CONCENTRATIONS OF AIRBORNE BACTERIA IN 100 U.S. OFFICE BUILDINGS

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ABSTRACT
This paper presents preliminary summary statistics of airborne bacteria from the US Environmental Protection Agency (USEPA) Building Assessment Survey and Evaluation (BASE) study. Air samples were collected with a single-stage agar impactor in 100 large office buildings from 1994 to 1998. Five groups of culturable bacteria were identified at two incubation temperatures: (1) Gram-positive rods, separately actinomycetes and *Bacillus* species, (2) Gram-positive cocci, (3) Gram-negative rods, (4) Gram-negative cocci, and (5) unknown bacteria. Bacterial concentrations were compared by: (1) incubation temperature (30°C and 55°C, respectively, for mesophilic and thermophilic bacteria), (2) sampling location (indoors and outdoors), and (3) season (summer and winter). Mesophilic bacteria accounted for more than 80% of total culturable bacteria, both indoors and outdoors. Total bacterial concentrations generally were higher and more variable outdoors but similar in summer and winter. Indoor concentrations showed more seasonal difference and Gram-positive cocci were somewhat higher in summer.

INDEX TERMS
Airborne bacteria, Culturable bacteria, Office buildings, Bioaerosols, BASE

INTRODUCTION
The USEPA conducted the Building Assessment Survey and Evaluation (BASE) study to collect baseline information on environmental factors, building characteristics, and occupants' perceptions of comfort and the indoor environment. In this cross-sectional study, data were collected in 100 large public and commercial office buildings from 1994 to 1998 in 25 states. Buildings meeting certain criteria were selected randomly (without regard to IAQ complaints), stratified into 10 climate zones, and studied once in summer or winter (Womble et al., 1996).

Bioaerosol data collected in the BASE study included air samples (culturable fungi and bacteria, and fungal spores), bulk samples (culturable fungi and bacteria), and dust samples (cat and dust mite allergens). This paper presents preliminary summary statistics on the concentrations of airborne culturable bacteria; allergen data are presented in a separate paper. Naturally occurring bacteria seldom cause human illness, although some are agents of hypersensitivity, infectious, or inflammatory diseases. Endotoxin (a component of the outer

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membrane of Gram-negative bacteria) is recognized as a health hazard in various occupations and has been associated with asthma severity (Park et al., 2001). Many bacteria are essential to human health (e.g., Gram-positive bacteria from human skin and scalp) and to the earth's ecology. The bacteria found in indoor air generally were shed by building occupants or entered with outdoor supply air. The risk of illness from environmental bacteria increases when they enter buildings in inappropriate numbers or multiply indoors (Otten and Burge, 1999). Sampling for culturable bacteria generally underestimates actual human exposure because non-culturable cells (often a large fraction of total bacteria) are not detected. However, this method is widely used to assess indoor air quality, and baseline information on the concentrations of culturable bacteria in occupied indoor environments is essential for proper interpretation of samples collected in investigations of problem buildings.

METHODS
COLLECTION METHOD
Air samples for culturable bacteria were collected using four single-stage, multiple-hole agar impactors (N-6, d_{50} cutpoint: 0.6 μm; Andersen Instruments, Smyrna, GA) for two sampling durations (2 and 5 min) at 28.3 ±1.4 L/min. Bacteria were collected on tryptic soy agar, which was incubated at 30°C (for mesophilic bacteria) and 55°C (for thermophilic bacteria). Bioaerosol samples were collected in the morning and afternoon, at one outdoor and three indoor locations at each building. The outdoor and one indoor sample were collected in duplicate. Samples for the two incubation temperatures and sampling durations were collected simultaneously at the indoor sites without duplicate samples (stopping two of the instruments after 2 min and allowing the others to run an additional 3 min). Otherwise, duplicate 2- and 5-min samples were collected simultaneously, first by sampling for 2 minutes onto four plates (two for each incubation temperature) followed by replacement of the media and re-sampling for 5 minutes. Bacterial results were reported as the number of colony-forming units (CFUs) of each bacterial group per plate and further adjusted by the volume of air sampled to obtain concentrations (CFU/m^3).

ANALYSIS METHOD
A total of 5201 bacterial samples, including 420 blanks (8%), were collected in the BASE study. Data of questionable quality were excluded for this analysis (Table 1) . Samples with concentrations below the respective detection limits (2-min samples: 18 CFU/m^3; 5-min samples: 7 CFU/m^3) were set to half of the detection limits.

