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Monitoring Microbial Populations on Wide-Body Commercial Passenger Aircraft

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Although exposure to bacteria has been assessed in cabin air previously, minimal numbers of samples have been collected in-flight. The purpose of this research was to comprehensively characterize bacterial concentrations in the aircraft cabin. Twelve randomly selected flights were sampled on Boeing-767 aircraft, each with a flight duration between 4.5 and 6.5 h. N-6 impactors were used to collect sequential, triplicate air samples in the front and rear of coach class during six sampling intervals throughout each flight: boarding, mid-climb, early cruise, mid-cruise, late cruise and deplaning. Comparison air samples were also collected inside and outside the 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 bacterial concentrations. A total of 513 airborne culturable bacterial samples were collected. During flight (mid-climb and cruise intervals), a model-adjusted geometric mean (GM) of 136 total colony-forming units per cubic meter of air sampled (CFU · m^{-3}) and geometric standard deviation of 2.1 were observed. Bacterial concentrations were highest during the boarding (GM 290 CFU · m^{-3}) and deplaning (GM 549 CFU · m^{-3}) processes. Total bacterial concentrations observed during flight were significantly lower than GMs for boarding and deplaning (*P* values <0.0001–0.021) in the modeled results. Our findings highlight the fact that aerobiological concentrations can be dynamic and underscore the importance of appropriate sample size and design. The genera analysis indicates that passenger activity and high occupant density contribute to airborne bacterial generation. Overall, our research demonstrates that the bacteria recovered on observed flights were either common skin-surface organisms (primarily gram-positive cocci) or organisms common in dust and outdoor air.

Keywords: aerobiology; aircraft; bacteria concentrations; cabin air quality; MIXED modeling

INTRODUCTION

In 2002, the National Academies of Science (NAS) completed a review of the airliner cabin environment (National Research Council Committee on Air Quality in Passenger Cabins of Commercial Passenger Aircraft, 2002). This characterization was of interest to several parties including aircraft manufacturers, passengers, regulators and flight personnel. Within the US, ~198 000 flight personnel and 73 000 aircrew personnel work (Bureau of Transportation Statistics, 2004) in the aircraft cabin environment. The NAS committee indicated that although bioaerosol concentrations observed inside the cabin environment were similar to other indoor environments, the number of samples was low, few replicates were collected and estimates of variability were poor. The potential for disease transmission on aircraft has been documented through anecdotal case events and outbreak investigations (Moser *et al.*, 1979; Driver *et al.*, 1994; Kenyon *et al.*, 1996; Olsen *et al.*, 2003; Mangili and Gendreau, 2005).

Several studies have been conducted to document the prevalence of bacteria on commercial passenger aircraft (Nagda *et al.*, 1989; Wick and Irvine, 1995;

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Lee *et al.*, 1999; Pierce *et al.*, 1999; Dumyahn *et al.*, 2000). Nagda et al. (1989) collected air samples for total bacteria, including *Staphylococcus* spp. on 69 smoking and 23 nonsmoking flights using a compact sieve plate sampler. Total bacteria averaged 163 colony-forming units per cubic meter (CFU $\cdot$ m^{-3}) [standard deviation (SD) 106 CFU $\cdot$ m^{-3}] in the smoking section and 131 CFU $\cdot$ m^{-3} (SD 89 CFU $\cdot$ m^{-3}) on nonsmoking flights and in the nonsmoking section of smoking flights. Wick and Irvine (1995) collected air samples of bacteria and fungi using a centrifugal air sampler on 42 flights including multiple aircraft types: Boeing-727 (B-727), B-737, DC10 and MD80. Bacterial levels ranged from 9 to 282 CFU $\cdot$ m^{-3}. Dumyahn *et al.* (2000) collected samples for bacteria using the portable Burkard culture plate sampler on B-777 aircraft. The geometric mean (GM) of the bacterial concentrations was 201 CFU $\cdot$ m^{-3}. (Lee *et al.* 1999) studied bacterial concentrations on three flights on B-747 aircraft. Using the Burkard air sampler, bacterial concentrations ranged from 33 to 93 CFU $\cdot$ m^{-3} and were observed to be higher during boarding compared to mid-flight time periods. Pierce *et al.* (1999) collected bacterial measurements on new B-777 aircraft on eight flights using the portable compact air sampler (Rodac). Measurements were collected at various times during both medium- and long-duration flights. The range of bacterial concentrations was 39–244 CFU $\cdot$ m^{-3}. Although each study design was unique, the emphasis of these studies was not typically bioaerosols and minimal numbers of bioaerosol samples were collected. For the most part, bacteria were not identified to the genus level in previous studies.

The NAS committee also indicated that more information regarding infectious transmission on aircraft was needed. The objectives of this work complement many of the research gaps identified by the National Academies. The primary objective was to comprehensively document bacterial concentrations at multiple locations and time periods on an aircraft. This analysis would produce variability estimates and a baseline of observed bacteria and allow for a comparison of the aircraft environment to other indoor working environments. Although this study was not designed to evaluate disease transmission on aircraft, a secondary objective was to pilot airborne viral sampling efforts which could potentially be used in later studies with disease transmission objectives.

