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Evaluating Fungal Populations by Genera/Species on Wide Body Commercial Passenger Aircraft and in Airport Terminals

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Given the potential health effects of fungi and the amount of time aircrew and passengers spend inside aircraft, it is important to study fungal populations in the aircraft environment. Research objectives included documenting the genera/species of airborne culturable fungal concentrations and total spore concentrations on a twin-aisle wide body commercial passenger aircraft. Twelve flights between 4.5 and 6.5 h in duration on Boeing 767 (B-767) aircraft were evaluated. Two air cooling packs and 50% recirculation rate (i.e. 50:50 mix of outside air and filtered inside air) were utilized during flight operations. Passenger occupancy rates varied from 67 to 100%. N-6 impactors and total spore traps 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 resulting in a total of 522 culturable and 517 total spore samples. A total of 45 surface wipe samples were collected using swabs onboard the aircraft and inside the airport terminals. A variety of taxa were observed in the culturable and total spore samples. A frequency analysis of the fungal data indicated that *Cladosporium*, *Aspergillus* and *Penicillium* were predominant genera in the culturable samples whereas *Cladosporium*, Basidiospores and *Penicillium/Aspergillus* were predominant in the total spore samples. Fungal populations observed inside the aircraft were comprised of similar genera, detected significantly less frequently and with lower mean concentrations than those observed in typical office buildings. Although sources internal to the aircraft could not be ruled out, our data demonstrate the importance of passenger activity as the source of the fungi observed on aircraft. Isolated fungal peak events occurred occasionally when concentrations of a particular genus or species rose sharply inside the cabin for a limited period. Overall, our research demonstrates that on the sampled flights the B-767 filtration system operated efficiently to remove fungal spores when two air cooling packs and 50% recirculation rate were utilized during flight operations.

Keywords: aircraft, fungal exposures, airplane, total spore fungi, genera analysis

INTRODUCTION

In 2003, the average US consumer spent 19% of his income on transportation expenses, with ~3% of this proportion spent on airfares. Air travel is a major transportation sector with nearly 600 million total enplaned passengers and 500 trillion passenger miles flown on aircraft annually throughout the United States (Bureau of Transportation Statistics, 2005). Approximately 198 000 flight personnel and 73 000 aircrew personnel work (Bureau of Transportation

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Statistics, 2004) in the aircraft cabin environment in the United States alone. Because of the amount of time spent inside the aircraft for both aircrew and passengers and the potential health effects of fungal exposure, it is important to study fungal populations in this environment.

Fungi are ubiquitous in both the indoor and outdoor environment. Health effects deriving from fungal exposure are primarily allergic responses, but infection may occur in immunocompromised individuals and toxic responses may also occur with intense exposure (Rogers, 2003). Exposure to some fungal species has been associated with exacerbation of asthma and other airway reactivity (Institute of Medicine Committee on the Assessment of Asthma and Indoor Air, 2000). Exposure to spores from fungal genera, such as *Alternaria* has led to allergic sensitization and symptoms of asthma and other allergic symptoms (Arbes *et al*., 2005; O'Driscoll and Denning, 2005). Patients with sensitization to fungi tend to have more severe asthma than those not sensitized (Sanchez and Bush, 2001).

To date, only four peer reviewed studies have documented fungal concentrations on passenger aircraft (Dumyahn and Muilenburg, 2000; Nagda *et al*., 1989; Pierce *et al*., 1999; Wick and Irvine, 1995). These select studies have relied on a small number (<5) of samples per flight to represent the entire aircraft environment. Nagda *et al*. (1989) sampled for fungal concentrations on 23 randomly selected, non-smoking flights using a compact sieve plate sampler and observed a mean culturable fungal concentration of less than 10 colony forming units per cubic meter of air sampled (cfu/m^3). Using a Reuter Centrifugal Air Sampler, Wick and Irvine observed a mean culturable fungal concentration of 43 cfu/m^3 on 36 flights on five types of aircraft (Wick and Irvine, 1995). Using a portable compact air sampler (Rodac), Pierce *et al*. (1999) studied fungal concentrations on eight flights aboard Boeing-777 aircraft, where culturable fungal concentrations ranged between 1 and 37 cfu/m^3. Dumyahn and Muilenburg (2000) collected fungal samples aboard Boeing-777 aircraft using the portable Burkard culture plate sampler and observed concentrations ranging between 1 and 500 cfu/m^3 (Dumyahn and Muilenburg, 2000). To date, no aircraft study has included spore trap samples for microscopic analysis, thus missing spores that might be dead or unable to grow in culture but still able to elicit health effects. Only the Dumyahn and Muilenburg (2000) study completed a cursory analysis of fungal genera with the culturable results, in spite of the fact that health effects can vary by genera and species.

