Concentrations and Size Distributions of Airborne Microorganisms in Guangzhou during Summer

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ABSTRACT

The concentrations and size distributions of airborne microorganisms were determined for outdoor air samples collected with cascade impactors on hazy summer days in Guangzhou. The percentage of airborne bacteria was markedly higher than that of fungi, and the concentrations of bacteria were much higher in a densely populated area (Guangzhou Medical College) than in a clean area (Guangzhou Liuhua Lake Park) or one with heavy traffic (Dongfengxilu Road). Moreover, the concentrations of bacteria at 8:00 and 23:00 were higher than those at 12:00 and 17:00. Sixteen species of bacteria belonging to eight genera were identified, and all were opportunistic pathogens. Of these, 11 species from four genera were Gram-positive, and five species from four genera were Gram-negative. *Staphylococcus*, *Bacillus*, *Enterobacter*, and *Serratia* were the dominant bacterial genera, and the most abundant species were *Staphylococcus hominis* (8–58%) and *Staphylococcus lugdunensis* (14 to 35%). Size distribution studies showed that 36 to 76% of the microorganisms deposited on stages 3 to 6 of the sampler (0.65–4.7 µm aerodynamic equivalent diameter), and particles of this size could penetrate the lower respiratory tract of humans.

Keywords: Microbial aerosol; Bacterial community composition; Size distribution; Clean area; Densely populated area; Traffic dense area.

INTRODUCTION

Airborne microorganisms (bioaerosols) are particles of biological origin (e.g., bacteria, fungi, pollen, viruses), and they are important constituents of the atmosphere because they have the potential to cause a variety of diseases in humans and animals and to damage vegetation (Lee *et al.*, 2003; Ho *et al.*, 2005). The presence of harmful airborne microorganisms in various indoor and outdoor environments

also has been associated with serious economic losses stemming from the contamination of food and the deterioration of medicines and building materials, etc. (Lee *et al.*, 2003; Ho *et al.*, 2005). Furthermore, several types of airborne microorganisms (e.g., fungi, bacteria, and algae) have been found to be effective cloud condensation nuclei (Chong *et al.*, 2010; Lee *et al.*, 2010), and therefore bioaerosols have the potential to affect weather and climate.

Bioaerosols are droplets or particles suspended in the air; they typically range in size from 0.3 to 30 µm in diameter (Koch, 1996), and their composition varies depending on their sources, the aerosolization mechanisms that produce them, and the environmental conditions along the pathways from the source to the sampling site. The bacteria most commonly encountered in natural environments range in size from 1 to 3 µm (Reponen *et al.*, 2001), but bacteria as

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small as 0.3 μm have been encountered (Koch, 1996). Most fungal spores range in size from 2 to 4 μm in aerodynamic equivalent diameter, but some specimens as large as 30 μm have been observed (Reponen *et al.*, 2001). If the bioaerosol is generated from dry, dusty surfaces or soils, the microorganisms tend to be suspended as single units or as rafts of larger particles (Lighthart, 1994).

Inhalation is the primary route of exposure to bioaerosols, with the average human inhaling approximately 10 m³ of air per day, and this can result in adverse health effects. For instance, the inhalation of airborne *Legionella pneumophila* or fungi of the genus *Aspergillus* can cause Legionnaires' disease. Fungal spores (even non-viable ones) retained in the upper respiratory tract can elicit allergic responses. The pathogenicity of the bioaerosol depends not only on the numbers and the species of microorganisms but also the sizes of the particles. Therefore, in order to correlate human disease with exposure to bioaerosols, it is critically important to measure the size distributions of bacteria and fungi in places where people are likely to be exposed. Inhaled aerosols larger than 10 μm often are retained in the nasal cavity but those 5 to 10 μm can penetrate into the bronchi (Stetzenbach, 1998). Most bio-particles that deposit in the alveoli and terminal bronchi range in size from 1 to 2 μm (Stetzenbach, 1998), and they mainly include bacteria and smaller fungal spores. Most previous studies have focused on the composition and concentrations of bioaerosols, and only a few of them have focused on the particle-size distributions of airborne microorganisms in outdoor environments (Fang *et al.*, 2006). Because of their small size and light weight, bioaerosols are readily transported, and therefore they can be rapidly dispersed in the environment. There are temporal as well as spatial variations in the concentrations of the bioaerosol; that is, the concentrations not only vary among areas but also over various time-scales, including seasons, months, days and hours (Lighthart, 1994).

Guangzhou has a high incidence of respiratory disease, and severe cases pneumonia of unknown origin (SARS, H_5N_1, H_3N_2, and so on) have occurred there. Therefore, information on the concentrations and size distributions of the airborne microorganisms in the city will help establish a base of knowledge for evaluating and eventually preventing both acute and chronic respiratory disease. In this study, we selected the hazy days in summer to conduct the field sampling; the aim of the study was to investigate the concentrations and size distributions of airborne bacteria and fungi and to obtain information on the spatial and temporal variations of the microbial aerosol concentrations.

MATERIALS AND METHODS

Sampling Sites

Guangzhou, the capital of Guangdong Province, is one of China's main industrial centers; it covers an area of 7,434 square kilometers and is home to over 10 million people. Guangzhou adjoins the South China Sea, and the city is crisscrossed by rivers and streams. It has a subtropical marine climate, with an annual average temperature of

21.8°C, rainfall of 1694 mm, and a frost-free period of 345 days. The city has a long summer and a warm winter; it is always green and flowers bloom throughout the year.