MATERIALS AND METHODS

A total of 12 flights were evaluated in this study. Two airlines with a majority ownership of B-767 aircraft within the US agreed to participate in the study. Flight segments were randomly selected based upon a complete list of flight schedules provided by the airlines. Eligible flights consisted of all B-767 flights with flight duration between 4.5 and 6.5 h. This duration allowed comparability and the evaluation of aircraft air derived from the aircraft ventilation system, rather than just the initial impact of the open doors from the boarding process. To minimize a seasonality effect, all flights were sampled during the same weather season (i.e. summer).

In the current climate of air travel, it was deemed inappropriate, if not impossible, to conduct a 'blind' study. Participating airlines were informed of the proposed sampling in advance of the selected flights. All team members (two teams, each with two members) wore photographic identification and 'uniform' clothing during the flights. Passengers were informed of the research study via announcements from the captain and flight attendant personnel.

Sampling intervals

Flight segments were divided into 10 sampling intervals: outside the airport terminal, inside the airport terminal (at both the origin and destination cities), boarding, mid-climb, early cruise, mid-cruise, late cruise and deplaning. On board the aircraft, simultaneous samples were taken in the front section of coach class (in the center section, in the first three rows excluding the bulkhead) and in the back section of coach class (in the center section, in the last three rows). Boarding samples were taken while passengers were actively boarding the aircraft, mid-climb samples were taken immediately after the aircraft reached $\sim$10 000 ft, 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. A parallel study completed for fungal concentrations on aircraft is presented elsewhere (McKernan *et al.*, 2007).

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 number 8550, TSI Instruments, Inc., Shoreview, MN, USA). Flight characteristics such as the occupancy rate (number of passengers to number of passenger seats available), air pack usage (environmental control system, 1 or 2) and the recirculation rate (0 or 50%) were also collected.

Airborne culturable bacteria

Airborne culturable bacterial measurements were obtained using an N-6 impactor, tryptic soy agar (TSA) media and a high-flow pump (PORTN-6, Thermo Andersen, Smyrna, GA, USA) calibrated at 28.3 liters per minute (l.p.m.). Prior to each round-trip flight, the N-6 impactors were autoclaved, and

between every sample, the N-6 impactor was cleaned with an alcohol wipe (70% isopropyl alcohol) (Allegro, Garden City, CA, USA) and allowed to dry. At each sampling interval on the aircraft, each team collected three sequential replicate samples of 4 min each, except during the deplaning interval. During deplaning, duplicate 2-min samples were collected, due to time restrictions. The optimal media selection and sampling times for the airborne samples were based upon results from four feasibility flights.

Surface sampling of bacteria

Surface sampling for culturable bacteria was also conducted inside the aircraft and inside the airport terminal. The surface samples were collected using sterile, cotton wool tipped sticks wetted with a Stuart solution (BBL™ Culture Swab, Becton Dickinson). Each sample was collected across a 5 cm × 5 cm surface area. The moistened cotton wool tipped stick was swabbed across the entire sample surface in a 'Z' pattern once and then stored in a sterile tube for submission to the laboratory. This collection technique has been documented elsewhere (American Industrial Hygiene Association Biosafety Committee, 1996). Each surface sample comprised two sterile cotton wool tipped sticks. On board the aircraft, one cotton wool tipped stick was collected on the armrest and one was collected on the tray table. Inside the airport terminal, one cotton wool tipped stick was collected on the armrest of a chair and one was collected on an end table in the gate area.

Sample handling and analysis

Culturable bacterial samples were kept cool and shipped on blue ice via an overnight mail carrier to the analytical laboratory $<$24 h after sample collection. Swab samples were swabbed onto TSA media. Airborne samples and swab-culturable samples were incubated at 25°C for 7–10 days. After the appropriate incubation period, bacterial colonies on the media were counted and identified. The lower limit of detection for culturable samples was 1 CFU per total volume of air sampled or 1 CFU per total area sampled.

As part of the laboratory's quality assurance procedures, samples were counted twice and compared for consistency. The laboratory was accredited by the American Industrial Hygiene Association (AIHA) and participated in the AIHA Environmental Microbiology Proficiency Analytical Testing program. Media field blanks and blinded blank samples for culturable bacteria were negative for growth.

Rhinovirus sampling

Since infectious agents were also of interest, piloting efforts for rhinovirus exposure were also completed. Recent research illustrates that rhinovirus can be detected not only in biological specimens but also on airborne filter samples when analyzed by reverse transcriptase–polymerase chain reaction (RT-PCR) (Myatt *et al.*, 2004). Rhinovirus samples were collected on select flights using 47-mm Teflon-coated glass fiber filters (Gelman Pallflex, T60A20) which were precleaned by solvent extraction prior to use. Two samples were collected during each flight, one during the boarding process and one for the remainder of the flight. Samples were collected using a high-flow pump calibrated at 28.3 l.p.m. for a total volume of ~710 l for the boarding sample and 7600 l for the in-flight sample. After sampling, the filters were shipped overnight on dry ice to researchers in the Indoor Environment Department of Lawrence Berkeley National Laboratory for analysis. Four blind blank samples were also submitted to the laboratory. Each filter was extracted for human rhinovirus (HRV) particles and subsequently analyzed for the presence of HRV by a quantitative RT-PCR (Russell *et al.*, 2002). This method detects the presence of HRV in aerosols regardless of culturability or viability. Filters were extracted the same day they were received into the laboratory. Extracts were stored at 4°C until analysis by the RT-PCR assay in two separate batches.

