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Original Article

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Prevalence, persistence, and phenotypic variation of *Aspergillus fumigatus* in the outdoor environment in Manchester, UK, over a 2-year period

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Abstract

Aspergillus fumigatus, an opportunistic fungal pathogen that causes invasive aspergillosis in immunosuppressed patients, is considered to be the world's most dangerous mould. It is widely distributed in the environment, and airborne asexual conidia serve as the main mode of transport for pulmonary lung infection. It is important to monitor seasonal airborne conidia levels when assessing the risk of acquiring this infection. In this study, air was sampled for total viable fungal spores and viable *A. fumigatus* conidia monthly over a 2-year period (2009 and 2010) close to Manchester, UK, city center. Total viable airborne fungal counts varied seasonally, peaking in the summer and autumn for both years and reaching levels of approximately 1100–1400 colony-forming units (CFU)/m^3; counts were strongly positively correlated to mean temperature ($R^2 = 0.697$). By contrast, *A. fumigatus* viable airborne counts were not seasonally associated; persistent low levels were between 3 and 20 CFU/m^3 and were not correlated with mean temperature ($R^2 = 0.018$). A total of 220 isolates of *A. fumigatus* were recovered on potato dextrose agar (PDA) at 45°C, and internal transcribed spacer sequencing and restriction digestion of a partial polymerase chain reaction amplicon of the β-tubulin gene (*benA*) of 34 randomly selected isolates were used to confirm the isolates as *A. fumigatus*. When the colony radial growth rates (Kr) were determined, the highest rates were observed on PDA, followed by Vogel's medium supplemented with phosphatidylcholine and Vogel's medium alone. Clinical isolates had a significantly higher mean colony Kr on PDA compared with environmental isolates.

Key words: *Aspergillus fumigatus*, airborne spore counts, seasonal variation.

Introduction

Aspergillus fumigatus is an opportunistic pathogen that is widely distributed in the environment. It is associated with decomposing plant material and ubiquitous worldwide [1–3]. Due to the small size of its asexual conidia, spores are readily transmissible in the air and have a low settling rate compared with other *Aspergillus* species [4]. Air is considered to be the primary medium for the transport of conidia [2]. These conidia are involved in pulmonary infections in

immunocompromised individuals and lead to a spectrum of diseases that range from pulmonary to systemic infection [5]. However, *A. fumigatus* is not the predominant fungal species in the airborne mycoflora [6–11].

Total airborne fungal counts are generally much higher in the summer and autumn months and are influenced by temperature and humidity. *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* spp. are usually the predominant fungal species in the airborne mycoflora, with peak concentrations typically ranging from 10^2 to 10^4 colony-forming units (CFU)/m^3 [6,12–14]. Few studies have specifically measured *A. fumigatus* airborne spore counts, and those that have give conflicting reports as to seasonal effects on spore concentration. Some studies have reported an increase in the autumn and winter months, possibly due to an abundance of decaying plant material, the result of autumn leaf fall, whereas other studies reported a lack of seasonal variation in airborne conidial numbers [2,15,16]. With the exception of locations that are close to composting facilities, where concentrations of approximately 1×10^5 CFU/m^3 have been reported [17,18], or locations close to building demolition work, which is associated with elevated counts [19–21], airborne counts have ranged from 0 to 70 CFU/m^3 [2]. In indoor environments, fungal spore concentrations are generally similar or lower than those observed outside. These fungal spores are associated with dust particles [22] and with potential reservoirs such as air-conditioning systems, carpets, and pets [23–27]. Monitoring and controlling airborne *A. fumigatus* are critical in hospital environments where the presence of *A. fumigatus* has been linked to nosocomial aspergillosis outbreaks [28–31]. Previous studies that monitored airborne *A. fumigatus* counts relied heavily on morphological features for presumptive identification; few studies used rDNA for verification. *Aspergillus fumigatus* belongs to the section *Fumigati*, which was recently investigated and found to contain 28 species [32], with several highly related strains being difficult to separate based on morphological parameters alone [33–38]. Therefore, it is possible that other related species may form a significant proportion of the population previously thought to have been *A. fumigatus*.

The aim of this study was to monitor the total airborne viable fungal count and the airborne putative *A. fumigatus* count over a 2-year period at an inner-city site in Manchester, UK, in order to increase our understanding of the variation in monthly levels of airborne *A. fumigatus* conidia. In addition, after presumptive identification of *A. fumigatus* strains by morphological examination, randomly selected strains were further characterized based on internal transcribed spacer (ITS) sequencing and restriction digestion analysis of a polymerase chain reaction (PCR) amplicon of the β-tubulin gene *benA* in order to verify the identity of the isolates, as morphotyping alone can lead to misidentification [37]. In addition, phenotypic variability within the population was also determined by measuring the colony radial growth rate (Kr) of isolates on three media.

Materials and methods

Dublin environmental and clinical strains of *A. fumigatus*

Clinical isolates were obtained from the *A. fumigatus* culture collection at Wythenshaw Hospital, Manchester, UK. Dublin environmental isolates were kindly supplied by H. Fuller, University of Dublin, and were isolated on a single day from a single site at the Belfield campus, University College Dublin, Ireland.