Three typical sites in the Yuexiu district, which is in the center of Guangzhou, were used for the outdoor bioaerosol sampling. Sampling was conducted during the summer (June to August) in 2008 to 2010 at three sites (1) Guangzhou Liuhua Lake Park (GLLP) where 60% of the area is a clear water lake, 35% is a green area, and 5% is various other types of surfaces. The sampling site was on a grassy area, ~20 m from tall trees, and there were no buildings around the site. (2) Guangzhou Medical College (GMC) where green areas make up ~10% of the campus grounds. In the courtyard where the samples were collected, there are laboratories, office buildings, roads, and some green vegetation. There is high flow of pedestrian traffic at this site but little automobile traffic. The sampling site there was on a grassy area ~25 m from a 13-floor building. (3) Dongfengxilu Road (DFR) which is an area with high motor vehicle traffic (~180 vehicles per minute), no more than 5% vegetative coverage, and serious air pollution problems. The sampling site was on the roadside near a tall business building.

Sampling Methods

Bioaerosols were collected with the use of a six-stage culturable FA-1 sampler (Applied Technical Institute of Liaoyang, China; this is similar to the six-stage Andersen cascade impactor, Thermo Scientific Waltham, MA, USA) at each sampling site. Each stage of the sampler held a plate with 400 holes of uniform diameter through which air was drawn at 28.3 L/min to impact onto petri-dishes containing agar growth media. Airborne particles were separated into six size fractions, and the aerodynamic cut-size diameters of the six stages were ≥ 7.0 μm (stage 1), 4.7 to 7.0 μm (stage 2), 3.3 to 4.7 μm (stage 3), 2.1 to 3.3 μm (stage 4), 1.1 to 2.1 μm (stage 5), and 0.65 to 1.1 μm (stage 6), respectively (Cripps *et al.*, 2000).

At each of the three sites, the sampler was mounted on a platform 1.5 m above ground level. Hazy days were selected for sampling in summer. The sampling period for each run was 10 min. Four separate samples were collected at four different time periods each day (8:00, 12:00, 17:00, and 23:00), and this schedule was followed for three consecutive days each week. Before sampling, the sampler was disinfected with 75% ethanol, and then 9.0 cm agar plates were inserted into the appropriate collection stage. Blood agar (BAP, bioMérieux, Marcy-l'Etoile, France) was used as bacterial culture medium while Sabouraud's agar (SDA, bioMérieux, Marcy-l'Etoile, France) was used for fungi. After sampling, the plates containing the culture media were immediately transported to the laboratory where the bacteria were cultured in an incubator for 1 to 2 d at 37°C and the fungi incubated for 3 to 5 d at 20 to 25°C.

Sample Analysis

The concentrations of airborne bacteria and fungi, that is, the colony forming units (CFU)/m³, were calculated by dividing the number of visible colonies on the plates by the volume of air sampled (m³). However, colony superposition

can occur when the microbial particles impact on the same spot after travelling through the same sieve pore, and therefore the numbers of colonies counted were revised by Eq. (1).

$$Pr = N \left(\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N_{-1}+1} \right) \quad (1)$$

where Pr is the revised colony number for each of the six impactor stages; N is the number of sieve pores in each stage of the sampler, and r is the number of colonies on the plate.

The airborne bacterial and fungal concentrations (C, in CFU/m³) were calculated using Eq. (2).

$$C = \frac{T \times 1000}{t \times F} \quad (2)$$

where T is the sum of the number of bacterial or fungal colonies on all stages; t (min) is the sampling time; and F (L/min) is the airflow rate.

Bacterial Identification

All bacterial colonies from samples were identified using API kits (Analytic Products, Inc. bioMérieux, Marcy-l'Etoile, France). For this procedure, the bacterial were first cultured on BAP at 37°C for 24 to 48 h. Samples for bacterial isolation were then subcultured on BAP to obtain pure cultures. Next, the pure cultures were separated by type, that is, Gram-positive cocci, Gram-positive rod, and Gram negative rod, according to their staining characteristics and morphology. Finally, they were identified by using the following API kits: API 20E, API 20NE, API Staph, API Coryne, API Strep, and API 50CH (bioMérieux, Inc.).

The API kits can be used to identify a wide range of microorganisms, and they are composed of plastic strips that generally contain 20 miniature tubes. Almost all of the bacterial groups and over 550 different species can be identified using these API tests. The identification is straightforward, and the API's tests are accurate. The identification system makes use of databases that contain extensive information on biochemical reactions characteristic of the microorganisms. In practice, the API test substrate is inoculated with a pure isolate in suspension. This process also rehydrates the dessicated medium in the miniature tubes; some of which are overlaid with sterile mineral oil. The results are read after incubation of 24 hr or less, depending on the API test, in a humid chamber. The color reactions are read (some of the tubes change color due to shifts in pH

and some with the aid of added reagents that react with metabolic end products), and the reactions (plus the oxidase reaction, which is done separately) are converted to a seven-digit code. The identifications were made using the proprietary software that came with the kits (bioMérieux, Inc., http://www.biomerieux.com/servlet/srt/bio/portail/home).

Data Analysis

All the experimental data were evaluated statistically using Excel 2007 and SPSS Version 17.0 (SPSS, Standard Version, IBM Corp., New York). The statistical tests used include: (1) the student's t-test, (2) the analysis of variance, and (3) linear regression. Results reported include the test statistic, the degrees of freedom, and P which is the probability that a statistical result of the magnitude observed would occur by chance alone.

RESULTS

Spatial and Temporal Variations of Airborne Microorganisms

Overall, the total microorganism concentrations (bacteria plus fungi) in the atmosphere ranged from 121 to 2442 CFU/m³, with the mean concentration ± standard devation of 1399 ± 167 CFU/m³ and median concentration 1378 CFU/m³, respectively (Table 1). Moreover, the mean concentrations ± standard devation and ranges varied among the three sampling sites as follows: 689 ± 40 CFU/m³ (121 to 1403 CFU/m³) at GLLP, 2092 ± 46 CFU/m³ (503 to 2442 CFU/m³) at GMC and 1413 ± 34 CFU/m³ (335 to 1686 CFU/m³) at DFR. The corresponding median concentrations were 668, 2057 and 1378 CFU/m³ at GLLP, GMC and DFR, respectively.

