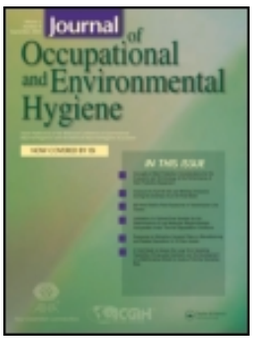

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Assessing Total Fungal Concentrations on Commercial Passenger Aircraft Using Mixed-Effects Modeling

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The primary objective of this study was to compare airborne fungal concentrations onboard commercial passenger aircraft at various in-flight times with concentrations measured inside and outside airport terminals. A secondary objective was to investigate the use of mixed-effects modeling of repeat measures from multiple sampling intervals and locations. Sequential triplicate culturable and total spore samples were collected on wide-body commercial passenger aircraft ($n = 12$) in the front and rear of coach class during six sampling intervals: boarding, midclimb, early cruise, midcruise, late cruise, and deplaning. Comparison samples were collected inside and outside airport terminals at the origin and destination cities. The MIXED procedure in SAS was used to model the mean and the covariance matrix of the natural log transformed fungal concentrations. Five covariance structures were tested to determine the appropriate models for analysis. Fixed effects considered included the sampling interval and, for samples obtained onboard the aircraft, location (front/rear of coach section), occupancy rate, and carbon dioxide concentrations. Overall, both total culturable and total spore fungal concentrations were low while the aircraft were in flight. No statistical difference was observed between measurements made in the front and rear sections of the coach cabin for either culturable or total spore concentrations. Both culturable and total spore concentrations were significantly higher outside the airport terminal compared with inside the airport terminal (p-value < 0.0001) and inside the aircraft (p-value < 0.0001). On the aircraft, the majority of total fungal exposure occurred during the boarding and deplaning processes, when the aircraft utilized ancillary ventilation and passenger activity was at its peak.

Keywords aircraft, culturable fungi, mixed effects models, total fungi, total spore fungi

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INTRODUCTION

Approximately 198,000 flight personnel and 73,000 aircrew personnel work [(1)] in the aircraft cabin environment in the United States. Yet, to date, only five peer reviewed studies have documented fungal concentrations on passenger aircraft.[(2–6)] These select studies have relied on a small number (<5) of samples per flight to represent the entire aircraft environment.

Nagda et al.[(2)] sampled for fungal concentrations on 23 randomly selected, nonsmoking flights. Using a compact sieve plate sampler, the mean culturable fungal concentration was less than 10 colony forming units per cubic meter of air sampled (CFU/m^3). Using a Reuter centrifugal air sampler, Wick and Irvine[(3)] observed a mean culturable fungal concentration of 43 CFU/m^3 on 36 flights on five types of aircraft. Using a portable compact air sampler (Rodac), Pierce et al.[(4)] studied fungal concentrations on eight flights aboard Boeing-777 aircraft where culturable fungal concentrations ranged between 1–37 CFU/m^3.[(4)] Dumyahn et al.[(5)] collected fungal samples aboard Boeing-777 aircraft using the portable Burkard culture plate sampler and observed concentrations ranging between 1–500 CFU/m^3. To date, no aircraft study has included total spore count monitoring, and only the Dumyahn et al. study completed a cursory fungal genus analysis with the culturable results.

In 2002, the National Academy of Sciences completed a review of the airliner cabin environment, including bioaerosol concentrations.[(7)] This review encompassed peer reviewed studies[(2–6)] and other non-peer reviewed works. The committee indicated that although bioaerosol concentrations observed on aircraft were similar to other indoor environments, the number of samples was low, few replicates were collected, and estimates of variability were poor.

Mixed-effects modeling has been used in several studies to model both the mean and covariance of an individual's exposure.[(8–10)] The primary advantage of mixed-effects modeling is that it allows an analysis of repeated measurements

from individuals and takes into account the potential dependencies among the repeated measures. Others have shown the value of mixed-effect models for analyzing repeated measures data in the presence of missing values.[11–13] Exposure variability is important when assessing the value of a particular exposure measure.[14] In many cases, mixed-effects modeling is used to analyze repeat measures from an individual, but it can also be applied to repeat measures from a sampling location.[15]

The objectives of this research were to create a baseline of total fungi (culturable and total spore) concentrations on wide-body commercial passenger aircraft; test specific hypotheses on cabin location and different in-flight time periods; investigate the use of mixed-effects modeling techniques and covariance models; and estimate variability for total fungi in the aircraft environment.

METHODS

Since it is representative of a large, twin-engine airplane that uses filtered recirculated air, the Boeing 767 (B-767) aircraft was selected for study. Two airlines operating within the United States with large fleets of B-767 aircraft agreed to participate in the study. A total of 12 B-767 flight segments were randomly selected based on a complete list of flight schedules provided by each airline. Eligible flights consisted of all B-767 flights with flight duration between 4.5 and 6.5 hr during the summer 2002 schedule. To minimize a seasonality effect, all flights were sampled during the same weather season (i.e., summer).

Sampling Intervals

Flight segments were divided into 10 sampling intervals. Samples were collected outside and inside the airport terminal at both the origin and destination cities. Samples were also collected during six sampling intervals onboard the aircraft: boarding, midclimb, early cruise, midcruise, late cruise, and deplaning. Boarding samples were taken while passengers were actively boarding the aircraft; midclimb samples were taken immediately after the aircraft reached approximately 10,000 feet; cruise samples were taken during the flight at three times selected to be approximately equally spaced based on the length of the flight; and deplaning samples were taken while passengers were actively disembarking from the aircraft.