In 2002, the National Academy of Sciences completed a review of the airliner cabin environment, including bioaerosol concentrations (National Research Council Committee on Air Quality in Passenger Cabins of Commercial Passenger Aircraft, 2002). This review encompassed the studies above 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. The committee also indicated that more information regarding infectious transmission on aircraft was needed.

Research objectives

Our research objectives coincide with many of the research gaps highlighted by the National Academy of Sciences. Research aims were to evaluate fungal populations before, during and after a wide body commercial passenger aircraft flight. Airborne concentrations of individual fungal taxa were identified within aircraft and airport terminals through an evaluation of both culturable fungi and spore trap samples. Surface fungal populations were also characterized inside the aircraft and compared to fungi on surfaces in airport terminals.

METHODS

All flights were completed on the Boeing 767 (B-767) aircraft, a twin-aisle wide body commercial passenger aircraft. Two airlines that had a large ownership of this aircraft agreed to participate in this study. A random sample of twelve flights with a duration between 4.5 and 6.5 h were sampled during the summer of 2002. Two air cooling packs and 50% recirculation rate (i.e. 50:50 mix of outside air and filtered inside air) were utilized during operations. In addition the B-767 incorporated a High Efficiency Particulate Air (HEPA) filter.

Sampling times and locations

Flight segments were divided into 10 distinct sampling intervals. Samples were collected outside the airport terminal and inside the airport terminal at both the origin and destination cities. Samples were also collected during six sampling intervals onboard the aircraft: boarding, mid-climb, early cruise, mid-cruise, late cruise and deplaning. Boarding samples were taken while passengers were actively boarding the aircraft, mid-climb 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. 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.

Airborne sampling for fungi

Both culturable and spore trap samples were collected to assess fungal populations. Both types of samples were collected to facilitate comparability to previous work, provide insight on both the culturable and non-culturable fungal populations, and to further differentiate some fungal categories to genera and species. 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, Georgia). During each interval, with the exception of deplaning, sequential triplicate culturable 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 Air-o-Cell cassettes (Zefon International, Ocala, FL) and high-volume pumps calibrated to 15 l/min (PORTN-6, Thermo Andersen, Smyrna Georgia). During each interval, with the exception of deplaning, sequential triplicate spore samples were collected, each of 10 min duration. During the short deplaning process, a single 10 min sample was collected.

Airborne culturable fungal results were assessed as total colony forming units (cfu) per sample and as the number of cfu of each identifiable genus and, in a few cases, species, per sample. Total spore results were assessed as total spores per sample and the number of spores per identifiable genus or larger grouping per sample. Given the diversity of fungal aerosol populations, each sample commonly included multiple fungal taxa. Culturable results were converted to a concentration in air by dividing by the air volume associated with the sample, resulting in the number of colony forming units per cubic meter of air (cfu/m^3). Spore sample results were converted to spores per cubic meter of air (spores/m^3) by adjusting for the proportion of the sample counted ($\sim$26%) and dividing by the sample volume. When analyzing results by identifiable genus/species or larger grouping, non-detectable values were treated as zero in all computations.

Surface sampling for fungi

Surface samples were collected using a sterile, cotton-wool tipped stick wetted with a Stuart solution (BBL™ Culture Swab, Becton Dickinson). Each sample was collected across a 5×5 cm surface area. The moistened cotton-wool tipped stick was swabbed across a sample surface in a 'Z' pattern once and then stored in a sterile tube for submission to the laboratory. This collection technique has been described in detail (American Industrial Hygiene Association Biosafety Committee, 1996). Each surface sample was comprised of two sterile cotton-wool tipped sticks. On the aircraft, one cotton-wool tipped stick was used to collect surface fungi on the armrest and one was used to collect surface fungi on the tray table. If time permitted, these samples were collected before the passengers boarded the aircraft. Otherwise, these samples were collected after the mid-climb sampling period. Inside the airport terminal, one cotton-wool tipped stick was collected on the armrest of a waiting chair and one sample was collected on an end table in the terminal. If end tables were not present, an alternate table or counter was sampled.

All culturable fungi samples were kept cool and shipped on blue ice via an overnight mail carrier to the analytical laboratory within 48 h after sample collection. Once at the laboratory, the cotton-wool tipped sticks from the surface samples were swabbed across DG-18 media Petri dishes. All culturable samples were incubated at 25°C for 7–10 days. After the appropriate incubation period, fungal 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. As part of the laboratory's quality assurance procedures, samples were counted twice and compared for consistency.