The bioaerosol concentrations in the clean area (GLLP) were significantly lower than those in the heavily trafficked area (DFR) or in the densely populated area (GMC; F(x, y), P < 0.001); the concentrations at DFR also were significantly lower than those at GMC (t(x), P < 0.001, Table 1). Furthermore, significantly higher concentrations of microorganisms were observed at 8:00 and 23:00 compared with 12:00 and 17:00 (P < 0.05), but no significant differences were found between 8:00 and 23:00, or 12:00 and 17:00 (P > 0.05, Fig. 1).

Concentrations of Airborne Bacteria and Fungi

The airborne bacteria at the three sampling sites accounted for ~87 to 94% of the total culturable microorganisms, and that percentage was significantly higher than that of

Table 1. Concentrations of culturable airborne microorganisms (colony forming units/cubic meter) at three sampling sites in Guangzhou, China.

Sampling Site[a]	Number of Samples	Mean ± SD[b]	Median	Geometric Mean	Minimum	Maximum
GLLP	432	689 ± 40	668	675	121	1403
GMC	432	2092 ± 46	2057	2088	503	2442
DFR	432	1413 ± 34	1399	1406	335	1686
All sites	1296	1399 ± 167	1378	1314	121	2442

[a] GLLP stands for Guangzhou Liuhua Lake Park, GMC is Guangzhou Medical College, and DFR is Dongfengxilu Road.
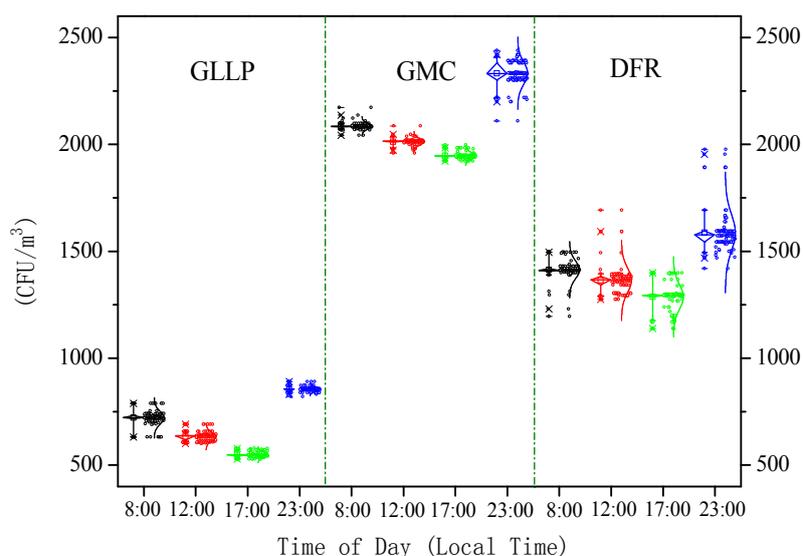[b] SD: standard deviation.

Fig. 1. Diurnal variations in airborne microorganism (bacteria plus fungi) concentration. GLLP stands for Guangzhou Liuhua Lake Park, GMC is Guangzhou Medical College, and DFR is Dongfengxilu Road.

airborne fungi ($P < 0.001$). Moreover, a higher percentage of fungi was observed at GLLP compared with GMC or DFR ($F(x, y)$, $P < 0.01$). Gram-positive bacteria accounted for ~67 to 90% of total microorganisms, and the percentage of Gram-positive bacteria was high than that of Gram-negative bacteria ($P < 0.001$). In addition, the percentage of Gram-positive cocci at the three sampling sites was significantly higher than that of rods ($P < 0.01$). A higher percentage of Gram-positive bacteria was observed at GMC compared with the other two sites ($P < 0.01$), and the lowest percentage of Gram-negative bacteria was found at GMC ($P < 0.01$, Table 2). The percentage of Gram-positive bacteria at DFR was higher than at GLLP ($P < 0.05$).

The concentrations and size distributions of airborne bacteria and fungi at three sites are summarized in Table 3. The percentage of bacteria was significantly higher than that of fungi on every stage of the impactor sampler from GLLP ($P < 0.001$). This also was true for stages 2 through 4 and stage 6 of the sampler at GMC ($P < 0.01$) and stages 2 through 5 of the sampler at DFR ($P < 0.01$). The percentage of Gram-positive bacteria was significantly higher than that of the Gram-negative bacteria on stages 1 through 5 of the impactor at GLLP ($P < 0.01$), on every stage except stage 5 at GMC ($P < 0.01$), and on stages 1 through 3 at DFR ($P < 0.01$).

Size Distributions of Airborne Bacteria

The concentrations and size distributions of airborne bacteria at the three sites are shown in Fig. 2. High concentrations of bacteria were observed on stages 2, 3, and 4 of the impactors; these stages combined to account for 64.7 to 92.0% of the total airborne bacteria. The size distributions of airborne bacteria also differed greatly among the three sites; that is, the highest percentage of culturable bacteria was detected on stage 2 at GMC (63.31%) and DFR (61.83%) but at GLLP the highest percentage was on stage 3 (38.83%).

With reference to the bacteria, 16 species belonging to 8 genera were cultured and identified from the entire set of samples. There were 11 species (4 genera) of Gram-positive bacteria and 5 species (4 genera) of Gram-negative bacteria that could be cultured from the three sites. These bacteria were all opportunistic pathogens; that is, no strict pathogens were identified from any of the samples.