Outside/Inside Airport Terminal Sampling

At the origin city airport, the field team collected a complete set of samples for both airborne culturable fungi and total fungal spores outside the airport terminal. A second set of samples was obtained inside the airport terminal in the vicinity of the selected flight gate. At the conclusion of each flight, airborne samples were collected in the same manner both inside and outside the airport terminal at the destination city airport.

Aircraft Sampling

Two complete sets of samples were taken in each aircraft, one in the front of coach class and one in the rear of coach class during each sampling interval. Two-member teams were seated in the center section within the first three rows of coach class and in the center section within the last three rows of coach class. Personal air outlets (i.e., gaspers) were closed at all times during the monitoring period. Additional details regarding the sampling process are presented elsewhere.[16]

Indoor Air Parameters

Selected comfort parameters including temperature, relative humidity, and carbon dioxide were collected gate to gate using a portable Q-trak instrument (model 8550; TSI Instruments, Inc., Shoreview, Minn.). Flight characteristics such as the occupancy rate (number of passengers to number of passenger seats available), airpack use (environmental control system, 1 or 2), and the recirculation rate (0 or 50%) were also collected.

Airborne Fungal Samples

Both agar and spore trap samples were collected to assess the fungal concentrations and to facilitate comparability with other work. Airborne culturable fungal samples were obtained using an N-6 impactor, DG-18 media, and a high-volume pump calibrated to 28.3 L/min (PORTN-6; Thermo Andersen, Smyrna, Ga.). During each interval, with the exception of deplaning, sequential triplicate culturable fungal samples were collected, each of a 4-min duration. During the short deplaning process, duplicate samples were collected, each of a 2-min duration.

Airborne total spore samples were obtained using an Air-o-Cell cassette (Zefon International, Ocala, Fla.) and the PORTN-6 high-volume pump calibrated to 15 L/min. During each interval, with the exception of deplaning, sequential triplicate spore samples were collected, each of a 10-min duration. During the short deplaning process, one sample was collected for a 10-min duration.

Sample Handling and Analysis

Culturable samples were kept cool and shipped on blue ice via an overnight mail carrier to the analytical laboratory within 48 hr after sample collection. Total spore samples were sent to the analytical laboratory after each roundtrip flight. The laboratory was accredited by the American Industrial Hygiene Association (AIHA) and participated in the AIHA Environmental Microbiology Proficiency Analytical Testing (EMPAT) program. Culturable fungal samples were incubated at 25°C for 7–10 days then counted and identified. Total spore samples were counted and analyzed for spore identification and counted using the 60× objective lens of a microscope. Twenty random passes were completed through the entire sample trace. All spores falling within the ocular micrometer were counted and identified.

Statistical Methods

SAS 9 Software (SAS Institute Inc., Cary, N.C.) was used for all statistical analyses. Airborne culturable fungi results were assessed as total colony forming units (CFU) per sample and total spore results were assessed as total spores per sample.

Samples reported as "no growth" or "no spore detected" were replaced with one-half of the limit of detection of one CFU per culturable sample and one spore per spore trap sample.[17] Overall, less than 20% of the culturable samples were below the limit of detection and less than three percent of the total spore samples were reported as "no spores detected." Culturable results were converted to a total concentration in air by dividing by the air volume associated with the sample, resulting in the number of CFU per cubic meter of air. Total spore sample results were converted to total spores per cubic meter of air (spores/m^3) using a sample-specific multiplication factor derived from the sample trace length, the trace length counted (microscope field diameter, number of transverses), and the sample volume. The distributions of both the airborne culturable fungi and total spore count concentrations were skewed to the right; therefore, the natural logarithm of the total concentration was used in all analyses.

Since samples were obtained at more than one sampling interval during each flight, and multiple samples were obtained during each sampling interval, airborne culturable and total spore count results were analyzed using mixed-effects modeling. In general, a mixed-effects model can be written as follows:

$$Y = X\beta + Z\gamma + \varepsilon$$

where X is the design matrix for the fixed effects, β is the parameter vector for the fixed effects, Z is the design matrix for the random effects, γ is the parameter vector for the random effects, and ε is the vector of random errors. The model assumes γ is multivariate normal with mean zero and covariance matrix G; ε is multivariate normal with mean zero and covariance matrix R; and independence between γ and ε. The covariance matrix of Y is given by $V = ZGZ' + R$. The MIXED procedure in SAS allows for the specification of different covariance structures through the RANDOM and REPEATED statements. In addition, the MIXED procedure does not require the data to be balanced.

Fixed effects considered included the sampling interval and, for samples taken on board the aircraft, location (front of coach section, rear of coach section), carbon dioxide (ppm), and occupancy rate. Individual fungal measurements and the time-weighted carbon dioxide concentrations were linked by interval on each flight. Random effects considered included the flight segment number (1, 2, . . . , 12) and the sampling interval (i.e., outside terminal, inside terminal, boarding, and so on).

To simplify the modeling process, separate models were specified for air samples obtained at the origin city (outside the airport terminal, inside the airport terminal, and during boarding); on board the aircraft (boarding, midclimb, early cruise, midcruise, late cruise, and deplaning); and at the destination city (during deplaning, inside the airport terminal, and outside the airport terminal). For example, the mixed-effects model for the origin city samples can be written as:

$$Y_{ijk} = \beta_j + \gamma_i + \varepsilon_{ijk}$$

where i (1, 2, . . . , 12) indicates the flight, j (0, 1, 2) indicates the sampling interval, and k (1, 2, . . . , n_{ij}) indicates the replicate. Five alternate covariance structures, denoted structures I–V, were specified by using different effects and options in the RANDOM and REPEATED statements (Table I).