Statistical methods

SAS v9 (SAS Institute, Cary NC) (SAS Institute, 2004) was used for the statistical analysis of the fungal data. The fungal taxa were evaluated in terms of detection rates (i.e. percent of samples in which genus/species detected), peak concentration and arithmetic mean concentration.

Since detection rates for a particular genus/species were fairly high, log-binomial modeling using the GENMOD procedure in SAS was used to compare detection rates among the sampling intervals. Sequential 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.

Ratio analysis between culturable and total spore results

To elucidate potential differences between the culturable and total spore fungal concentrations, a ratio analysis was completed for the predominant genera.

Since microscopic examination of the total spore samples cannot differentiate between *Aspergillus* and *Penicillium*, these genera were combined for the culturable samples to allow comparison between sampling methods. CFUs from sequential, triplicate samples for a particular sampling interval were summed by genus and then divided by the total sample volume to estimate the culturable numerator. The denominator was calculated similarly using the total spore samples. The ratio of the culturable and total spore concentrations was calculated for each sampling interval and each sampling location (outside airport terminal, inside airport terminal, front and rear of aircraft).

Comparison with building assessment survey evaluation (BASE) study

To place fungal populations in the aircraft environment in perspective with respect to other interior environments, we compared our data to those collected by the US Environmental Protection Agency (EPA) in the BASE studies. In 1994, the EPA embarked on a four year study to measure indoor environmental quality in randomly selected, non-complaint buildings. This is the largest normative study of indoor air quality parameters in the US. Researchers used the N-6 impactor to assess culturable fungal concentrations in 100 buildings and Burkard samplers to assess total spore concentrations in 44 buildings (Macher *et al*., 2001). We acquired the complete BASE fungal data from the EPA and were able to conduct direct comparisons by genus to our airline data (Environmental Protection Agency, 2005). To facilitate this comparison, some genus/species combinations were grouped into a larger generic category within the two datasets. Since our data were collected in the summer, we only included BASE data from the summer season.

RESULTS

A total of 522 culturable samples and 517 total spore samples were collected on 12 aircraft flights as well as inside and outside origin and destination airport terminals. A total of 45 surface swab samples were collected from surfaces onboard the aircraft and inside the airport terminals. A variety of taxa were observed in the airborne culturable, surface culturable and total spore samples (Table 1). The generic composition of airborne fungal populations varied by location and sampling interval (Table 2); whereas the surface populations components varied by location (Table 3).

Airborne culturable analysis

Detection rates for the fungi illustrated that a few select fungal genera, *Cladosporium*, *Aspergillus* and *Penicillium*, were predominant in the airborne culturable samples (Table 2). The peak concentration analysis was conducted by graphing the various taxa by flight (data not shown). Given the high non-detection rates observed for particular taxa, arithmetic means are presented (Table 4) instead of geometric means.

For both *Cladosporium* and *Penicillium*, both mean concentrations and detection rates revealed bathtub-shaped trends, being highest outside the airport terminal and lowest during the cruise intervals on board the aircraft (Table 4). *Cladosporium* was detected significantly more often outside the airport terminal than inside the airport terminal (origin city airport: P-value = 0.0049; destination city airport: P-value = 0.0050) and significantly more often outside the airport terminal than inside the aircraft during boarding (P-value = 0.0010) or deplaning (P-value = 0.0086). Detection rates for *Cladosporium* were similar inside the airport terminal and onboard the aircraft during boarding and deplaning. Onboard the aircraft, *Cladosporium* was detected significantly more often in the boarding and deplaning intervals compared to intervals when the aircraft was in the air (P-value < 0.01).

Detection rates for *Penicillium* during cruise intervals were significantly lower than those observed during the boarding process (P-value < 0.01) and the mid-climb interval (P-value < 0.05). The detection rate for *Penicillium* during the late cruise interval was significantly lower than the detection rate during the deplaning process (P-value < 0.05). *Aspergillus* detection rates during the mid-climb and cruise intervals were all significantly lower than during the boarding process (P-value < 0.01). The detection rates for *Aspergillus* during the cruise intervals were significantly lower than the mid-climb interval (P-value < 0.05) and the deplaning process (P-value < 0.01).

Aspergillus species analysis

Samples which detected *Aspergillus* were first analyzed by genera. Since fourteen species were observed in the study, a second analysis was completed by species. Each individual species of *Aspergillus* was analyzed and graphed over the various sampling intervals by flight. On three flights, *Aspergillus* was not observed outside or inside the airport terminal at the origin city, but was detected in 31% of samples collected inside the aircraft.

