contamination/poisoning, especially for food that was placed open after preparation for sometime.

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