All of the structures considered assume that the flights were independent and fit a compound symmetric structure within each sampling interval. The first structure specified a common covariance between samples obtained in different intervals and allowed the parameters of the compound symmetric structure within each interval to vary across the intervals. The second was similar to the first, but specified common parameters for the compound symmetric structure within each interval.

The third and fourth were similar to the first two but specified a covariance of zero between samples obtained in different intervals. Finally, the fifth structure specified a compound symmetric structure overall. The restricted maximum likelihood (REML) method was used to estimate the covariance parameters and the Kenward-Rogers (KR) method was used to compute degrees of freedom. Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC), which are functions of the maximized residual log likelihood, penalized for the effective number of covariance parameters, were used to select between models with different covariance structures.

Models having the smallest AIC or BIC were deemed best. Once a structure was selected, levels of the fixed effects were compared using the Tukey-Kramer adjustment for multiple comparisons. Since the dependent variable was natural log transformed, results are presented as adjusted geometric means after exponentiating the adjusted means. Model residuals were analyzed for normality and deemed appropriate.

RESULTS

Samples were collected on 12 randomly selected B-767 flights. Passenger occupancy rates on the flights ranged from 67–100%. For each flight, the pilots turned on the recirculation fans (i.e., approximately 50% outside bleed air and 50% filtered recirculated air) and operated both airpacks during the entire duration of the flight.

Table II presents the percent and range of the detectable concentrations for both the culturable and total spore fungi results summarized by sampling interval. All outside airport terminal samples were positive (reported detectable concentrations) for airborne culturable fungi and airborne total spores. A majority of samples taken during the remaining sampling intervals were positive for airborne culturable fungi, with samples taken inside the airport terminal and during boarding and deplaning more likely to be positive than samples taken during the midclimb and cruise intervals. Nearly all samples taken on board the aircraft were positive for spores.

Table III presents estimated correlation coefficients of the natural log transformed concentrations for replicate samples taken within the same sampling interval (diagonal entries) and between samples taken during different sampling intervals

TABLE I. Covariance Structures Used for Modeling Airborne Fungal Concentration

Structure	$\mathrm{cov}(Y_{ijk}, Y_{i'j'k'})$[A]: $i=i'$, $j=j'$, $k=k'$	$i=i'$, $j=j'$, $k\neq k'$	$i=i'$, $j\neq j'$	$i\neq i'$	SAS Code: PROC MIXED METHOD=REML; CLASS FLIGHT INTERVAL; MODEL LN_TOTAL_CONCENTRATION = INTERVAL/SOLUTION DDFM=KR;
I[B]	$\sigma_b^2 + \sigma_j^2 + \phi_j$	$\sigma_b^2 + \phi_j$	σ_b^2	0	RANDOM INTERCEPT/SUBJECT=FLIGHT; REPEATED/GROUP=INTERVAL SUBJECT = FLIGHT TYPE=CS;
II	$\sigma_b^2 + \sigma^2 + \phi$	$\sigma_b^2 + \phi$	σ_b^2	0	RANDOM INTERCEPT/SUBJECT=FLIGHT; REPEATED / SUBJECT = INTERVAL(FLIGHT) TYPE=CS;
III	$\sigma_j^2 + \phi_j$	ϕ_j	0	0	REPEATED/GROUP=INTERVAL SUBJECT = FLIGHT TYPE=CS;
IV	$\sigma^2 + \phi$	ϕ	0	0	REPEATED/SUBJECT = INTERVAL(FLIGHT) TYPE=CS;
V	$\sigma^2 + \phi$	ϕ	ϕ	0	REPEATED/SUBJECT = FLIGHT TYPE=CS;

[A] Y_{ijk} refers to the natural log transformed air level from flight i, at sampling interval j, and replicate k; $\mathrm{cov}(Y_{ijk}, Y_{y'j'k'})$, the covariance matrix for Y, is given by $ZGZ' + R$.

[B] For Structure I, the RANDOM statement specifies that G is $\sigma_b^2 I$, where I is the 1×1 identity matrix. The SUBJECT=FLIGHT option in the REPEATED statement specifies that R is a block diagonal matrix with blocks that are determined by the flights. The GROUP=INTERVAL option in the REPEATED statement specifies that the block for a particular flight is also a block diagonal matrix where the sub-blocks are determined by the sampling intervals. With the options as specified here, the TYPE=CS option in the REPEATED statement specifies that the structure for each sub-block is compound symmetry, with different parameters (i.e., σ_j^2 and ϕ_j) for each sub-block.

TABLE II. Percent and Range of Detectable Samples for Airborne Culturable and Total Spore Fungal Concentrations

Location	Interval	N Flights	Airborne Culturable Fungi[A]: N Samples	N > LOD (%)	Range[C] (CFU/m^3)	Airborne Total Spore[B]: N Samples	N > LOD (%)	Range[C] (spores/m^3)
Airport	Outside airport terminal	12	35	35 (100)	123–2840	36	36 (100)	317–22,000
Airport	Inside airport terminal	12	36	34 (94)	8.8–482	36	36 (100)	25.3–1230
Aircraft	Boarding	12	64	60 (94)	8.7–3350	69	68 (99)	24.7–1850
Aircraft	Midclimb	12	72	57 (79)	8.6–131	72	71 (99)	24.9–349
Aircraft	Early cruise	12	72	49 (68)	8.7–183	72	69 (96)	24.7–175
Aircraft	Midcruise	12	72	47 (65)	8.7–43.9	72	68 (94)	24.7–174
Aircraft	Late cruise	12	72	49 (68)	8.7–61.0	72	71 (99)	24.7–179
Aircraft	Deplaning	12	33	32 (97)	17.5–1310	23	22 (96)	26.7–642
Airport	Inside airport terminal	11	33	32 (97)	8.7–303	33	33 (100)	25.2–706
Airport	Outside airport terminal	11	33	33 (100)	116–3590	32	32 (100)	199–12,200

[A] The minimum detectable concentration of airborne culturable fungi was approximately 8.8 CFU/m^3 = 1 CFU / (113.4 L × 0.001 m^3/L) for all samples except deplaning for which the minimum detectable concentration was approximately 17.6 CFU/m^3 = 1 CFU / (56.8 L × 0.001 m^3/L) based on median air volumes of 113.4 L and 56.8 L for nondeplaning and deplaning samples, respectively.