Staphylococcus, Bacillus, Enterobacter, and *Serratia* were the dominant bacterial genera; they accounted for 81 to 89% of the microorganisms. *Staphylococcus* was the most common genus, and members of this genus made up 45.13% of the microbial population at GLLP, 85.13% at GMC, and 74.00% at DFR. The most common *Staphylococcus* species were *S. hominis* and *S. lugdunensis*; they accounted for 8 to 58% and 15 to 35% of total microorganisms, respectively. The dominant bacterial species at GLLP were *S. lugdunensis,* and *Staphylococcus simulands* while at GMC *Bacillus*

Table 2. Percentages of various types of airborne microorganisms at three sampling sites in Guangzhou.

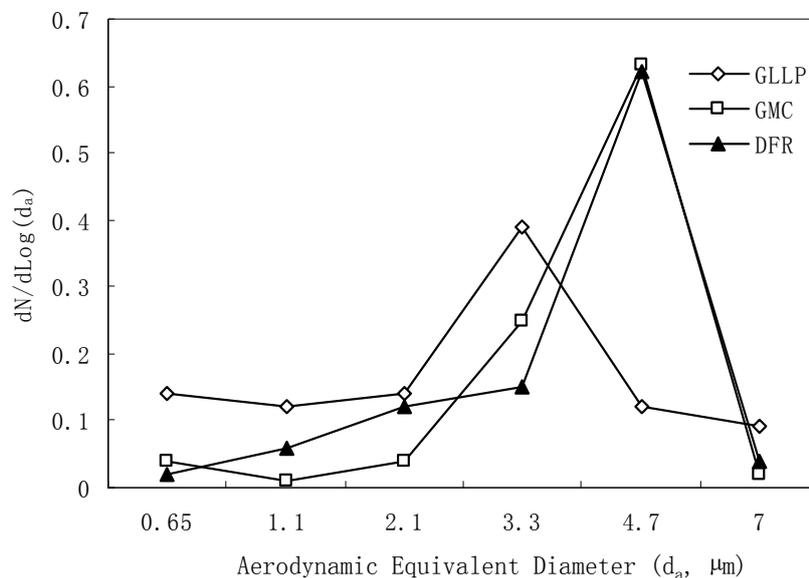| Sampling Site[a]	Bacteria (%)			Fungi (%)
	Gram-positive		Gram-negative	
	Cocci	Rods		
GLLP	47.18	19.49	20.51	12.82
GMC	85.98	2.87	0.51	10.64
DFR	74.50	3.50	15.00	7.00

[a] GLLP stands for Guangzhou Liuhua Lake Park, GMC is Guangzhou Medical College, and DFR is Dongfengxilu Road.

Table 3. The type composition of airborne microorganisms in six-stage sampler impactor samples from three sampling sites in Guangzhou.

Sampling Site[a]		Bacteria (%)			Fungi (%)
		Gram-positive		Gram-negative	
		Cocci	Rods		
GLLP	1[b]	2.6	5.1	0.5	4.1
	2	9.2	0.5	1.1	1.0
	3	19.5	5.1	9.3	3.1
	4	4.6	5.7	1.5	0.5
	5	8.7	2.1	-	1.0
	6	2.6	1.0	8.1	3.1
	Total	47.2	19.5	20.5	12.8
GMC	1	1.5	0.7	-	2.2
	2	57.9	1.1	0.1	1.0
	3	20.4	0.2	-	0.8
	4	2.7	0.2	0.1	1.7
	5	0.8	-	0.2	4.1
	6	2.7	0.7	-	0.8
	Total	86.0	2.9	0.4	10.6
DFR	1	2.3	0.5	1.0	2.3
	2	56.0	0.3	1.2	0.5
	3	8.5	1.3	4.0	1.8
	4	4.8	0.2	6.0	0.5
	5	2.3	1.0	2.3	0.3
	6	0.6	0.2	0.5	1.6
	Total	74.7	3.5	15.0	7.0

[a] GLLP stands for Guangzhou Liuhua Lake Park, GMC is Guangzhou Medical College, and DFR is Dongfengxilu Road.
[b] 1–6 represents stage 1, 2, 3, 4, 5, 6.

Fig. 2. Size distributions of total airborne bacteria determined from six-stage impactor samplers deployed at three sampling sites. Abbreviations for the station locations are the same as for Fig.1.

subtilis and *S. hominis* were the most common, followed by *S. lugdunensis*. At DFR, *S. lugdunensis*, *S. hominis*, and *Enterobacter cloacae* were the most abundant bacteria.

The bacteria showed some interesting variations in abundance as a function of their aerodynamic size. At GLLP, the percentage of Gram-positive bacteria on stages 3 to 6 was significantly higher than on stages 1 and 2 combined ($P < 0.001$), and the same pattern held true for the Gram-negative bacteria. In contrast, at GMC the percentages of Gram-positive bacteria and Gram-negative bacteria on stages 1 plus 2 were similar to those on stages 3 to 6 ($P > 0.05$). At DFR, the percentage of Gram-positive bacteria on

stages 1 plus 2 was much higher than on stages 3 to 6 (P < 0.001); however, Gram-negative bacteria showed the opposite pattern.

In terms of the variations in species abundance as a function of size, *S. lugdunensis* and *B. subtilis*, were the dominant bacteria on stages 3 to 6 at GLLP while *S. simulans* was the most common bacterium on stages 1 plus 2 at that site. At GMC, *S. lugdunensis* was the most abundant species on stage 3 to 6 while *S. hominis* was the most common on stages 1 plus 2. At DFR, *E. cloacae* was the dominant bacterial species on stage 3 to 6 while *S. lugdunensis* and *S. hominis* were the most common on stages 1 plus 2 (Table 4).