Aspergillus species, such as *Aspergillus niger*, *Aspergillus penicillioides* and *Aspergillus restrictus* were evident sporadically during multiple flights. *A.niger* was more evident in outside airport terminal samples (9%, $n = 68$) than inside the aircraft during cruise samples (1%, $n = 216$). *A.penicillioides*, which prefers drier environments, was present in 3% ($n = 216$) of mid-flight samples and 3% ($n = 68$) of outside

Table 1. Observed genera and species by sample type

Genus/species	Culturable air	Total spore air	Culturable surface
Acremonium	●	–	○
Alternaria	●	●	●
Arthrinium	●	●	○
Ascospores	–	●	–
Aspergillus	●	–	●
Aspergillus flavus	●	–	○
Aspergillus fumigatus	●	–	●
Aspergillus japonicus	●	–	○
Aspergillus nidulans	●	–	○
Aspergillus niger	●	–	○
Aspergillus niveus	●	–	○
Aspergillus ochraceus	●	–	○
Aspergillus parasiticus	●	–	○
A.penicillioides	●	–	●
A.restrictus	●	–	○
A.sydowii	●	–	○
Aspergillus ustus	●	–	○
Aspergillus versicolor	●	–	●
Aureobasidium pullulans	●	–	○
Bactrodesmium	Δ	●	Δ
Basidiospores	●	●	–
Botrytis cinerea	●	○	○
Cercospora	–	●	–
Chaetomium	○	●	○
Cladosporium	●	●	●
Curvularia	○	●	○
Curvularia lunata	●	–	○
Drechslera/Bipolaris	○	●	○
Emericella (Aspergillus) nidulans	●	–	○
Epicoccum nigrum	●	●	●
Eurotium	●	–	○
Exophiala jeanselmei	●	–	○
Fusarium	●	Δ	○
Ganoderma	–	●	–
Mucor plumbeus	●	–	○
Mucor racemosus	●	–	○
Myxomycetes	○	●	○
Nigrospora	●	●	●
Oidiodendron griseum	●	–	○
Paecilomyces variotii	●	–	○
Penicillium	●	–	●
Penicillium/Aspergillus-like	–	●	–
Phoma	●	–	○
Pithomyces	●	●	○
Pithomyces atro-olivaceus	●	–	○
Pithomyces chartarum	●	○	○
Rhizopus oryzae	●	–	○
Rhizopus stolonifer	●	–	○
Rhodotorula glutinis	●	–	●
Rusts	–	●	–
Scopulariopsis	●	●	○
Scopulariopsis acremonium	●	–	○
Scopulariopsis brevicaulis	●	–	○
Scopulariopsis candida	●	–	○
Smuts	–	●	–
Spegazzinia	Δ	●	Δ
Sporobolomyces salmonicolor	○	–	●
Stachybotrys	○	●	○
Stemphylium	Δ	●	Δ
Sterile fungi	●	–	●
Syncephalastrum racemosum	●	–	○
Taeniolella	○	●	○
Tetraploa	–	●	–
Torula herbarum	○	●	○
Trichoderma koningii	●	–	○
Tritirachium sp.	●	–	○
Ulocladium botrytis	●	○	○
Wallemia sebi	●	–	○
Yeasts	●	–	●
Zygosporium echinosporum	●	–	○
Zygosporium masonii	●	–	○

● genus/species detected by at least one sample; ○ analysis possible, but not observed; – analysis for genera/species not applicable for sample type; Δ analysis possible, but rarely recovered.

samples. Average maximum concentrations and mean detection rate of *A.penicillioides* were highest during the boarding process. Similarly, *A.restrictus* was most common inside the airport terminal and during the boarding and deplaning processes.

A.penicilliodes, *A.restrictus* and *Aspergillus sydowii* were observed in the aircraft during boarding, mid-climb, and the three cruise sampling intervals on Flight 12 but not inside or outside the airport terminals at the origin city. A similar scenario was observed for all *Aspergillus* species on three other flights. During flight 10, *A.penicilliodes* was observed inside the origin airport terminal, during boarding and again during deplaning and inside the destination airport terminal. *A.penicillioides* was not recovered from outdoor samples.