[B] The minimum detectable concentration of airborne total spore count fungi is approximately 25.2 spores/m^3 = 3.81 × 1 spore / (151.1 L × 0.001 m^3/L) for all samples, based on a median air volume of 151.1 L and a median multiplication factor of 3.81.

[C] Range is for samples positive for growth or spores.

TABLE III. Estimated Correlations of Natural Log Transformed Concentrations for Replicate Samples Obtained Within Each Sampling Interval and for Samples Obtained in Different Sampling Intervals

	OC Out	OC In	BD	MC	ECR	MCR	LCR	DP	DC In	DC Out
OC Out	**0.88** **0.89**	0.06	0.05							
OC In	0.13	**0.80** **0.76**	0.03							
BD	0.15	0.17	**0.07** **0.32**	0.02	0.02	0.03	0.02	0.02		
MC	0.19			**0.20** **0.20**	0.03	0.04	0.03	0.02		
ECR	0.19		0.28		**0.02** **0.36**	0.05	0.04	0.03		
MCR	0.20		0.28	0.29		**−0.12** **0.36**	0.05	0.04		
LCR	0.21		0.31	0.31	0.32		**−0.05** **0.23**	0.03		
DP	0.13		0.18	0.18	0.19	0.21		**0.21** **0.27**	0.34	0.39
DC In	0.47								**0.78** **0.60**	0.37
DC Out	0.48								0.48	**0.95** **0.88**

Notes: Main diagonal entries in **boldface** type; off-diagonal entries in regular type. Correlations for total culturable fungi concentrations (CFU/m^3) are provided in the upper right corner of each cell and correlations for total spore concentrations (spores/m^3) are provided in the lower left corner of each cell. OC = origin city, Out = outside airport terminal, In = inside airport terminal, BD = boarding, MC = midclimb, ECR = early cruise, MCR = midcruise, LCR = late cruise, DP = deplaning, DC = destination city.

(off-diagonal entries). Correlation coefficients for the total culturable fungal and total spore concentrations are provided in the upper right corner and lower right of each cell in the table, respectively.

Five different covariance structures were evaluated in each analysis of the total fungi concentration data (Table I). In general, the best-fitting covariance structures were those that allowed the parameters of the compound symmetric structure within each sampling interval to vary (i.e., Structures I and III that specify interval-specific variances and covariances). Depending on the outcome being modeled (culturable or total spore) and the situation (modeling origin city samples, aircraft samples, or destination city samples), the best-fitting structure specified either a positive (Structure I) or zero (Structure III) covariance between replicate samples obtained during different sampling intervals.

Airborne Culturable Fungal Concentrations

The distributions of the culturable fungal concentrations within each sampling interval are graphically depicted in Figure 1 and summarized in Table IV along with the modeling results.

Origin City Model

Samples collected outside the airport terminal were not correlated with samples collected inside the airport terminal (estimated correlation (r): 0.06) and neither were correlated with samples collected during the boarding process (r: 0.05 and 0.03, respectively), indicating that these environments were somewhat independent (Table III) Strong correlations were observed among replicate samples taken outside the airport (r = 0.88) and inside the airport (r = 0.80) but not during the boarding process (r = 0.07). Accordingly, modeled results presented in Table IV for the origin city utilized covariance Structure III which assumed independence among the sampling intervals.

The geometric mean (GM) airborne total culturable fungi concentration for samples taken outside the origin airport terminal was significantly higher than the GM concentration for samples taken inside the origin airport terminal (p-value = 0.0003) and on the aircraft during boarding (p-value < 0.0001). Log transformed concentrations observed inside the origin airport terminal and during boarding were more variable than concentrations observed outside the origin airport terminal (total variance of natural log transformed data: 0.68, 1.64 and 1.77 for outside, inside and boarding samples, respectively).

Aircraft Model

Culturable samples taken during different sampling intervals on board the aircraft (boarding through deplaning) were generally not correlated (Table III, range 0.02–0.05). Similarly, triplicate samples collected within each aircraft