DISCUSSION

Many previous studies have been conducted in the recent past to determine the concentrations of airborne microorganism in different environments (e.g., Pastuszka *et al.*, 2000; Shelton *et al.*, 2002; Adhikari *et al.*, 2004; Fang *et al.*, 2005). Results of those studies showed that *Staphylococcus*, *Bacillus* and *Micrococcus* were the most commonly observed genera, and our results are consistent

with those findings. Moreover, our results showed that the bioaerosol concentrations in the atmosphere varied greatly during the summer in Guangzhou, and this could be related to the micro-environmental conditions, sampling times, and hazy weather (Shaffer *et al.*, 1997). As shown in Table 5, the bacterial concentrations at Guangzhou were substantially higher than those reported for various other cities of the world (Giorgio *et al.*, 1996; Pastuszka *et al.*, 2000), but they were lower than those observed elsewhere in China (Hu *et al.*, 1995; Cai *et al.*, 1998; Song *et al.*, 1999; Xie *et al.*, 2004; Fang *et al.*, 2007; Tian *et al.*, 2008; Wang *et al.*, 2008). The relatively low concentrations of airborne bacteria in Guangzhou compared with other sites in China are probably due at least in part to the abundance of sunlight there; that is, ultraviolet light is known to inhibit the growth of bacteria (Cox *et al.*, 1989; Lighthart *et al.*, 1995; Shaffer *et al.*, 1997).

Airborne bacterial concentrations can be affected by ground disturbances, including those caused by the movements of pedestrians and motor vehicles. These activities can stir up dust and cause the ejection of small soil particles from ground surfaces (Ju *et al.*, 2003). This is relevant to our studies because bacteria commonly exist in the atmosphere in association with other types of particles

Table 4. Species composition of airborne microorganisms in six-stage impactor samples from three sampling sites in Guanzhou.

Microorganism	GLLP[a] (%)			GMC (%)			DFR (%)		
	Total	1–2[b]	3–6	Total	1–2	3–6	Total	1–2	3–6
Gram-positive bacteria	66.67	17.43	49.24	88.85	61.15	27.70	78.00	59.00	19.00
Micococcus spp.	0.51	–	0.51	0.52	–	0.52	0.50	–	0.50
M. luteus	0.51	–	0.51	0.52	–	0.52	0.50	–	0.50
Staphylococcus spp.	45.13	11.79	33.34	85.13	58.58	26.55	74.00	58.25	15.75
S. epidermidis	7.69	–	7.69	–	–	–	2.00	0.50	1.50
S. hominis	8.21	3.59	4.62	57.55	51.57	5.98	30.25	25.00	5.25
S. lugdunensis	14.87	0.51	14.36	24.69	6.53	18.16	34.50	30.5	4.00
S. capitis	0.51	–	0.51	–	–	–	0.25	–	0.25
S. simulans	13.85	7.69	6.16	1.95	0.48	1.47	5.00	1.75	3.25
S. saprophyticus	–	–	–	0.21	–	0.21	0.75	0.25	0.50
S. warneri	–	–	–	0.73	–	0.73	1.25	0.25	1
Streptococcus spp.	1.54	–	1.54	–	–	–	–	–	–
S. mitis	1.54	–	1.54	–	–	–	–	–	–
Bacillus spp.	17.95	5.64	12.31	2.78	2.57	0.21	3.00	0.75	2.25
B. cereus	6.67	5.13	1.54	2.57	2.57	–	1.00	–	1.00
B. subtilis	11.28	0.51	10.77	0.21	–	0.21	2.00	0.75	1.25
No identification	1.54	–	1.54	0.42	–	0.42	0.50	–	0.50
Gram-negative bacteria	20.51	1.53	18.98	0.51	0.17	0.34	15.00	2.25	12.75
Enterobacter spp.	9.23	0.51	8.72	–	–	–	7.70	0.90	6.80
E. cloacae	9.23	0.51	8.72	–	–	–	7.70	0.90	6.80
Klebsiella spp.	0.51	–	0.51	0.51	0.17	0.34	0.56	–	0.56
K. pneumoniae	0.51	–	0.51	0.51	0.17	0.34	0.56	–	0.56
Pseudomonas spp.	2.56	0.51	2.05	–	–	–	2.02	0.90	1.12
P. putica	2.05	0.51	1.54	–	–	–	1.46	0.90	0.56
P. aeruginosa	0.51	–	0.51	–	–	–	0.56	–	0.56
Serratia spp.	8.21	0.51	7.70	–	–	–	4.72	0.45	4.27
S. marcescens	8.21	0.51	7.70	–	–	–	4.72	0.45	4.27
Fungi	12.82	5.12	7.70	10.64	3.21	7.43	7.00	2.75	4.25

[a] GLLP stands for Guangzhou Liuhua Lake Park, GMC is Guangzhou Medical College, and DFR is Dongfengxilu Road.
[b] 1–2 represents stages 1 plus 2 of the cascade impactor; 3–6 denotes the sum of stages 3, 4, 5 and 6.

Table 5. Concentrations of culturable airborne microorganisms at selected sites.