Total spore analysis

Select fungal categories, *Cladosporium*, Basidiospores, *Penicillium/Aspergillus* and *Ascospores* were predominant in the total spore samples (Tables 2 and 4). *Cladosporium* was ubiquitous in the spore samples, as it was observed in almost all outside terminal samples (96%, $n = 68$) and at every airport sampled (Table 4). Basidiospores, which are

Table 2. Genus by location and sampling interval

	Outside airport terminal[a]		Inside airport terminal[b]		Inside aircraft during boarding/deplaning		Inside aircraft during in-flight intervals[c]	
	Genus	% Detected[d]	Genus	% Detected[d]	Genus	% Detected[d]	Genus	% Detected[d]
	(*n* = 68)		(*n* = 69)		(*n* = 92)		(*n* = 288)	
Total spore samples	*Cladosporium*	96	Basidiospores	62	Basidiospores	*77*	Basidiospores	52
	Basidiospores	94	*Cladosporium*	59	*Cladosporium*	*74*	*Cladosporium*	47
	Ascospores	93	*Penicillium*/ *Aspergillus*-like	54	*Penicillium*/ *Aspergillus*-like	*64*	*Penicillium*/ *Aspergillus*-like	40
	Penicillium/ *Aspergillus*-like	60	Ascospores	25	Hyphal fragments	*36*	Hyphal fragments	16
	Gandoderma	51	Hyphal fragments	17	*Alternaria*	*22*		
	Hyphal fragments	50	Unknown	12	*Curvularia*	*16*		
	Unknown	47	*Alternaria*	10	Unknown	*13*		
	Alternaria	38			*Gandoderma*	*12*		
	Epicoccum	15			Ascospores	*11*		
	Smuts	10						
	(*n* = 68)		(*n* = 69)		(*n* = 97)		(*n* = 288)	
Culturable samples	*Cladosporium*	100	*Cladosporium*	77	*Cladosporium*	81	*Cladosporium*	40
	Penicillium	88	*Penicillium*	54	*Aspergillus-combined*	61	Yeasts	16
	Sterile Fungi	76	*Aspergillus-combined*	46	*Penicillium*	28	*Aspergillus-combined*	16
	Yeasts	56	Sterile fungi	39	Sterile Fungi	27	*Penicillium*	11
	Alternaria	47	Yeasts	20	Yeasts	24		
	Aspergillus-combined	43	*Alternaria*	12	*Alternaria*	24		
	Botrytis cinerea	22	*Wallemia sebi*	12	*Epicoccum nigrum*	12		
	Basidiomycete	21			*Wallemia sebi*	12		
	Phoma	21						
	Rhodotorula glutinis	21						
	Aureobasidium pullulan	18						
	Epicoccum nigrum	18						
	Eurotium-combined	18						
	Fusarium	16						

Only taxon results that were observed ⩾10% are represented in the table.
[a]Data represents 68 outdoor samples from 24 airport terminals taken before/after 12 flights.
[b]Data represents 69 indoor samples from 24 airport terminals taken before/after 12 flights.
[c]Inside Aircraft samples included the mid-climb, early cruise, mid-cruise and late cruise sampling intervals.
[d]Percent of total samples which detected genus.

Table 3. Surface samples genus frequency by location

Inside airport terminal (n = 21)		Inside aircraft (n = 24)	
Genus	% Detected	Genus	% Detected
Yeasts	24	*Rhodotorula glutinis*	17
Aspergillus versicolor	5	Yeasts	12
Cladosporium sphaerospermum	5	*Penicillium*	8
Epicoccum nigrum	5	*Aspergillus fumigatus*	4
Rhodotorula glutinis	5	*Alternaria tenuissima*	4
Sterile fungi	5	*Aspergillus penicillioides*	4
		Aspergillus versicolor	4
		Sporobolomyces salmonicolor	4

All observed taxa are presented.

produced by mushrooms, bracket fungi and puffballs, were observed most frequently outdoors (94%, $n = 68$) compared to inside the airport terminal (62%, $n = 69$) in-flight samples (52%, n = 216) and boarding/deplaning intervals (77%, n = 92). Only *Cladosporium* was observed more frequently than *Basidiospore* in samples collected outside airport terminals. *Penicillium/Aspergillus* spores were observed in samples collected outside (61%, n = 68), inside airport terminals (69%, n = 62), during boarding/deplaning (64%, $n = 92$) and during in-flight intervals (37%, n = 216).

An analysis using the GENMOD procedure with a log-binomial model was completed on the detection rates (i.e. the percentage of samples which detected a particular taxa) and is presented in Table 4. Statistical significance was observed between the detection rates for *Cladosporium* outside and inside the airport terminals (P-value = 0.036). *Cladosporium* detection rates observed during the boarding and deplaning intervals were also significantly different than detection rates observed outside the airport (P-value = 0.024). The detection rates for *Cladosporium* observed during the boarding and deplaning intervals were similar to detection rates observed inside the airport terminal.