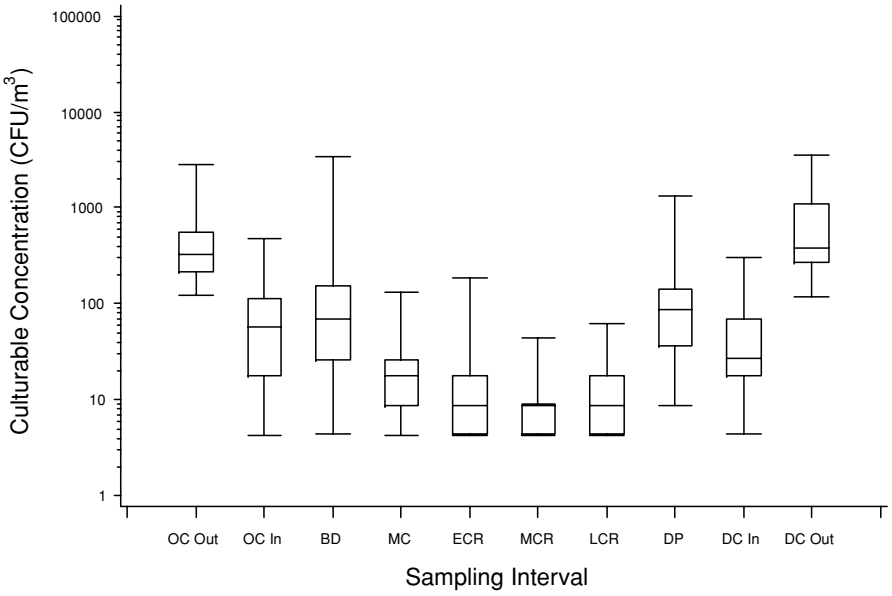

FIGURE 1. Distribution of the total concentration of airborne culturable fungi by sampling interval. Horizontal lines indicate the minimum, 25th percentile, median, 75th percentile, and maximum. Note: OC = origin city, Out = outside airport terminal, In = inside airport terminal, BD = boarding, MC = midclimb, ECR = early cruise, MCR = midcruise, LCR = late cruise, DP = deplaning, DC = destination city.

interval were also not highly correlated with the highest observed correlations occurring during the midclimb (r: 0.20) and deplaning (r: 0.21) intervals.

However, the covariance structure that did not assume independence among the sampling intervals was selected (Structure I), based on the AIC and BIC criteria, to model the culturable results obtained on board the aircraft. Carbon dioxide concentration and occupancy rate were not significantly related to airborne culturable fungal concentrations on the aircraft. Carbon dioxide was also evaluated and deemed not

TABLE IV. Modeling Results for Airborne Culturable Fungi Total Concentration (CFU/m^3)

Model Sampling Interval	N Flights	N Samples	Unadjusted GM	Unadjusted GSD	Model Adjusted GM	Model Adjusted 95% CI	Modeled Variance[A]
Origin city							
Outside airport terminal	12	35	385	2.2	393	238–650	0.68
Inside airport terminal	12	36	53.4	3.5	53.4[B]	25.0–114	1.64
Boarding	12	64	65.7	3.8	64.9[B]	43.1–97.7	1.77
Aircraft[C]							
Boarding	12	64	65.7	3.8	65.2	44.2–96.3	1.74
Midclimb	12	72	16.0	2.7	16.0[D]	11.2– 22.7	0.93
Early cruise	12	72	10.3	2.2	10.3[D]	8.3–12.7	0.63
Midcruise	12	72	8.6	1.9	8.6[D]	7.8–9.6	0.40
Late cruise	12	72	10.4	2.2	10.4[D]	8.8–12.2	0.59
Deplaning	12	33	77.5	2.8	76.5	48.8–120	1.00
Destination city							
Deplaning	12	33	77.5	2.8	71.6[E]	44.2–116	1.08
Inside airport terminal	11	33	36.0	3.0	37.4[E]	19.5–71.7	1.19
Outside airport terminal	11	33	530	2.6	550	300–1020	0.93

[A] Modeled variance of the natural log transformed fungi concentrations for samples taken in the specified sampling interval.
[B] Modeled GM significantly less than outside origin airport terminal GM (Tukey-Kramer adjusted p-value < 0.001).
[C] Model adjusts for location in coach cabin (front, rear).
[D] Modeled GM significantly less than boarding and deplaning GMs (Tukey-Kramer adjusted p-value < 0.0001).
[E] Modeled GM significantly less than outside destination airport terminal GM (Tukey-Kramer adjusted p-value < 0.0001).

an effect modifier. There was no difference between culturable fungal concentrations in the front and rear locations within the coach section of the aircraft (p-value = 0.95). The concentration of culturable fungi in the air was significantly higher during boarding and deplaning compared with the midclimb and cruise sampling intervals. The airborne concentration of culturable fungi was most variable during boarding.

In an analysis limited to data from the cruise intervals, the estimated within-flight variance ($\hat{\sigma}_w^2 = 0.53$) was much higher than the estimated between-flight variance ($\hat{\sigma}_b^2 = 0.01$) for the natural log transformed culturable fungal concentrations. When flight was considered to be a fixed effect in the cruise only model, culturable fungal geometric means were not significantly different among the flights (overall F test: $F_{11,201} = 1.34$, p-value = 0.20). In other words, once the aircraft reached cruising altitude, the flights were indistinguishable from one another with respect to culturable fungal concentrations.

Destination City Model

Concentrations observed during deplaning were somewhat correlated with concentrations observed both inside (r: 0.34) and outside (r: 0.39) the destination airport terminal. In contrast to the origin airport terminal, concentrations observed inside the destination airport terminal were also moderately correlated with concentrations observed outside the destination airport terminal (r: 0.37). Replicate samples taken during deplaning (two each from the front and rear of coach class) were less correlated than triplicate samples collected either inside or outside of the destination airport terminal (Table III). Accordingly, covariance Structure I was used to model the culturable results from the destination city. The geometric mean culturable fungal concentration observed outside the destination airport terminal was significantly higher than the geometric mean concentration observed inside the destination airport terminal and on the aircraft during deplaning. Concentrations observed inside the destination airport terminal and during deplaning were slightly more variable than concentrations observed outside the destination airport terminal (total variance of natural log transformed data: 1.08, 1.19, and 0.93 for deplaning, inside and outside samples, respectively).