Composite bacterial concentrations (i.e., one indoor and one outdoor measurement for each building) were obtained by first averaging duplicate samples, then summing the plate counts for all bacterial groups and samples: 24 indoor and 8 outdoor samples.
> **Number of indoor samples**: (2 sampling durations, 2-/5-min) (2 incubation temperatures, 30°/55°C) (2 sampling times, AM/PM) (3 sampling locations) = **24**
> **Number of outdoor samples**: (2 sampling durations, 2-/5-min) (2 incubation temperatures, 30°/55°C) (2 sampling times, AM/PM) = **8**

The total indoor and outdoor plate counts were divided by the respective total volumes of air collected at each building to determine the composite concentrations. Air volumes varied but were approximately 8 m^3 for all outdoor samples and 24 m^3 for all indoor samples.
> **Indoor sample volume**: [(12 samples) (2 min) (0.283 m^3/min)] + [(12 samples) (5 min) (0.283 m^3/min)] = **23.8 m^3**
> **Outdoor sample volume**: [(4 samples) (2 min) (0.283 m^3/min)] + [(4 samples) (5 min) (0.283 m^3/min)] = **7.9 m^3**

The respective minimum concentrations that could be detected indoors and outdoors for composite samples were 0.08 and 0.13 CFU/ m^3. Results are presented separately for thermophilic and mesophilic bacteria and for their sums (total culturable bacteria).

Table 1.	Air samples for culturable bacteria collected in the BASE study (N and %)		
Total number of samples collected		**5201**	**100%**
Samples under detection limit		1816	35 %
Maximum samples used in analyses		4359	84 %
Samples excluded from analyses		842	16 %
Blank samples		420	8 %
Unacceptable data		338	7 %
Samples spoiled by laboratory		45	1 %
Overgrown samples		38	<1 %
Samples not analyzed		1	<0.1%

RESULTS

Concentrations for all blank samples were below the detection limit (i.e., little or no growth was observed on the plates). The precision of duplicate samples was evaluated by calculating the Relative Standard Deviation (RSD = $[|a1 - a2| / (a1 + a2)](2)^{\frac{1}{2}}$, where a1 and a2 were the concentrations of co-located duplicate samples). The average RSD for 1413 paired samples was 0.28 (median = 0.18, mode = 0, maximum = 1.35).

The concentrations of the five bacterial groups are summarized in Tables 2 (by location and season) and in Tables 3A–3C (by location and incubation temperature). Minimum concentrations are not shown but almost always were below the detection limit. Approximately 44% of 2-min samples (N=977) and 38% of 5-min samples (N=836) were below the detection limit, and 1% and 4%, respectively, were overgrown. Due to the exclusion of some measurements, a category may have fewer than 100 buildings. Using mesophilic bacteria as an example (Table 3B), only 98 buildings had complete indoor data and 84 buildings had complete outdoor data.

Average concentrations of total bacteria were higher outdoors than indoors (respectively, 470 and 280 CFU/m^3) (Table 2). Outdoor concentrations were similar in summer and winter (respectively, 474 and 465 CFU/m^3), while indoor concentrations showed more seasonal difference (respectively, 306 and 252 CFU/m^3).

 Indoors, Gram-positive cocci and rods comprised similar fractions of the total bacterial concentration:
   Unknowns (38%), Gram-positive cocci (**29%**), Gram-positive rod (**23%**), Gram-negative rods (5%), and Gram-negative cocci (4%).
 Outdoors, Gram-negative rods were found twice as often as Gram-positive cocci:
   Unknown (55%), Gram-positive rods (**25%**), Gram-positive cocci (**11%**), Gram-negative rods (7%), and Gram-negative cocci (3%).

Bacterial group	Indoors			Outdoors		
Table 2. Concentrations (CFU/m^3) of airborne culturable bacteria by location (indoors/outdoors) and season (summer/winter/combined)						
	Summer	Winter	**Combined seasons**	Summer	Winter	**Combined seasons**
All Gram + rods	63	68	**66**	107	127	**117**
(Actinomycetes)	(22)	(22)	**(22)**	(32)	(25)	**(29)**
(*Bacillus* species)	(29)	(30)	**(29)**	(50)	(64)	**(57)**
(Other Gram + rods)	(12)	(16)	**(14)**	(26)	(38)	**(32)**
GM + cocci	101	60	**82**	58	48	**53**
GM − rods	16	14	**15**	31	31	**31**
GM − cocci	12	13	**12**	12	14	**13**
Unknowns	114	98	**106**	266	246	**256**
Total bacteria	306	252	**280**	474	465	**470**

A seasonal difference was observed indoors for Gram-positive cocci (summer: 101 CFU/m^3; winter: 60 CFU/m^3), which also was the only group for which the mean concentration was higher indoors than outdoors (respectively, 82 and 53 CFU/m^3) (Table 2). Higher outdoor concentrations of Unknown bacteria and Gram-positive rods (especially *Bacillus* species) contributed strongly to the otherwise higher outdoor bacterial concentrations. The large number of isolates that could not be identified readily (Unknowns) illustrates one of the difficulties of relying on culturing of environmental air samples to evaluate exposure to biological agents.