Significant differences were observed in *Basidiospore* detection rates observed outside the airport terminal compared to inside the airport terminal (origin city airport: P-value = 0.036, destination city airport: P-value = 0.002). *Basidiospore* detection rates observed during the boarding/deplaning intervals and detection rates observed inside the airport terminal were not significantly different. Significant differences were observed in *Basidiospore* detection rates observed during the boarding interval compared to detection rates from the mid-climb and the cruise intervals (P-values < 0.05). Significant differences were not observed in detection rates of *Penicillium/Aspergillus* among measurements observed outside the airport terminal, inside the airport terminal, or inside the aircraft during boarding and deplaning.

Ratio analysis between culturable and total spore results

A ratio analysis was completed to compare culturable and total spore concentrations by flight, sampling interval and location. For a given taxon, ratios less than 1 were expected because spore traps capture both viable and non-viable spores regardless of culturability. However, in a number of cases, ratios exceeded 1. Outside airport terminals, 43% of the combined samples produced ratios greater than 1 for *Penicillium/Aspergillus* (i.e. a higher culturable concentration). The highest observed ratio was 5.29. For *Cladosporium*, 30% of the combined samples had ratios greater than 1 outside airport terminals. For *Penicillium/Aspergillus* inside the aircraft, 21% of the samples had ratios greater than 1. For *Cladosporium*, 26% of the ratios were greater than one inside the airport terminal whereas 13% were greater than one inside the aircraft. The highest ratio was 3.44, which occurred inside the aircraft in the front of the cabin.

Comparison with building assessment survey evaluation (BASE) study

Overall, the airline and BASE study datasets were similar for the culturable fungal data. The most common five genera/species with respect to percent detected for culturable samples inside the aircraft cabin and inside the buildings were identical, although the rank order differed (Table 5). Detection rates for the predominant culturable fungi inside the aircraft were significantly lower than the detection rates in the BASE study for all taxa except *Aspergillus*. The *Aspergillus* detection rates were not significantly different between the two studies.

We determined that a positive hole correction factor was not appropriate for the airline dataset; however, we recognize that the BASE study data had applied the correction factor to the culturable samples. Therefore, we applied the positive hole correction factor to the airline data in order to calculate the mean concentrations presented in Table 5. The mean concentrations for the culturable individual

Table 4. Percent detect and arithmetic mean concentration by sampling interval for predominant fungal taxa

Location	Airborne culturable fungi										Airborne total spore									
Sampling interval	N samples	*Cladosporium*			*Penicillium*			*Aspergillus*			N samples	*Cladosporium*			*Pen/Asp-like*			*Basidiospores*		
		%[a]	AM[b]	SD	%	AM	SD	%	AM	SD		%	AM	SD	%	AM	SD	%	AM	SD
Origin city																				
Outside airport	35	100	430	646	86	63	173	34	7	13	36	92	1470	2769	72	168	245	92	1628	2150
Inside airport	36	78[c]	28	34	56[c]	51	104	42	9	18	36	64[c]	39	43	61	126	231	69[c]	58	66
Boarding	64	83[c]	29	35	33[c,d]	8	22	66[c]	115	435	69	74[c]	42	42	65	94	239	78[c]	71	94
Aircraft																				
Boarding	64	83	29	35	33[f]	8	22	66	115	435	69	74	42	42	65	94	239	78	71	94
Mid-climb	72	50[e,f]	9	15	22	5	16	28[e]	5	13	72	64	31	42	49[e]	20	24	54[e]	23	30
Early cruise	72	40[e,f]	6	10	7[e,g]	3	19	13[e,f,g]	1	3	72	33[e,f,g]	10	16	40[e,f]	15	22	54[e]	23	31
Mid-cruise	72	33[e,f,g]	3	6	7[e,g]	1	2	11[e,f,g]	1	4	72	39[e,f,g]	13	19	33[e,f,g]	12	25	47[e,f]	18	27
Late cruise	72	38[e,f]	5	8	7[e,f,g]	1	2	14[e,f,g]	2	5	72	50[e,f,g]	15	17	38[e,f,g]	18	32	54[e]	19	21
Deplaning	33	79	32	30	18[e]	4	9	52	46	104	23	74	47	40	61	60	85	74	51	60
Destination city																				
Deplaning	33	79[h]	32	30	18[h,i]	4	9	52	46	104	23	74[h]	47	40	61	60	85	74	51	60
Inside airport	33	76[h]	25	27	52[h]	15	33	52	10	14	33	55[h]	49	103	45	17	26	55[h]	28	36
Outside airport	33	100	389	405	91	29	25	52	14	22	32	100	1148	2420	47	67	111	97	341	375

[a]% denotes the percent of samples in the sampling interval that detected the genus/species.
[b]AM denotes the arithmetic mean (CFU/m^3 for culturable and spores/m^3 for total spore results) for the sampling interval; SD denotes the standard deviation.
[c]In the origin city model, significantly different from outside airport terminal (p-value < 0.05).
[d]In the origin city model, significantly different from inside airport terminal (p-value < 0.05).
[e]In the aircraft model, significantly different from boarding interval (p-value < 0.05).
[f]In the aircraft model, significantly different from deplaning interval (p-value < 0.05).
[g]In the aircraft model, significantly different from mid-climb interval (p-value < 0.05).
[h]In the destination city model, significantly different from outside airport terminal (p-value < 0.05).
[i]In the destination city model, significantly different from inside airport terminal (p-value < 0.05).