Airborne Total Spore Count

The distributions of the total spore concentrations are depicted graphically in Figure 2 and summarized in Table V along with the modeling results.

Origin City Model

Total spore concentrations from the three environments (outside the airport terminal, inside the airport terminal and on the aircraft during the boarding process) were not highly correlated (Table III). Triplicate samples collected during the boarding interval were appreciably less correlated than triplicate samples collected both outside the airport terminal and inside the airport terminal. Modeling results presented in Table V for the origin city used the covariance Structure III.

The GM airborne total spore concentration observed outside the origin airport terminal was significantly higher than the GM concentration observed inside the origin airport terminal (p-value < 0.0001) and on the aircraft during boarding (p-value < 0.0001). Concentrations observed outside and inside

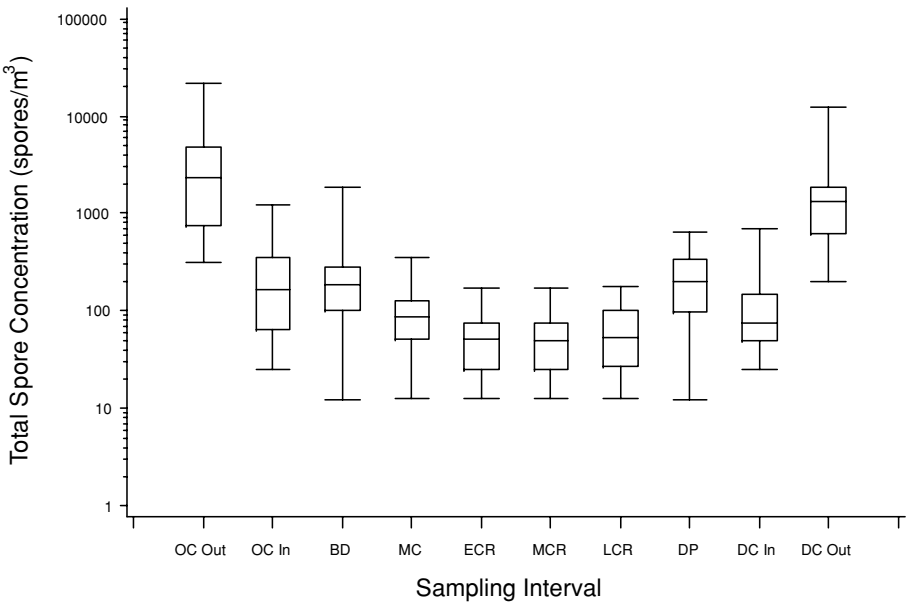

FIGURE 2. Distribution of the total concentration of airborne total spores by sampling interval. Horizontal lines indicate the minimum, 25th percentile, median, 75th percentile, and maximum. Note: OC = origin city, Out = outside airport terminal, In = inside airport terminal, BD = boarding, MC = midclimb, ECR = early cruise, MCR = midcruise, LCR = late cruise, DP = deplaning, DC = destination city.

TABLE V. Modeling Results for Airborne Total Spore Concentration (spores/m^3)

Model Sampling Interval	N Flights	N Samples	Unadjusted GM	Unadjusted GSD	Model Adjusted GM	Model Adjusted 95% CI	Modeled Variance[A]
Origin city							
Outside airport terminal	12	36	2150	3.1	2150	1050–4400	1.37
Inside airport terminal	12	36	146	3.0	146[B]	74.8–286	1.30
Boarding	12	69	172	2.4	174[B]	121–249	0.80
Aircraft[C]							
Boarding	12	69	172	2.4	175[D,E]	110–278	1.04
Midclimb	12	72	81.0	2.0	81.0[D]	62.8–105	0.50
Early cruise	12	72	49.9	1.9	49.9	37.5–66.6	0.49
Midcruise	12	72	44.9	2.0	44.9	33.8–59.6	0.47
Late cruise	12	72	57.5	1.9	57.5	45.3–73.0	0.40
Deplaning	12	23	156	2.6	150[D]	86.1–261	1.12
Destination city							
Deplaning	12	23	156	2.6	151[F]	92.5–248	0.92
Inside airport terminal	11	33	84.1	2.3	89.2[F]	50.9–156	0.91
Outside airport terminal	11	32	1220	3.0	1280	724–2270	0.90

[A] Modeled variance of the natural log transformed fungi concentrations for samples taken in the specified sampling interval.
[B] Modeled GM significantly less than outside origin airport terminal GM (Tukey-Kramer adjusted p-value < 0.0001).
[C] Model adjusts for location in coach cabin (front, rear).
[D] Modeled GM significantly higher than cruise GMs (Tukey-Kramer adjusted p-value < 0.01).
[E] Modeled GM significantly higher than midclimb GM (Tukey-Kramer adjusted p-value < 0.01).
[F] Modeled GM significantly less than outside destination airport terminal GM (Tukey-Kramer adjusted p-value < 0.0001).

the origin airport terminal were more variable than concentrations observed during boarding (total variance of natural log transformed data: 1.37, 1.30, and 0.80 for outside, inside, and boarding samples, respectively).

Aircraft Model

Total spore concentrations collected during different intervals were only somewhat correlated (r: range 0.13–0.32, Table III). Replicate total spore concentrations within a particular sampling interval were also somewhat correlated (r: range 0.20–0.36). Structure I was the best-fitting covariance structure. Carbon dioxide and occupancy rate were not significantly related to the concentration of total spores after adjusting for interval and location; therefore, neither was included in the final model. Carbon dioxide was also evaluated and deemed not an effect modifier. There was no difference between the front and rear locations within the coach section of the aircraft (p-value $= 0.62$).