RNA isolated from HRV type 89 (American Type Culture Collection) was used as a positive control. A serial dilution of the external quantitation standard was included with each group of samples that were amplified. The standard curve formed by the amplification of a dilution series of HRV quantitation standard shows the detection limit of the assay to 5 fg of HRV.

Statistical methods

SAS 9 Software® (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Airborne culturable bacteria results were assessed as total CFU per sample. We considered applying the positive hole correction factor to the culturable bacterial 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. Since this practice may actually overcompensate total counts, we deemed the positive hole correction factor not appropriate for the data. Samples reported as 'no growth' were replaced with one-half of the limit of detection of one CFU per culturable sample (Hornung and Reed, 1990). Culturable airborne bacterial results were converted to a total concentration in air by dividing by the air volume associated with the sample, resulting in the number of CFUs per cubic meter of air ($CFU \cdot m^{-3}$). The distribution of the airborne culturable bacteria was 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 bacterial results were analyzed using mixed-effects modeling via the MIXED procedure in SAS. The dependent variable was the natural log-transformed concentration. Fixed effects considered included the sampling interval (i.e. outside terminal, inside terminal, boarding, etc.) and, for samples taken on board the aircraft, location (front of coach section, rear of coach section), carbon dioxide (p.p.m.) and occupancy rate. Individual bacterial measurements and the time-weighted carbon dioxide concentrations were linked by interval on each flight. Random effects considered included the flight number (i.e. 1, 2, . . ., 12). In order 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, mid-climb, early cruise, mid-cruise, late cruise and deplaning) and at the destination city (during deplaning, inside the airport terminal and outside the airport terminal). The underlying covariance structure assumed that the flights were independent and fit a compound symmetric structure within each sampling interval. Variance components were allowed to vary by sampling interval. The structure included a common covariance between samples collected during different intervals. Alternate structures were evaluated using Akaike's information criterion and Schwarz's Bayesian criterion, but did not improve the model. 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 GMs after exponentiating the adjusted means. Model residuals were analyzed and determined to be approximately normal.

Selected genus/species were evaluated in terms of the detection rate (i.e. the percent of samples in which the genus/species was detected). Log-binomial modeling using the GENMOD procedure in SAS was used to compare detection rates for selected genus/species among the sampling intervals. Replicate air samples were collected during each sampling interval and multiple sampling intervals were associated with each flight, therefore the REPEATED statement was used to account for the correlated nature of the data. The flight number identified the subjects and an exchangeable correlation structure was specified. Convergence was not attained in a few models due to one or more intervals with detection rates of 100%; in these models exact logistic regression using the LOGISTIC procedure in SAS was used to compare the detection rates among the sampling intervals (Derr, 2000). The EXACT statement was used to obtain an exact logistic regression analysis for the sampling intervals that conditioned on flight number, treated as a class variable in the model, in order to control for possible within-flight correlation. Since nondetection rates were higher at the genus/species level, GM and GSD were estimated using maximum likelihood methods available in the LIFEREG procedure in SAS. Samples that did not detect a particular genus/species were considered to be left censored at 1 CFU per volume of air sampled.

Building assessment study evaluation study comparisons

Bacterial results observed during flight in this study were compared to published results from the EPA building assessment study evaluation (BASE) study (Macher *et al.*, 2005; Tsai and Macher, 2005). The BASE study provides normative bacterial concentrations in noncomplaint indoor office environments. High occupant density spaces, such as elevators were not studied. The BASE study used the N-6 impactor with TSA media to assess culturable bacterial concentrations in 100 buildings. To facilitate this comparison, we reorganized our taxa to fit the six taxa codes utilized by the BASE study (gram-positive rods, gram-positive cocci, gram-negative rods, gram-negative cocci, unknown and total bacteria). Summaries of the airborne culturable bacterial samples obtained during the cruise intervals on the flights were informally compared to summaries reported by the BASE study (Tsai and Macher, 2005). The comparison was also limited to BASE samples which were 5 min in duration and incubated at 30°C. Although our airborne bacterial samples were collected for 4 min and were incubated at 25°C , we felt these conditions were still comparable. Since the BASE study results employed the positive hole correction factor, we also applied this factor for this comparison.

RESULTS

A total of 513 airborne culturable bacterial samples were collected on 12 randomly selected B-767 flights. Passenger occupancy rates ranged from 67 to 100% on the flights. The recirculation fans were set to 50% outside bleed air and 50% filtered recirculated air. Two air packs were operated during all flights.

Airborne culturable bacteria

The distributions of the total culturable bacterial concentrations within each sampling interval are graphically depicted in Fig. 1. Table 1 presents the percent and range of the detectable concentrations for culturable bacteria summarized by sampling interval. Less than 1% of samples collected reported nondetectable concentrations for airborne culturable bacteria.