Table 5. Comparison of top five genera/species from airline data and BASE study

	Genera/species observed	Airline data Rank	% detected	Mean	BASE data Rank	% detected	Mean	*P*-value[e]
Inside aircraft[a]/indoor[b]								
Culturable	*Cladosporium*	1	40	6	1	66	77	0.0001
	Aspergillus	2.5	16	2	5	26	4	0.065
	Yeasts	2.5	16	2	4	29	7	0.012
	Penicillium	4	11	2	3	52	16	<0.0001
	Sterile fungi[d]	5	7	1	2	59	14	<0.0001
Total spore	Basidiospores	1	52	21	2	26	44	<0.0001
	Cladosporium	2	47	17	1	52	226	0.40
	Penicillium/Aspergillus-like	3	40	16	4	19	16	0.0099
	Hyphal fragments	4	16	4	–[f]	0	0	<0.0001[g]
	Unknown	5	8	3	12	3	1	0.17
Outdoor[c]								
Culturable	*Cladosporium*	1	100	546	1	96	683	0.15[g]
	Penicillium	2	88	56	3	82	73	0.33
	Sterile fungi [d]	3	77	12	2	83	95	0.37
	Yeasts	4	56	12	6	31	34	0.0026
	Alternaria	5	47	11	5	50	23	0.69
Total spore	*Cladosporium*	1	96	1318	2	97	4201	0.84
	Basidiospores	2	94	1022	1	98	1159	0.25
	Ascospores	3	93	531	3	95	2367	0.71
	Penicillium/Aspergillus-like	4	60	120	5	74	502	0.17
	Ganoderma	5	51	74	–[f]	0	0	<0.0001[g]

[a]Airline data represents 288 samples from 12 flights during mid-climb, early cruise, mid-cruise and late cruise sampling intervals only.
[b]BASE study culturable data represents 311 samples from 52 buildings; BASE study total spore results represent 170 samples from 29 buildings.
[c]Airline data represents 68 outdoor samples from 24 airport terminals taken before/after 12 flights. BASE study culturable data represents 102 outdoor samples from 52 buildings; BASE study total spore results represent 56 outdoor samples from 29 buildings.
[d]BASE data category 'non-sporulating' was considered equivalent to airline data category 'sterile fungi.'
[e]p-value for airline percent detected versus BASE percent detected obtained using the two sample z-test. Standard errors were estimated using the GENMOD procedure in SAS with a repeated subject effect to account for the correlated nature of the data.
[f]– indicates taxa was not observed in BASE study and was therefore not assigned a rank.
[g]*P*-value obtained using Fisher Exact-test.

taxa were lower inside the aircraft compared to mean concentrations collected inside the BASE buildings. Outdoor culturable results from both studies were similar in composition. The outdoor yeast detection rate was significantly different between the BASE and airline data.

Differences were also observed between the two datasets for total spore concentrations. Three of the five most frequent genera/species observed for total spores were the same for the aircraft cabin and the BASE buildings, although the rank order differed (Table 5). Mean concentrations for *Penicillium/Aspergillus-like* were equivalent for the two studies. Mean concentrations for Basidiospores and *Cladosporium* were higher in the BASE study compared to the mean concentrations observed in-flight samples for the aircraft data. Detection rates for Basidiospores and *Penicillium/Aspergillus-like* were significantly different between the studies, as the aircraft cabin observed higher detection rates.

Surface samples

The genera recovered from the surface wipe samples are presented in Table 3. A majority of the surface swab samples (62%) had no detectable fungal recoveries. Only eight fungal genera were observed in the 17 samples in which fungal taxa were observed. Three of these genera were observed on both surfaces inside the airport terminal and inside the aircraft. For example, *Rhodotorula glutinis*, a common yeast contaminant, was observed in 17% of the surface samples inside the aircraft, and 5% of the surface samples inside the airport terminal.