The concentration of total spores in the air was significantly higher during boarding, midclimb, and deplaning compared with the cruise sampling intervals. The boarding airborne spore concentration was also higher compared to the midclimb intervals. The airborne concentration of total spores was most variable during boarding and deplaning, when aircraft conditions were changing.

In an analysis limited to data obtained during the cruise intervals, the estimated within-flight variance ($\hat{\sigma}^2_w = 0.31$) was higher than the estimated between-flight variance ($\hat{\sigma}^2_b = 0.13$) for natural log transformed total spore concentrations. When flight was considered to be a fixed effect in the cruise only model, significant differences total spore GMs were observed among the flights (overall F test: $F_{11,201} = 8.35$, p-value $= < 0.0001$). In contrast to the culturable results, once the aircraft reached cruising altitude, some differences were observed among the flights with respect to total spore concentrations.

Destination City Model

Total spore samples collected during different intervals at the destination city (deplaning, inside the airport terminal and outside the airport terminal) were moderately correlated (Table III). Triplicate samples taken outside the destination city airport terminal were more correlated ($r = 0.88$) with each other than triplicate samples collected inside the airport terminal ($r = 0.60$) or the replicate samples collected during deplaning (one each from the front and back of coach class, $r = 0.27$). Structure I was the best-fitting covariance structure. The GM airborne total spore concentration for samples taken outside the destination airport terminal was significantly higher than the GM for samples taken inside the destination airport terminal and on the aircraft during deplaning. Variability was similar for the three sampling intervals (total variance of natural log transformed data 0.92, 0.91, and 0.90 for deplaning, inside and outside samples, respectively).

DISCUSSION

Overall, our data indicate that the in-flight aircraft environment contains statistically lower concentrations of total fungal propagules relative to outdoor air. Total culturable and spore concentrations during cruise were consistently lower than concentrations measured outside airport terminals or during the boarding interval indicating that spores entering the aircraft while on the ground either settle or are removed quickly by the filtration/ventilation system. While on the aircraft, both culturable and total spore samples were highest during boarding and deplaning intervals when the aircraft was attached to the airport terminal and human activity was at its peak. However, even these samples contained fewer fungi than those collected outside the terminal. No statistically significant differences were observed between measurements made in the front and back sections of the coach cabin for either culturable or total spore concentrations. This result does not support anecdotal reports of contaminant rearward drift due to the rear aircraft exhaust valve.

The authors considered applying the positive hole correction factor to the culturable fungal results prior to statistical analysis. However, the laboratory counted all culturable colonies on the agar plates rather than just those colonies where the impactor jets were aligned. In this situation, the positive hole correction factor may actually overcompensate total counts; therefore, it was not appropriate for this study data. Nonetheless, a supplemental analysis was performed to determine whether these findings were influenced by the decision not to apply the positive hole correction factor. Results obtained with the corrected data did not differ appreciably from results with the uncorrected data. On average, corrected concentrations from the midclimb and cruise intervals were only 1% higher than uncorrected values. Larger differences were observed inside the airport terminal, during deplaning and boarding, and outside the airport terminal where corrected concentrations were 2%, 2%, 6%, and 12% higher than uncorrected concentrations, respectively. Results presented do not employ the positive hole correction factor; consequently, peak concentrations may be underestimated in some cases.

Although there are currently no regulatory standards for airborne bioaerosols, there are some publications that can provide perspective for our results. Shelton et al.[18] examined 12,026 culturable samples from both indoor and outdoor environments throughout the United States. While the authors' median outside airport terminal concentration of 360 CFU/m^3 was comparable to the outdoor median concentration of 540 CFU/m^3 reported by Shelton et al., the median concentration of 8.8 CFU/m^3 observed during the cruise intervals on the aircraft was much lower than the median indoor concentration of 82 CFU/m^3 Shelton et al. reported.

In 1994, the Environmental Protection Agency (EPA) embarked on a 4-year Building Assessment Survey Evaluation (BASE) study to measure indoor environmental quality in nonconplaint buildings.[19] To assess culturable fungal concentrations, the BASE study collected six indoor air samples from each of 100 buildings using the N-6 impactor. The BASE total culturable mean indoor concentration was 95 CFU/m^3 whereas the authors' mean cruise concentration was 14 CFU/m^3. Both of these studies demonstrate that the total culturable fungal concentrations observed inside the aircraft are low when compared with other indoor environments.

The current literature for normative total spore concentrations is rather sparse. Baxter et al.[20] recently reported results from "clean" commercial buildings in southern California. Using the Air-O-Cell cassettes with sampling times ranging from 5 to 10 min, the mean concentration inside defined "clean" commercial buildings was approximately 1100 spores/m^3(values estimated from Figure 1).[20] The BASE study collected six indoor Burkard spore trap samples from each of 44 buildings and reported a median total concentration of 55 spores/m^3 and an intra-class correlation coefficient (ICC) of 0.57.[15] The median total spore concentration for samples collected on board the aircraft was 52 spores/m^3, which was similar to the BASE study result even though the aircraft samples were collected during a single season (i.e., summer) and the BASE samples were collected during all four seasons.

The ICC is the ratio of the between-building variance to the total variance (i.e., the sum of the between- and within-building variances) and is close to 1 when the within-building variance is low relative to the between-building variance and close to zero when the between-building variance is low relative to the within-building variance. Analogous ICCs for the airport terminals were 0.76 and 0.60 for the origin and destination city airport terminals, respectively. ICCs for total spore samples taken inside the aircraft, however, are much lower (Table III, range 0.20–0.36) reflecting higher within-aircraft variability compared with between-aircraft variability. Since the aircraft included in the study were all the same type (i.e., B-767s) and utilized the same ventilation configurations during the study (i.e., 50% recirculation rate and two airpacks), it is reasonable to expect the between-aircraft variability to be low.