More bacteria (in all groups) grew at the moderate incubation temperature of 30°C in both locations (Tables 3B and 3C) and seasons (data not shown). Therefore, the concentrations of mesophilic bacteria were similar to those for total bacteria (Tables 3A and 3B). Indoor and outdoor distributions among the bacterial groupings for both incubation temperatures were similar, but Gram-positive rods comprised the largest fraction of thermophilic bacteria in both locations.

The concentration of total cultural bacteria was higher outdoors in all climate zones (data not shown). Stratification by season showed that outdoor bacterial concentrations were higher in all zones in winter and 7 of 10 zones in summer.

DISCUSSION
The large RSD for duplicate samples indicates that there was a high degree of variability in the concentration of culturable bacteria; therefore, single samples may be poor indicators of bacterial air concentrations. For nonbiological agents (e.g., formaldehyde or airborne particles), large RSDs may reflect sampling problems (e.g., inconsistent equipment performance). For biological agents, differences between duplicate samples provide estimates of both sampling and random ("chance") errors. Assuming that the two samplers performed comparably, the differences observed for duplicate samples can be attributed primarily to random variation rather than instrument bias. Investigators have observed differences in air concentrations of culturable microorganisms over time and space of three to four orders of magnitude (AIHA, 1996), even greater than what was observed in the BASE buildings.

Table 3. Concentrations (CFU/m^3) of airborne culturable bacteria by location (indoors/outdoors) and incubation temperature (sum/30°C/55°C)

Table 3A. Total bacteria (sum of mesophilic and thermophilic bacteria)

	Variable	N	Mean	Std. Dev	Maximum	Median
Indoors	Gram + rods	100	66	19	166	60
	Gram + cocci	98	81	53	293	67
	Gram – rods	98	15	9	81	12
	Gram – cocci	98	12	8	59	10
	Unknowns	100	106	81	422	88
Outdoors	Gram + rods	85	117	111	748	78
	Gram + cocci	84	53	68	407	21
	Gram – rods	84	31	59	367	14
	Gram – cocci	84	13	9	60	10
	Unknowns	85	256	436	3678	138

Table 3B. Mesophilic bacteria (isolated at 30°C)

	Variable	N	Mean	Std. Dev	Maximum	Median
Indoors	Gram + rods	98	44	15	108	39
	Gram + cocci	98	81	53	293	67
	Gram – rods	98	15	9	81	12
	Gram – cocci	98	12	8	59	10
	Unknowns	98	95	79	411	78
Outdoors	Gram + rods	84	84	98	725	54
	Gram + cocci	84	53	68	407	21
	Gram – rods	84	31	59	367	14
	Gram – cocci	84	13	10	60	10
	Unknowns	84	244	437	3667	129

Table 3C. Thermophilic bacteria (isolated at 55°C)

	Variable	N	Mean	Std. Dev	Maximum	Median
Indoors	Gram + rods	100	23	5	58	21
	Gram + cocci	2	6	5	10	6
	Gram – rods	2	6	5	10	6
	Gram – cocci	2	6	5	10	6
	Unknowns	100	13	13	122	10
Outdoors	Gram + rods	85	34	33	194	22
	Gram + cocci	2	4	2	5	4
	Gram – rods	1	5	NA	5	5
	Gram – cocci	3	4	1	5	5
	Unknowns	85	15	13	110	10

For this paper, we calculated composite indoor and outdoor concentrations assuming that samples from two times of day and three randomly selected locations provided reasonable estimates of exposures throughout an 8-hour workday. Combining all samples effectively lowered the detection limit to 0.13 CFU/m^3 outdoors and 0.08 CFU/m^3 indoors, as compared

with the limits for individual 2- or 5-min samples (respectively, 18 and 7 CFU/m^3).

In addition to providing a more representative estimation of occupant exposures, collection of multiple samples allows investigators to examine the variation in bioaerosol concentrations over time and space. In future work, we plan to compare the types and concentrations of bacteria in samples collected in the morning and afternoon as well as the agreement among the three indoor sampling locations. The extensive information on building characteristics and occupant perceptions in the database also will be linked with the information on biological agents in air and bulk samples to identify features that may be associated with indoor environmental quality.

CONCLUSION AND IMPLICATIONS

Outdoor concentrations of airborne bacteria generally were higher than those indoors but similar in summer and winter. Bacterial concentrations indoors showed more seasonal difference, which may be due to changes in occupant dress and activities as well as ventilation patterns during the cooling and heating seasons. Concentrations of bacteria associated with normal human flora (e.g., Gram-positive cocci) were more abundant in indoor air and in summer whereas those associated with soil and plant surfaces (e.g., Gram-positive and –negative rods) were more abundant in outdoor air, with little seasonal difference. Likewise, mesophilic bacteria comprised a larger proportion of total culturable bacteria than thermophiles in this as in other studies of residential and office environments. The preliminary results in this paper provide baseline information on the concentrations of culturable bacteria in commercial and public buildings in the United States. The results may change somewhat when the full dataset becomes available and questions about some of the entries are resolved.

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