Site	Mean (Colony forming units per cubic meter)
Marseilles	883 (791 ± 598 bacteria, 92 ± 92 fungi)
Porquerolles Island	88 (42 ± 70 bacteria, 46 ± 55 fungi)
Upper Silesia	10^2 to 10^3 (10^2 in offices, 10^3 in homes)
Guangzhou	1399 ± 167
Shenzhen	3541 (2172 to 5856)
Lanzhou	24385 (7942 to 56167)
Beijing	2217 ± 121
Shanghai	5528 (3356 to 10648)
Shenyang	5909
Taiyuan	4150

(Lighthart *et al.*, 1994). Furthermore, vegetative cover can suppress the production of dust, and the amount of vegetative cover and the types of vegetation can influence the concentrations of bacteria in the atmosphere in other ways (Song *et al.*, 1999; Ju *et al.*, 2003). While plants do shed some bacteria into the atmosphere (Ju *et al.*, 2003). On the other hand, some plants emit volatile organic compounds that can be toxic to microorganisms, thus potentially reducing bioaerosol loadings; for example, eucalyptus, aloe, cactus, and spurge all emit natural disinfectants (Xie *et al.*, 1999).

There was more human activity (e.g., walking, running) and also more motor vehicle traffic at GMC and DFR compared with GLLP. These disturbances can generate airborne particulate matter which in turn can act as supportive material for bioaerosols, and this may explain why the bioaerosol concentrations at those two sites were higher than at GLLP. In addition, the large amount of surface covered by water at GLLP (60%) is consistent with lower loadings of suspended dust and therefore fewer particles in the air available to support bacteria. Moreover, the greater green area at GLLP compared with the other sites means that there is less area available there for dust production, and at the same time, the plants can exert the negative effects on microorganisms just mentioned. In these ways, the larger amount of green area at GLLP can help explain the lower concentrations of airborne microorganisms at that relatively clean site.

Temporal variations of the airborne microorganism concentrations showed that peak concentrations at the three sites occurred at 8:00 am and 23:00: this finding is different from the results reported by some other researchers (Lighthart *et al.*, 1997, Shaffer *et al.*, 1997, Zhu *et al.*, 2003). Besides the human activities, traffic patterns, and vegetative coverage just mentioned, meteorological conditions and the flux of ultraviolet light from the sun also can influence the concentrations of airborne bacteria. Gradients in temperature and barometric pressure affect wind speeds, and strong winds can break-up the stable air layer near the Earth's surface. Variations in temperature and wind strength determine the depth of the surface mixed layer, and this directly affects the number of particles in the lower levels of the atmosphere. As noted above, ultraviolet energy is a natural disinfectant (Hu *et al.*, 1989; Shaffer *et al.*, 1997), and therefore some diurnal variability in bioaerosol populations is to be expected because of the daily cycle in the solar energy flux.

The first sampling period in our study was at 08:00, and this is close to morning rush hour when both human and motor vehicle activities peaked. Meanwhile, temperature inversions and the low levels of solar radiation at that time of day are consistent with the relatively high concentrations of bacteria that were observed. When the sampling period changed from 08:00 to 12:00, the air temperatures gradually increased, and this would likely lead to an increase in the depth of the surface mixed layer and the dispersal of bacterial particles as previously mentioned. In addition, the airborne bacteria would be exposed to strong UV radiation at 12:00, and that, too, could decrease the number of viable bacteria (Cox *et al.*, 1989; Lighthart *et al.*, 1995; Shaffer *et al.*, 1997). There was no significant difference among the airborne bacteria concentrations at the three sites at 12:00 and 17:00 despite the fact that human activities, traffic, and vegetation were quite different among these sites. Therefore, surface temperature and ultraviolet light may have been important influences on the concentrations of airborne bacteria; however, further investigations should be performed to study the relationships between bacterial concentrations and these factors, not only in Guangzhou but also at other sites.

We found that Gram-positive bacteria were more abundant than Gram-negative bacteria, and Gram-positive cocci were more common than Gram-positive bacilli; these findings are consistent with the results of other studies (Che, 1988; Ju *et al.*, 2003). Previous studies conducted in Beijing, Shanghai, and other cities in China showed that the dominant bacterial genera were typically *Micrococcus*, *Bacillus*, *Staphylococcus*, and *Pseudomonas* (Che, 1988; Song *et al.*, 1999; Ju *et al.*, 2003). In Guangzhou, the dominant bacterial genera at the clean Liuhua Lake Park site were *Staphylococcus*, *Bacillus*, *Enterobacte*r, and *Serratia*. The Gram-positive *Micrococcus* spp. and the Gram-negative *Pseudomonas* spp. composed only a small percentage of the bacteria at Guangzhou. The dominant bacterial genera in densely populated area of the Medical College were *Staphylococcus*, *Bacillus*, *Micrococcus*, and *Klebsiella* while those in Dongfengxilu Road area were *Staphylococcus*, *Enterobacter*, *Serratia*, and *Bacillus*; these results, except the low percentage of *Micrococcus* in our investigations, are more-or-less consistent with previous studies (Chen *et al.*, 2008).

We found that the size distributions of bacteria at the three sites were different, and this could be due to the different sources of various bacteria (Reponen *et al.* 1992),

but meteorological and environmental factors also may have contributed to these differences. Aerosol particles > 4.7 μm can reach the upper respiratory tracts of humans and other animals but smaller particles (0.65 to 4.7 μm) penetrate further into the lower respiratory tract. The size of a single bacterium is approximately in this lower size range, typically 0.5 to 2.0 μm, and particles of this size would be expected to deposit on stage 5 or stage 6 of the FA-1 sampler.

The impactor data indicate that the size of the bacteria changed as a result of the aerosolization processes and transport, and these factors evidently increased the variance in the size distributions as well. The fact that the size distributions of the bacterial aerosol differed among sites (Table 4) suggests that the bacteria may form aggregates with a variety of atmospheric constituents, including organic carbon, elementary carbon, NH_4^+, SO_4^{2-}, NO_3^-, metals, polycyclic aromatic hydrocarbons, etc. (Cao *et al.*, 2004; Wang *et al.*, 2006; Lai *et al.*, 2010). It is important from a human health standpoint to note that 36 to 76% of the airborne microorganisms deposited on stages 3 to 6 (0.65 to 4.7 μm) of the sampler, and if inhaled, these bacteria would likely be able to reach the lower respiratory tract of humans.