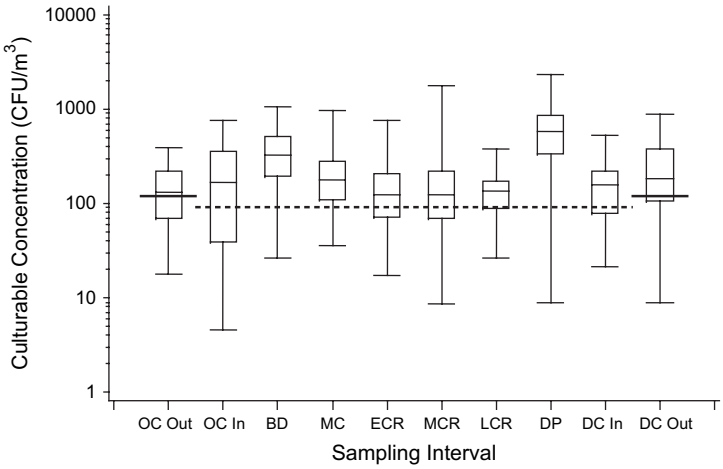

Fig. 1. Distribution of the total concentration of airborne culturable bacteria by sampling interval ($n = 513$). Two samples that reported no bacterial growth were replaced with one-half the limit of detection of 1 CFU per volume of air sampled. Horizontal lines indicate the minimum, 25th percentile, median, 75th percentile, and maximum. BD, boarding; DC, destination city; DP, deplaning; ECR, early cruise; In, inside airport terminal; LCR, late cruise; MC, mid-climb; MCR, mid-cruise; OC, origin city; Out, outside airport terminal. Extended horizontal lines indicate the BASE study (Tsai and Macher, 2005) mean total culturable bacteria concentration for samples collected inside (dashed line) and outside (bold line) buildings.

Table 1. Descriptive statistics and modeling results for airborne culturable bacteria total concentration (CFU m^{-3})

Model sampling interval	*N* flights	*N* samples	$N >$ LOD (%)[a]	Range (CFU m^{-3})[b]	Unadjusted		Model adjusted		Modeled variance[c]
					GM	GSD	GM	95% CI	
Origin city									
Outside airport terminal	12	35	35 (100)	17.6–389	109	2.2	107[d]	71.3–161	0.65
Inside airport terminal	12	36	35 (97)	8.7–764	115	4.0	115	48.6–271	2.11
Boarding	12	69	69 (100)	26.7–1060	297	2.2	295	246–354	0.60
Aircraft[e]									
Boarding	12	69	69 (100)	26.7–1060	297	2.2	290	222–379	0.71
Mid-climb	12	72	72 (100	35.2–969	170	2.1	170[f]	127–226	0.50
Early cruise	12	72	72 (100)	17.4–750	131	2.1	131[f]	105–165	0.55
Mid-cruise	12	72	72 (100)	8.7–1740	122	2.4	122[f]	92.1–161	0.73
Late cruise	12	65	65 (100)	26.6–378	126	1.7	126[f]	102–157	0.27
Deplaning	12	29	28 (97)	181–2290	518	2.7	549	329–914	1.17
Destination city									
Deplaning	12	29	28 (97)	181–2290	518	2.7	531	349–808	0.94
Inside airport terminal	11	30	30 (100)	21.2–532	134	2.3	154[g]	83.7–285	0.91
Outside airport terminal	11	33	33 (100)	8.8–879	185	2.4	193[g]	128–291	0.81

CI, confidence interval; GSD, geometric standard deviation; LOD, limit of detection; *N*, number.
[a]The minimum detectable concentration of airborne culturable bacteria was approximately 8.8 CFU m^{-3} = 1 CFU/(113.4 l × 0.001 m^3 l^{-1}) based on a median air volume of 113.4 l.
[b]Range is for samples positive for growth.
[c]Estimates obtained using the MIXED procedure in SAS. The outcome variable was the natural log-transformed bacteria concentration. The underlying covariance structure was compound symmetric within each sampling interval, but the variance components were allowed to vary by sampling interval. The structure included a common covariance between samples collected during different intervals.
[d]Modeled GM significantly less than boarding GM (Tukey–Kramer adjusted *P* value: <0.001). Note that the difference between the boarding and inside airport terminal GMs was marginally significant (Tukey–Kramer adjusted *P* value: 0.071).
[e]Model adjusts for location in coach cabin (front, rear).
[f]Modeled GM significantly less than boarding and deplaning GMs (Tukey–Kramer adjusted *P* values: <0.0001–0.021).
[g]Modeled GM significantly less than deplaning GM (Tukey–Kramer adjusted *P* value: <0.01).

In the modeled results (Table 1), GM total bacterial concentrations observed during flight (mid-climb and cruise intervals) were significantly lower than GMs for boarding and deplaning (*P* values <0.0001–0.021). The GM total bacterial concentrations observed outside the airport terminals were significantly lower than GM concentration observed during boarding (*P* value <0.001) or deplaning

(P value <0.01). The GM total bacterial concentrations observed inside the airport terminal were significantly lower than deplaning (P value <0.01) and marginally significantly lower than boarding (P value $= 0.071$). Total bacterial concentrations were more variable inside the airport terminal at the origin city airport and during the deplaning interval on board the aircraft; however, at the destination city airport, variances were similar for the different sampling intervals. The estimated modeled variances for the sampling intervals illustrate the heterogeneity of variances and the importance of specifying a model that allows for heterogeneity.

Total airborne bacterial concentrations did not differ between the front and back locations within the coach cabin; however, a visual inspection of the data indicated that the locations might differ depending on the sampling interval. When an interaction term between location and sampling interval was considered, statistically significant differences were observed for some of the sampling intervals. The largest difference between the front and back locations was observed during the boarding interval (front GM 375 CFU $\cdot$ m^{-3} versus back GM 223 CFU $\cdot$ m^{-3}, P value $=$ 0.0055) with smaller differences observed during the early-cruise (front GM 107 CFU $\cdot$ m^{-3} versus back GM 162 CFU $\cdot$ m^{-3}, P value $= 0.012$) and late-cruise intervals (front GM 110 CFU $\cdot$ m^{-3} versus back GM 145 CFU $\cdot$ m^{-3}, P value $= 0.010$). A difference between front and back was observed during deplaning (front GM 740 CFU $\cdot$ m^{-3} versus back GM 392 CFU $\cdot$ m^{-3}, P value $= 0.085$) but due to low sample size, this difference was not statistically significant. After adjusting for sampling interval and location, carbon dioxide concentrations and occupancy rate were not significant in the aircraft model.