DISCUSSION

The qualitative analysis indicates that, for the most part, a few dominant genera are present outside the airport terminal, inside the airport terminal and inside the aircraft. Given the ubiquitous nature of the

genera *Cladosporium*, *Aspergillus* and *Penicillium*, the prevalence of these genera was expected, as were the large categories of *Ascospores* and Basidiospores. It was noteworthy that the maximum concentration of *Penicillium* outside the airport terminal was more than two times higher than the maximum concentration of *Penicillium* collected during the boarding/deplaning process and cruise intervals, even though *Penicillium* is frequently associated with elevated indoor fungal concentrations.

The genus and species analysis suggested that fungi are entrained into the aircraft cabin directly from outside air and inside the terminal during both the boarding and deplaning processes. Isolated fungal peak concentrations (i.e. when a genus concentration spiked inside the cabin) occasionally occurred during cruise intervals. The exact source of the sudden spikes is unclear, but passenger activity may either resuspend settled fungi or release additional spores from clothing.

The qualitative analysis indicates that genera, such as *Cladosporium* and *Penicillium* are present both inside and outside the airport terminal. Therefore, while the aircraft is attached to the airport terminal handling system or portable air units, it is likely that some fungi from both inside and outside the terminal are migrating onto the aircraft. A third probable source for the fungi recovered in aircraft is likely shedding from passengers' clothing and carry-ons. The presence and patterns of *Aspergillus* (both as a combined variable and as separate species) strongly suggest passenger activity as the potential source rather than outside air on select flights. Shedding from clothing is exaggerated during activity and re-aerosolization, which could explain the rising fungal concentrations during the boarding and deplaning process.

Basidiospores were observed repeatedly and were dominant during cruise sampling intervals. Typically, Basidiospores are released primarily from outdoor sources, and are unlikely to be fungi growing in the aircraft environment. Other possible reasons for their prevalence in the cabin environment are that they were brought in through ventilation systems or carried in on passenger clothing.

The spore trap sampling provided insight into the total spore concentrations (both culturable and non-culturable). For example, *Ascospores* and Basidiospores often fail to germinate in culture, or fail to produce recognizable spores. There were situations where the ratio of culturable results to spore results was greater than 1. These occurrences may be related to situations of higher frequencies of small single spores a proportion of which, due to the differences in efficiencies of the samplers, may slip past the spore sampler but could be captured by the N-6 impactor, which has a lower cut point. Another explanation could be the varying limits of detections between the two analytical techniques.

After evaluating our results in terms of the BASE study, it appears that the aircraft cabins we sampled were very similar in terms of the kinds of fungi present in typical office buildings for both culturable and total fungi. Our culturable and total spore mean concentrations were lower, which indicates that the aircraft cabins contained lower concentrations than private and public office buildings.

In general, our results confirm previously published fungal studies on aircraft as outlined in the NAS report (National Research Council Committee on Air Quality in Passenger Cabins of Commercial Passenger Aircraft, 2002). However, our research presents its conclusions with the largest sample size to date and provides a comprehensive analysis using the fungal taxa. When evaluating the aircraft cabin fungal research in its summation, low fungal concentrations have been observed repeatedly. Researchers may wish to focus future efforts on other aircraft aspects, such as exposures during ground-based operations.

A natural progression of our work is to consider how our results may contribute to the disease transmission paradigm on aircraft. Our research demonstrates that on our sampled flights the B-767 filtration system operated efficiently to remove fungal spores when two air cooling packs and 50% recirculation rate were utilized during operations. While these results only relate to fungal-sized particles, it illustrates the time periods when the risk of potential airborne exposure will be higher. Our work would suggest that researchers focus disease prevention efforts on the exposure time period before the air is entrained into the filtration system. We believe controlling the microclimates (i.e. the airflow surrounding passengers sitting within immediate proximity to each other) is crucial to minimize disease transmission on aircraft.

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. For example, we could not determine whether the *Cladosporium* in the outdoor air was the same species as inside the aircraft cabin. We also recognize that bacterial and viral exposures on aircraft are also of considerable importance, and this research is currently underway. Another approach might be to use a panel of PCR probes for common outdoor fungi to track their entry into the aircraft environment.

Since our data came from flights during the summer months, our results may not be generalizable to other seasons when 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.

CONCLUSIONS

Culturable fungal populations observed inside the aircraft are comprised of similar genera, detected significantly less frequently and have lower mean concentrations than those observed in typical office buildings. Thus, airline cabins are actually quite clean in terms of fungi. Our data demonstrate the importance of passenger activity as the source of the fungi observed on aircraft. Isolated fungal peak events occur occasionally when concentrations of a particular genus or species rise sharply inside the cabin for a limited period. Overall, our research demonstrates that on the sampled flights the B-767 filtration system operated efficiently to remove fungal spores when two air cooling packs and 50% recirculation rate were utilized during operations.

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