Observed correlations between replicate measurements made both within and between the cruise intervals were moderate or very low. Low concentrations observed during the cruise intervals, particularly for culturable fungi, that had geometric means only slightly above the limit of detection are likely responsible for the low observed correlations. It is difficult to estimate a correlation when the measurements have low variability and are clustered around the same value. This is a situation where a statistical analysis may be misleading if the aerobiologic nature of the data is ignored.

In analyses that considered only samples taken on board the aircraft during the cruise intervals, statistically significant differences in culturable fungal concentrations were not observed between the flights. Within-flight variability greatly exceeded between-flight variability for culturable fungal concentrations observed during the cruise intervals. On the other hand, significant differences in total spore fungal concentrations were observed between the flights. Since the total spore samples are reflective of nonviable and viable components of fungi, the authors hypothesize that the statistical differences

observed are indicative of nonviable fungal accumulation on the aircraft. Since miles flown or time in flight since the last comprehensive cleaning of the aircraft were not obtained, the authors could not verify this hypothesis.

Obtaining replicate samples is particularly important for estimating exposure to bioaerosols since there can be considerable variability within an environment. Determining the appropriate sample size becomes complicated because it involves determining both the number of environments (e.g., buildings, aircraft, etc.) and the number of samples for each environment. The estimated correlations for replicate samples obtained in the same sampling interval presented in Table III could be used to guide future investigations.

Although several hundred culturable and total spore samples were obtained, samples were collected only on 12 flights and the statistical analysis needed to take this into account. The MIXED procedure in SAS includes built-in covariance structures (e.g., compound symmetry, autoregressive, etc.) that are sufficient for many analyses. The nested nature of the replicate samples described here, with multiple sampling intervals per flight and replicates taken within each sampling interval, required a nonstandard covariance structure. Significant work was completed to create and evaluate the five covariance structures presented (Table I). The SAS code is presented in Table I for the structures that were tested as examples of using the options in the RANDOM and REPEATED statements to obtain non-standard structures.

Modeling results are shown in Tables IV and V based on the best-fitting covariance structure, as determined by the AIC and BIC criteria; however, the main results were not dependent on the choice of the covariance structure. In all structures tested, the outside terminal geometric mean was statistically significantly higher than both the inside terminal and aircraft geometric means (Tukey-Kramer adjusted p-values < 0.0001); boarding and deplaning geometric means were statistically significantly higher than midclimb and cruise geometric means. On board the aircraft, the front geometric mean was not significantly different from the back geometric mean.

The statistical significance for some comparisons, however, did depend on the specified covariance structure. For example, Tukey-Kramer p-values for the difference between the deplaning geometric mean and the inside airport geometric mean for culturable fungi varied from 0.0043 to 0.16 depending on the covariance structure (the best-fitting covariance structure produced a p-value of 0.081). The most significant p-value of 0.0043, in this case, was associated with covariance Structure V, which specifies an overall compound symmetric structure that assumes a common variance and covariance within each sampling interval. This structure likely is not appropriate given the lower correlation observed among replicate samples obtained during deplaning compared to the airport terminal.

Limitations

Samples collected on board the aircraft were subject to left-censoring at the limit of detection of 1 CFU per culturable sample or 1 spore per total spore sample. Censoring was less of an issue for the total spore samples with no more than 6% censoring during any particular sampling interval. Higher censoring rates were observed for the culturable samples with censoring rates ranging from 3% to 35% on board the aircraft. Methods for dealing with data that are both correlated and censored are becoming more readily available[21,22] but currently exclude models with nested random effects. Substituting one-half the limit of detection for the censored values has been shown to produce biased estimates and decreased standard errors, particularly when the censoring rate is above 10%.[22] It is unlikely that the main finding of significantly decreased fungal concentrations observed when the aircraft is in flight is an artifact of the imputation procedure; however, as previously discussed, variance and covariance estimates for the culturable results on board the aircraft were likely affected by the observed censoring.

Although evaluating total fungal concentrations in terms of CFU/m^3 is common practice, it should be recognized that such an analysis does categorize all fungi together into one group. Since an individual's allergic response to fungi is specific to a genus/species, the identity of a specific genus may be as important as the total concentration observed. A genus/species analysis of the culturable and total spore aircraft samples is presented elsewhere.[16] The authors recognize that bacterial and viral exposures on aircraft are also of considerable importance. The analysis of airborne bacterial and viral samples from these same flights is currently under way.

Seasonality affects outdoor fungal concentrations, with summer and fall concentrations typically being the highest.[18] Since our data came from flights during the summer months, these results may not be generalizable to other seasons when concentrations tend to be lower. It is also unclear whether the results of this study have external validity to other aircraft models or other B-767 flights under different airpack or recirculation settings.

CONCLUSIONS

Mixed-effects modeling was used to analyze a nested data set. Five covariance structures were tested and evaluated to determine the appropriate models for analysis. Overall, both total culturable and total spore fungal concentrations were low while the wide-body passenger aircraft was in-flight. No statistical difference was observed between measurements made in the front and back sections of the coach cabin for either culturable or total spore concentrations. Total fungal concentrations were highest outside the airport terminal compared with inside the airport terminal and on board the commercial passenger aircraft. On the aircraft, the majority of total fungal exposure occurred during the boarding and deplaning processes when the aircraft was attached to ancillary ventilation systems and passenger activity was at its peak.

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