Table 2 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 (off-diagonal entries). Bacterial measurements observed inside the airport terminals were highly correlated [estimated correlation (r): 0.80 and 0.86]. However, bacterial measurements observed during the boarding, mid-climb, cruise and deplaning intervals (Table 2) were not so highly correlated and correlations among these sampling intervals were also low.

In an analysis limited to bacterial data from the cruise intervals, within-flight variability was much higher than between-flight variability for culturable bacterial concentrations (estimates of natural log-transformed within-flight variability $\hat{\sigma}^2_w=0.44$ and between-flight variability $\hat{\sigma}^2_b=0.11$). When flight was considered to be a fixed effect in the cruise-only model, GM culturable bacterial concentrations were significantly different among the flights (overall F-test: $F_{11,194} = 5.54$, P value <0.0001).

Bacterial speciation analysis

A variety of taxa were observed in the airborne culturable bacteria samples (Table 3). No airborne samples were analyzed with colonies described as unknown. Both gram-positive and gram-negative bacteria were observed in the airborne samples. Since the majority of samples were only analyzed at the genus level, interpretation of the taxon results is limited. The predominant types of bacteria observed inside the aircraft were *Micrococcus luteus*, *Staphylococcus*, *Bacillus* and gram-negative bacteria (Table 3). All taxa observed inside the aircraft were also observed inside the airport terminals.

Table 2. Estimated correlations of natural log-transformed total culturable bacteria concentrations (CFU m^{-3}) for replicate samples obtained within each sampling interval (main diagonal entries, in shaded boxes) and for samples obtained in different sampling intervals (off-diagonal entries)

	OC out	OC in	BD	MC	ECR	MCR	LCR	DP	DC in	DC out
OC out	0.46	0.05	0.10							
OC in		0.80	0.05							
BD			0.07	0.10	0.09	0.08	0.13	0.06		
MC				0.32	0.11	0.10	0.16	0.08		
ECR					0.09	0.09	0.15	0.07		
MCR						0.14	0.13	0.06		
LCR							0.30	0.10		
DP								0.23	0.21	0.22
DC in									0.86	0.23
DC out										0.19

BD, boarding; DC, destination city; DP, deplaning; ECR, early cruise; In, inside airport terminal; LCR, late cruise; MC, mid-climb; MCR, mid-cruise; OC, origin city; Out, outside airport terminal. Correlations are estimated from the covariance matrix of the mixed-effects model presented in Table 1 and do not have associated P values. Estimated correlations were obtained using the MIXED procedure in SAS. The underlying covariance structure was compound symmetric within each sampling interval, but the variance components were allowed to vary by sampling interval. The structure included a common covariance between samples collected during different intervals.

Table 3. Percent of samples detecting airborne taxa[a] by location and sampling interval

Airport terminal				Aircraft			
Outside (n = 68)		Inside (n = 66)		Boarding/deplaning (n = 98)		Cruise intervals (n = 209)	
Genus or group	%	Genus or group	%	Genus or group	%	Genus or group	%
Gram-negative bacteria	90	*Staphylococcus*	92	*Micrococcus luteus*	99	*Staphylococcus*	97
Rhodococcus	75	*Micrococcus luteus*	82	*Staphylococcus*	98	*Micrococcus luteus*	92
Methylobacterium	71	Gram-negative bacteria	64	Gram-negative bacteria	88	Gram-negative bacteria	58
Flavobacterium	63	*Bacillus*	62	*Bacillus*	71	*Bacillus*	42
Micrococcus luteus	63	*Rhodococcus*	53	*Rhodococcus*	59	*Rhodococcus*	28
Bacillus	54	*Flavobacterium*	45	*Flavobacterium*	48	*M. roseus*	27
Staphylococcus	53	*Methylobacterium*	26	*M. roseus*	35	*Flavobacterium*	26
Actinomycetes	40	*M. roseus*	23	*Shewanella putrefaciens*	28	*Pseudomonas* sp. non-*aeruginosa*	20
Pseudomonas sp. non-*aeruginosa*	18	*Pseudomonas* sp. non-*aeruginosa*	21	*Pseudomonas* sp. non-*aeruginosa*	27	*Shewanella putrefaciens*	11
Stenotrophomonas maltophilia	13	Actinomycetes	17	*Methylobacterium*	26	*Methylobacterium*	11
Shewanella putrefaciens	12	*Shewanella putrefaciens*	15	Actinomycetes	10		

n, number of samples; %, percent of samples positive for the genus or group.
[a]Genera and groups limited to percent detected greater than or equal to 10%.

Micrococcus luteus and *Staphylococcus* are commonly found on skin and mucus membranes so their presence inside the aircraft was expected. Detection rates of *M. luteus* and *Staphylococcus* were similar within all aircraft sampling intervals and did not change significantly from boarding through deplaning (Table 4). *Staphylococcus* was detected at significantly greater frequencies inside the airport terminal compared to detection frequencies observed outside the terminal (P value <0.05).