All of the bacteria cultured were opportunistic pathogens, and their presence in the body could stress the immune system. When the immune system is compromised, the body's ability to fight infection is impaired and it becomes more susceptible to disease. In addition, a person's perception of the air quality can be influenced by the numbers of opportunistic pathogenic bacteria that can lead to respiratory problems. In summary, the observed differences in the concentrations of airborne bacteria and their size distributions at the three sites in Guangzhou have important implications for human respiratory disease, and the relationships between bioaerosols and human health deserve increased attention in future studies.

CONCLUSIONS

An investigation of airborne bacteria in different outdoor environments of Guangzhou was conducted in the summers of 2006, 2007, and 2008. We found that (1) the major bacterial genera in Guangzhou were *Staphylococcus*, *Bacillus*, *Enterobacte*r, and *Serratia*; these accounted for 72 to 87% of total airborne microorganisms. The most common bacterial species were both from the genus *Staphylococcus*; these were *S. hominis* and *S. lugdunensis*, and they represented 8 to 46% and 14 to 34% of the total airborne microorganism, respectively; (2) 36 to 76% of the microorganisms deposited in stages 3 to 6 of the sampler (0.65 to 4.7 μm), and if inhaled, particles of this size could penetrate the lower respiratory tract of humans. (3) relatively low concentrations of bacteria were observed at 12:00 and 17:00 at all of the sampling sites, and this is likely due to combined influences of environmental factors, especially the high surface temperatures and strong ultraviolet radiation.

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REFERENCES

Adhikari, A., Sen, M.M., Gupta-Bhattacharya, S. and Chanda, S. (2004). Airborne Viable, Non-Viable, and Allergenic Fungi in a Rural Agricultural Area of India: A 2-Year Study at Five Outdoor Sampling Stations. *Sci. Total Environ.* 326: 123–141.

Cai, Q.R., Pan, Y.W. and Zhang, X.J. (1998). Trends of Variation of Atmospheric Germs in Taiyuan. *Taiyuan Sci. Technol.* 3: 8–11. (in Chinese)

Cao, J.J., Lee, S.C., Ho, K.F., Zou, S.C., Fung, K., Li, Y., Watson, J.G. and Chow, J.C. (2004). Spatial and Seasonal Variations of Atmospheric Organic Carbon and Elemental Carbon in Pearl River Delta Region, China. *Atmos. Environ.* 38: 4447–4456.

Che, FX. (1988). Spatial and Temporal Distribution of Atmosphere Bacteria over Beijing-Tianjin Area. *Chin. J. Publ. Health* 8: 151. (in Chinese).

Chen, X.Y., Bi, X.H., Sheng, G.Y., Fu, J.M. and Li, B. (2008). The Distributive Features of Indoor and Outdoor Bacterium Aerosol in Residential Area of Guangzhou City. *Chin. Trop. Med.* 8: 739–742. (in Chinese)

Cox, C.S. (1989). Airborne Bacteria and Viruses. *Sci. Prog.* 73: 469–499.

Cripps, A., Riebe, M., Schulze, M. and Woodhouse, R. (2000). Pharmaceutical Transition to Non-CFC Pressurized Metered Dose Inhalers. *Respir. Med.* 94: S3–S9.

Fang, Z.G., Ouyang, Z.Y., Hu, L.F., Wang, X.K. and Miao, H. (2004). Progresses of Airborne Microbial Communities in Urban Ecosystem. *Acta Ecol. Sin.* 24: 315–322 (in Chinese).

Fang, Z.G., Ouyang, Z.Y., Hu, L.F., Wang, X.K., Zheng, H. and Lin, X.Q. (2005). Culturable Airborne Fungi in Outdoor Environments in Beijing, China. *Sci. Total Environ.* 350: 47–58.

Fang, Z.G., Ouyang, Z.Y., Zhao, J.Z., Wang, X.K. and Zheng, H. (2006). Community Composition and Dynamics of Airborne Bacteria in Beijing. *Acta Microbiol. Sin.* 46: 618–623 (in Chinese).

Fang, Z.G., Ouyang, Z.Y., Zheng, H., Wang, X. and Hu, L. (2007). Culturable Airborne Bacteria in Outdoor Environments in Beijing, China. *Microb. Ecol.* 54: 487–

496.

Ho, J. and Duncan, S. (2005). Estimating Aerosol Hazards from an Anthrax Letter. *J. Aerosol Sci.* 36: 701–719.

Hu, Q.X., Che, F.X., Zhang, S.L., Chen, Z.S., Xu, G.Q., Xu, X.Z., Shi, C.Y. and Meng, L.Y (1989). The Concentration of Airborne Microbes in Beijing–Tianjin Area. *Environ. Sci.* 10: 30–35 (in Chinese).

Hu, Q.X., Cai, Z.L. and Lu, Z.X. (1995). Relation between Airborne Bacteria and Fungal Particles in Shenyang Atmosphere. *Shanghai Environ. Sci.* 14: 29–32 (in Chinese).

Ju, T.Z., Suo, A.N., Tian, Y.J. and Feng, K.K. (2003). Analysis on Aerobiologia in Lanzhou. *Ind. Saf. Environ. Prot.* 3: 17–19 (in Chinese).

Koch, A.L. (1996). What Size Should a Bacterium be? A Question of Scale. *Annu. Rev. Microbiol.* 50: 317–348.