Bacilli are gram-positive bacteria which produce highly resistant endospores, enabling them to live in habitats with varying temperatures, pHs and moisture levels. The majority of *Bacillus* species are harmless but select species can cause infections, especially in immunocompromised individuals. *Bacilli* were observed inside and outside airport terminals and inside the aircraft during each sampling interval. The detection rates of *Bacillus* were significantly lower during the mid-climb and cruise intervals compared to the boarding detection rates (P value <0.05). Overall, mean peak concentrations for *Bacillus* concentrations were fairly consistent throughout the flights. In contrast, the detection frequencies were highly variable from flight to flight (data not shown).

Detection rates for gram-negative bacteria were significantly higher during boarding compared to detection rates observed inside the airport terminal (P value <0.05). Detection rates observed during boarding were also significantly higher than the detection rate observed during mid-climb and all three cruise intervals (P value <0.05). The detection frequencies observed during the mid-climb interval were also significantly greater than those observed during the three cruise intervals (P value <0.05).

BASE study comparisons

In general, gram-positive cocci were dominant in both the airline and BASE studies. Gram-negative rods were not as common in the BASE study. The average total bacteria concentration during cruise was higher than the average total bacteria concentration observed in the BASE study (Table 5). Figure 1 also displays the differences between the two studies graphically. Due to differences in analytical categories, a more detailed taxa comparison could not be completed.

Surface-culturable bacteria

A total of 48 surface swab samples were collected from surfaces on board the aircraft and inside the airport terminals. The genera recovered by location are presented in Table 6. Detectable recoveries were observed on 87.5% of the bacterial swab samples. Samples were reported by genus/species or as gram-negative or gram-positive bacteria. Except for *Streptococcus* and *Micrococcus roseus*, all genera observed inside the aircraft were also observed on surfaces inside the airport terminal. The bacteria observed on surface swabs are indicative of human commensals.

Rhinovirus samples

Rhinovirus samples were collected on the last seven selected flights in the study. All filters were negative for HRV particles.

Table 4. Percent detect and GM concentration by location and sampling interval for selected genera

Location sampling interval	*N* flights	*N* samples	Airborne bacteria											
			Staphylococcus			*Micrococcus luteus*			*Bacillus*			Gram negative		
			%	GM	GSD	%	GM	GSD	%	GM	GSD	%	GM	GSD
Origin city														
Outside airport terminal	12	35	46	8.1	2.7	54[b]	11	3.1	46	7.4	3.4	83	19	2.3
Inside airport terminal	12	36	89[a]	35	3.7	75[b]	29	4.6	67[a]	11	2.2	67[b]	15	3.0
Boarding	12	69	99[a]	100	2.4	100	88	2.8	74[a]	13	2.1	91	23	2.0
Aircraft														
Boarding	12	69	99	100	2.4	100	88	2.8	74	13	2.1	91	23	2.0
Mid-climb	12	72	94	69	2.9	96	39	2.4	57[c]	9.3	2.0	75[c]	12	1.9
Early cruise	12	72	97	60	2.7	94	29	2.4	42[c,d]	6.8	2.1	60[c,d,e]	9.6	2.0
Mid-cruise	12	72	93	58	3.2	90	23	2.4	42[c,d,e]	7.0	2.1	58[c,e]	9.3	1.9
Late cruise	12	65	100	61	2.2	92	28	2.1	43[c,d]	7.4	1.9	55[c,d,e]	8.6	1.7
Deplaning	12	29	97	200	2.3	97	170	2.4	66	25	2.3	79	29	2.2
Destination city														
Deplaning	12	29	97[f]	200	2.3	97[f]	170	2.4	66	25	2.3	79	29	2.2
Inside airport terminal	11	30	97[f]	61	2.5	90	27	2.7	57	9.0	1.8	60[g]	11	2.7
Outside airport terminal	11	33	61	14	3.5	73	18	2.8	64	11	2.4	97	29	2.2

% denotes the percent of samples in the sampling interval that detected the genus/species. GM denotes the GM concentration (CFU m^{-3}) for the sampling interval; GSD denotes the geometric standard deviation. Since nondetection rates were higher at the genus/species level, GM and GSD were estimated using maximum likelihood methods available in the LIFEREG procedure in SAS. Samples that did not detect a particular genus/species were considered to be left censored at 1 CFU per volume of air sampled.

[a]In origin city model, significantly greater than outside airport terminal (P value <0.05).
[b]In origin city model, significantly less than boarding (P value <0.05).
[c]In aircraft model, significantly less than boarding (P value <0.05).
[d]In aircraft model, significantly less than deplaning (P value <0.05).
[e]In aircraft model, significantly less than mid-climb (P value <0.05).
[f]In destination city model, significantly greater than outside airport terminal (P value <0.05).
[g]In destination city model, significantly less than outside airport terminal (P value <0.05).

Table 5. Comparison of select bacterial populations between the airline study and the BASE study

Bacterial category	Airline results[a] (n = 209)				BASE study results[b] (n = 98)			
	Mean[c]	SD	Maximum	Median	Mean[c]	SD	Maximum	Median
Gram-positive cocci	153.5	203.2	2312.2	107.7	39.7	27.1	146.4	32.8
Gram-negative rods	19.0	24.5	246.0	9.2	3.1	5.2	38.0	1.7
Total bacteria	179.6	211.6	2384.8	128.5	100.3	49.6	275.5	91.4

n, number of samples.
[a]Airline data represent data from cruise intervals only.
[b]BASE results from Table 4b Mesophilic bacteria of Tsai and Macher (2005).
[c]Average airborne culturable bacteria (CFU m^{-3}).

DISCUSSION

The bacteria recovered throughout this study were either common skin-surface organisms (primarily gram-positive cocci) or organisms common in dust and outdoor air. None were respiratory pathogens that could be used as an indicator of the risk for transmission of respiratory illness in the aircraft environment.

Although monitoring for airborne bacteria is often conducted to identify a bacterial source or complete a general indoor air investigation, the results do not provide a complete bacterial picture. Culturable bacteria are only a fraction of all airborne bacteria. Obligate pathogens, bacteria with highly specific nutritional requirements and nonliving bacteria are not recovered from culturable techniques. The skin-surface organisms that are recovered are found, in general, in relatively large particles (skin scales or fragments), i.e. >7.5 μm (Tham and Zuraimi, 2005), whereas respiratory pathogens would be more likely generated as droplet nuclei (2–5 μm). Because of this size difference, ventilation controls will differ for these types of aerosols.

In spite of limitations of airborne culturable sampling for bacteria, the data may provide an indication of the extent of release of skin-surface organisms in occupied environments and could indicate active sources within an environment that deserve attention.

Table 6. Percent of samples detecting surface taxa by location

Genus	Inside airport terminal (n = 24) % detected	Inside aircraft (n = 24) % detected
Staphylococcus	54.2	58.3
Bacillus	41.7	45.8
Flavobacterium	45.8	33.3
Micrococcus luteus	45.8	33.3
Stenotrophomonas maltophilia	29.2	29.2
Pseudomonas sp. non-*aeruginosa*	20.8	20.8
Micrococcus roseus	0	4.2
Shewanella putrefaciens	25	4.2
Streptococcus	0	4.2
Microbacterium sp.	4.2	0
Methylobacterium	4.2	0
Rhodococcus	12.5	0
Gram-positive bacteria[a]	8.3	0
Gram-negative bacteria[b]	58.3	45.8

% denotes the percent of samples in the location that detected the genus/species.
[a]Denotes the percent of samples identified as gram-positive bacteria, not identified at a genus/species level.
[b]Denotes the percent of samples identified as gram-negative bacteria, not identified at a genus/species level.

Our trend analysis of *M. luteus* and *Staphylococcus* illustrate how data may be used to evaluate air movement patterns and the relationship between activity and bacterial shedding.

Micrococcus luteus, M. roseus and *Staphylococcus* concentrations tended to increase when passengers move about the aircraft, indicating that these have a human source. Detection frequencies of these taxa were higher inside the terminal compared to outside, very high during boarding or deplaning and only slightly lower during the cruise intervals. At the same time, *Rhodococcus* and *Methylobacterium* were highest outdoors and during boarding, but concentrations decreased and these taxa were detected less frequently during cruise. Outdoor contaminants may be captured into the aircraft during boarding/deplaning and then settle out of the air during cruise. The observed trends for *Rhodococcus* and *Methylobacterium* are similar to total fungal concentration trends observed in our previous work (McKernan *et al.*, 2007).

Bacterial concentrations were higher in the front of the cabin compared to the back during boarding. This is likely to be a result of passenger activity during the boarding process. During the time of the study, aircraft were boarded back to front, so standing lines were common in the front of coach class. During cruise, bacterial concentrations were uniformly low, with slightly higher concentrations in the back of the cabin. This could be reflective of a slight rearward drift of bacteria-bearing particles or passenger movement to the restrooms in the back of the aircraft.

Compared to all other sampling intervals, total bacteria concentrations were highest during deplaning, followed by the boarding process due to increased passenger activity. It is our hypothesis that the total bacteria concentration was higher during deplaning because more activity occurs in a shorter time frame.

In general, our data are comparable to previous studies of bacteria in aircraft when viewed in context of the samplers and sampling times used (Nagda *et al.*, 1989; Wick and Irvine, 1995; Dechow *et al.*, 1997; Lee *et al.*, 1999; Dumyahn *et al.*, 2000). Overall, the bacterial concentrations we observed should not cause adverse health effects for healthy individuals traveling on aircraft. However, there were isolated observations for particular taxa which could create problems for individuals who are immunocompromised. Health effects are possible in these immunocompromised individuals due to variation in individual susceptibility toward particular species.

In their published review, Tsai and Macher (2005) proposed that 175 CFU $\cdot$ m^{-3} (the BASE 90th percentile) could be used as an upper bound for an interpretation guideline for bacteria for offices and similar nonmanufacturing workplaces. If an interpretation guideline for airborne bacteria is considered further (which may or may not be appropriate), we recommend the incorporation of both an activity-level and occupant density component.

To facilitate appropriate comparisons with the BASE study, the positive hole correction factor was applied to the culturable airline data in Table 5. 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. Accordingly, we performed a supplemental analysis to determine whether the airline/BASE comparisons were influenced by the decision 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 cruise intervals were only 3% higher than uncorrected values. Larger differences were observed during boarding and deplaning, where corrected concentrations were 6 and 7% higher than uncorrected concentrations, respectively. For consistency, results presented in the airline/BASE study comparisons in Table 5 employ the positive hole correction factor.