Lai, S.C., Ho, K.F., Zhang, Y.Y., Lee S.C., Huang, Y. and Zou, S.C. (2010). Characteristics of Residential Indoor Carbonaceous Aerosols: A Case Study in Guangzhou, Pearl River Delta Region. *Aerosol Air Qual. Res.* 10: 472–478.

Lee, N., Hui, D., Wu, A., Chan, P., Cameron, P., Joynt, G.M., Ahuja, A., Yung, M.Y., Leung, C.B., To, K.F., Lui, S.F., Szeto, C.C., Chung, S. and Sung, J.J. (2003). A Major Outbreak of Severe Acute Respiratory Syndrome in Hong Kong. *New Engl. J. Med.* 348: 1986–1994.

Lighthart, B. (1994). Physics of Microbial Bioaerosols, In *Atmospheric Microbial Aerosols: Theory and Applications*, Lighthart, B. and Mohr, A.J. (ed.), Chapman and Hall, New York, p. 5–27.

Lighthart, B. and Mohr, A. (1994). *Fundamental Principles Describing Atmospheric Microbial Aerosols: Theoretical and Applied Aspects*, Chapman-Hall, New York.

Lighthart, B. and Stetzenbach, L.D. (1994). Distribution of Microbial Bioaerosols, In *Atmospheric Microbial Aerosols: Theory and Applications*, Lighthart, B. and Mohr, A.J. (ed.), Chapman and Hall, New York, p. 68–98.

Lighthart, B. and Shaffer, B.T. (1995). Airborne Bacteria in the Atmospheric Surface Layer: Temporal Distribution above a Grass Seed Field. *Appl. Environ. Microbiol.* 61: 1492–1496.

Lighthart, B. (1997). The Ecology of Bacteria in the Alfresco Atmosphere. *FEMS Microbiol. Ecol.* 23: 263–274.

Mancinelli, R.L. and Shulls, W.A. (1978). Airborne Bacteria in an Urban Environment. *Appl. Environ. Microbiol.* 35: 1095–1101.

Pastuszka, J.S., Paw, U.K.T., Lis, D.O., Wlazlo, A. and Ulfig, K. (2000). Bacteria and Fungal Aerosol in Indoor Environment in Upper Silesia, Poland. *Atmos. Environ.* 34: 3833–3842.

Reponen, T.A., Grinshpun, S.A., Conwell, K.L., Wiest, J. and Anderson, M. (2001). Aerodynamic Versus Physical Size of Spores: Measurement and Implication on Respiratory Deposition. *Grana* 40: 119–125.

Shaffer, B.T. and Lighthart, B. (1997). Survey of Culturable Airborne Bacteria at Four Diverse Locations in Oregon: Urban, Rural, Forest, and Coastal. *Microb. Ecol.* 34: 167–177.

Shelton, B., Kirkland, K.H., Flanders, W.D. and Morris, G.K. (2002). Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States. *Appl. Environ. Microbiol.* 68: 1743–1753.

Lee, S., Ghim, Y.S., Kima, S.W. and Yoon, S.K. (2010). Effect of Biomass Burning and Regional Background Aerosols on CCN Activity Derived from Airborne *in-situ* Measurements. *Atmos. Environ.* 44: 5227–5236.

Song, L.H., Song, W.M. and Shi, W. (1999). Study on Airborne Bacteria Pollution in Shanghai. *Shanghai Environ. Sci.* 18: 258–260 (in Chinese).

Stetzenbach, L.D. (1998). Microorganisms and Indoor Air Quality. *Clin. Microbiol. Newsletter* 20: 157–161.

Szweda, P., Kotłowski, R. and Kur, J. (2005). New Effective Sources of the *Staphylococcus simulans* Lysostaphin. *J. Biotechnol.* 117: 203–213.

Tian, Y.J., Ju, T.Z., Feng, K.K., Ma, Y.P., Suo, A.N. and Zhang, J.H. (2008). Analysis of Air Bacterium Pollution in Function Area and Sterilization Effect of Urban Greenbelt System in Lanzhou City. *Environ. Monit. China* 19: 45–47 (in Chinese).

Wang, C.H., Xie, X.B., Zeng, H.Y., Ouyang, Y.S., Zheng, Z.Q. and Chen, Y.Y. (2008). Monitoring Analysis of Air Microbial Pollution Conditions in Shenzhen City. *J. Microbiol.* 28: 93–97 (in Chinese).

Wang, X.H., Bi, X., Sheng, G. and Fu, J. (2006). Hospital Indoor $PM_{10}/PM_{2.5}$ and Associated Trace Elements in Guangzhou, China. *Sci. Total Environ.* 336: 124–135.

Xie, H.L., Li, S.R., Yuan, X.Y., Liu, G.W., Gao, X.M., Cao, Y. and Jia, J. (1999). Study on the Disinfection of Plant Volatile Secretion to the Microorganism Content in the Air. *J. Henan Agric. Univ.* 33: 127–133 (in Chinese).

Xie, X.B., Ouyang, Y.S., Chen, Y.B., Huang, X.M., Peng, H. and Jia, Y.Y. (2004). Investigation on Airborne Microbial Pollution in Walking Street in Guangzhou. *Chin. J. Health Lab. Technol.* 14: 353–354 (in Chinese).

Zhu, C.S., Cao, J.J., Tsai, C.J. Shen, Z.X., Ho, K.F., Liu, S.X. (2010). The Indoor and Outdoor Carbonaceous Pollution during Winter and Summer in Rural Areas of Shaanxi, China, *Aerosol Air Qual. Res.* 10: 550–558.

Zhu, H., Phelan, P.E., Duan, T., Raupp, G.B., Fernando, H.J.S. and Che, F. (2003). Experimental Study of Indoor and Outdoor Airborne Bacterial Concentrations in Tempe, Arizona, USA. *Aerobiologia* 19: 201–211.