The total bacterial concentrations we observed in the aircraft were significantly higher than the proposed BASE recommendation as our 90th percentile

during cruise was 302 CFU · m^{-3} while during boarding and deplaning the 90th percentile was 851 CFU · m^{-3}. The higher bacterial concentrations observed are likely the result of a higher occupant density in an aircraft compared to an office building. Other studies (Luoma and Batterman, 2001; Toivola *et al.*, 2004) have indicated that indoor culturable bacterial concentrations are related to occupant density and levels of human activity.

This study also emphasizes the difficulty of detecting environmental viral samples on commercial aircraft. Since the buffering solution used for the RT-PCR extracts was potentially harmful when mixing, it was not appropriate for use 'in the field' (i.e. a hotel room where traveling staff would be working). Therefore, samples could not be buffered until received by the laboratory—the elapsed time varied depending on the destination of the flight. Second, the filters were collected at one location on the aircraft during a seasonal time period when rhinovirus infections were not at their peak. For these reasons, the likelihood of capturing a positive viral sample using these methods was low. Since traditional techniques seem rather limited in this application, novel techniques, such as sensors, may be useful. Other investigators have used computational fluid dynamics tools to identify the number and optimal placement for sensors on aircraft (Zhang *et al.*, 2007).

It is also interesting to compare bacterial trends to previously published fungal trends on aircraft (McKernan *et al.*, 2007, 2008). As shown in Fig. 2, total bacterial concentrations were generally higher than fungal concentrations. Bacterial and fungal concentrations in the aircraft were highest during boarding and deplaning. Both bacterial and fungal concentrations declined during the cruise intervals, at least partially reflecting the operation of the B-767 aircraft filtration system. Concentrations may also decline as a result of reduced passenger activity, thereby less aerosolization from clothing, belongings or other aircraft sources. Bacterial concentrations were highest during boarding and deplaning and were lower outside the airport terminal. Total fungal concentrations were highest outside the airport terminal with lower concentrations observed during boarding and deplaning.

During cruise, total fungal and bacterial concentrations exhibited higher within-flight than between-flight variability (bacteria, $\hat{\sigma}^2_b=0.11/\hat{\sigma}^2_w=0.44$; culturable fungi, $\hat{\sigma}^2_b=0.01/\hat{\sigma}^2_w=0.53$ and total spore, $\hat{\sigma}^2_b = 0.13/\hat{\sigma}^2_w = 0.31$). This means that the cruise samples collected during each flight exhibited more variability than the samples collected across the 12 flights. High temporal variability between bioaerosol samples collected at the same location over a short time period is quite common (American Industrial Hygiene Association Biosafety Committee, 1996; Toivola *et al.*, 2002; Tsai and Macher, 2005; McKernan *et al.*, 2007). Our findings highlight the fact that aerobiological concentrations can be dynamic and underscore the importance of appropriate sample size and design for both bacteria and fungi.

Limitations

One of the primary limitations of the qualitative analysis is the fact that much of the analysis was completed at the generic level and did not include species identification. There are more than one species in nearly all of the genera recovered and it was impossible to differentiate between them. The species analysis would have facilitated a more thorough evaluation of potential intestinal bacteria.

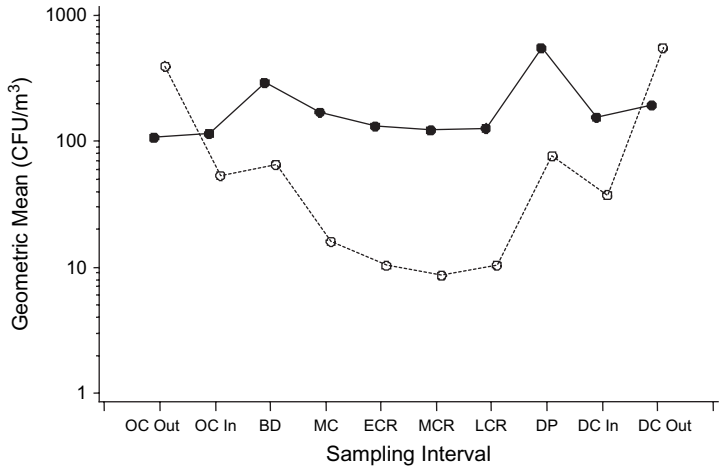

Fig. 2. Culturable air concentrations (CFU m^{-3}) by sampling interval. Black line represents bacterial concentrations; dashed line represents fungal concentrations (McKernan *et al.*, 2007, 2008). BD, boarding; DC, destination city; DP, deplaning; ECR, early cruise; In, inside airport terminal; LCR, late cruise; MC, mid-climb; MCR, mid-cruise; OC, origin city; Out, outside airport terminal.

Since our data came from flights during the summer months, our results may not be generalizable to other seasons when outdoor concentrations tend to be lower. An additional limitation is that our study design could not be blinded. It is also unclear whether the results of this study have external validity to other aircraft models or other B-767 flights under different ventilation settings.

The bacteria recovered throughout this study were either common skin-surface organisms (primarily gram-positive cocci) or were organisms common in dust and in outdoor air. None were respiratory pathogens that could be used as an indicator of the risk for transmission of respiratory illness in the aircraft environment. Bacterial concentrations were highest during the boarding and deplaning process. The analysis of specific genera supports our hypothesis that passenger activity and high occupant density contribute to airborne bacterial generation. This study also illustrates the difficulty of collecting environmental viral samples on commercial aircraft.

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