Determination of total fungal-viable and *A. fumigatus*–viable airborne spore concentrations in Manchester

Total airborne viable fungal counts were determined at monthly intervals over a 2-year period (1 March 2009 through 28 February 2011) outside the Michael Smith Building on the University of Manchester campus (GPS +53°27′53.7834″, −2°13′41.5884″) using a Super 10/180-SAS Isolator DuoSAS 360 (Cherwell Laboratories, UK). The lid was sterilized by autoclaving or wiping with 70% (v/v) industrial methylated spirits, and the air was sprayed with 70% (v/v) industrial methylated spirits while airflow ran for 30 s prior to air sampling. Air was aspirated through a 219-hole sieve head plate onto a potato dextrose agar (PDA; Formedium, UK) plate (90 mm diameter) supplemented with 0.05% (w/v) chloramphenicol (Sigma-Aldrich, UK) to suppress bacterial growth. The samples were collected at approximately 1.5 m above the ground between 10:00 a.m. and 12:00 p.m. when it wasn't raining. A total volume of 500 L of air was sampled in standard mode, six times in succession. Three plates were incubated at 45°C and three at 25°C and inspected for growth after 3 days; the number of colonies visible to the naked eye was counted and numbers corrected using the manufacturer's positive-hole conversion (Cherwell Laboratories, Bicester, UK). The total concentration of airborne-viable fungal spores per cubic meter of air was determined from the total fungal counts at 25°C. The total concentration of airborne-viable putative *A. fumigatus* spores was calculated from the counts at 45°C. Putative *A. fumigatus* colonies were subcultured using a sterile toothpick placed onto PDA in tissue culture flasks and universal tubes. Only one main morphological type was recovered during the 2-year sampling period at 45°C and provisionally identified as *A. fumigatus* by

macroscopic and microscopic characteristics based on the redefinition of *Aspergillus* section *Fumigati* as proposed by Hong et al. [38]. In total, 220 putative *A. fumigatus* isolates were collected over the 2-year period. Randomly selected isolates were further characterized by ITS rDNA sequencing as previously described [39] and by PCR-restriction fragment length polymorphism (RFLP) of a β-tubulin (*benA*) amplicon [36].

Cultivation of A. fumigatus isolates

Aspergillus fumigatus isolates were grown in 500-ml vented tissue culture flasks that contained 50 ml of PDA. The media in the flasks were streaked with spores and incubated at 37°C for a minimum of 7 days or until confluent growth was obtained. Spore suspensions were prepared by adding 20 ml of sterile 0.05% (v/v) Tween 20 (Sigma-Aldrich, Dorset, UK), tightening the lid, and shaking vigorously to dislodge the spores. The spore suspensions were separated from mycelial fragments and conidiophores by filtering through a double layer of sterile lens tissue (Whatman, UK). Spore suspensions were mixed with an equal volume of sterile glycerol and stored at −80°C until needed.

Colony Kr determination

Colony Kr measurements were performed as described by Robson et al. [40]. Petri dishes containing 20 ml of PDA or modified Vogel's medium [41] containing 1% (w/v) glucose instead of sucrose and ammonium nitrate (replacing ammonium sulfate in the presence or absence 0.2% (w/v) soy bean lecithin) were centrally inoculated by dipping the tip of a sterile cocktail stick into the spore suspension and gently piercing the center of the plate. Plates were incubated at 37°C and readings taken twice a day by measuring the diameter of each colony in two perpendicular directions. The colony Kr was determined by plotting colony radius against time and calculating the slope of the regression line.

ITS rDNA sequencing and PCR-RFLP

Fungal genomic DNA was extracted from mycelia grown for 16–18 h on a rotary shaker (New Brunswick, UK) at 250 rpm in modified Vogel's medium containing 1% (w/v) glucose in liquid culture at 37°C. Mycelia were harvested by vacuum filtration through Whatman 54 filter paper, washed with distilled water, transferred to a 15-ml polypropylene tube, and flash frozen in liquid nitrogen. Mycelia were either immediately used for DNA extraction or stored at −80°C until needed. Frozen mycelia were either ground in liquid nitrogen to a powder using a mortar and pestle precooled to −20°C or freeze-dried by lyophilization for 2 days. Genomic DNA was extracted using the DNeasy Plant minikit (Qiagen, UK) according to the manufacturer's instructions.

ITS rDNA of *A. fumigatus* was amplified as previously described [39] using the primers ITS-1 (5′ TCCGTAGGTGAACCTGCGG) and ITS-4 (5′ TCCTCCGCTTATTGATATGC). PCR was performed using 50 µl of reaction mixture that contained approximately 100 ng of genomic DNA. The final concentration of components in the PCR reaction was 1× PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, pH 8.8, and 0.1% stabilizer), 1.5 mM $MgCl_2$, 0.02 mM of ITS-1 and ITS-4 primers (Eurofins, UK), 0.2 mM of dNTPs (Bioline, UK), and 0.5 U of *Taq* DNA polymerase (Bioline, UK). The PCR was carried out under the following conditions: denaturation at 95°C for 1 min, followed by annealing at 55°C for 1 min for 40 cycles, and finally extension at 72°C for 1 min. PCR products were purified using a Qiaquick PCR purification column (Qiagen, UK) according to the manufacturer's instructions and sequenced in house.

The ITS region is known to be inadequate for discriminating between closely related species within the *Aspergillus* section *Fumigati* such as *A. fumigatus*, *A. lentulus*, and *Neosartorya udagawae*. However, sufficient variation in the intragenic regions between these species has been shown in the β-tubulin encoding gene *benA* to enable the discrimination of these closely related species [36]; this was used to further verify their identity. PCR was performed using 50 µl of reaction mixture containing approximately 100 ng of genomic DNA. The final concentration of components in the PCR reaction was 1× PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, pH 8.8, and 0.1% stabilizer), 1.5 mM $MgCl_2$, 0.2 pM of each primer (βtubI and βtubII [Eurofins, UK]), 0.2 mM of dNTPs, and 0.5 U of *Taq* DNA polymerase. PCR was carried out under the following conditions: initial denaturation at 94°C for 2 min followed by three cycles at 94°C for 30 s, at 55°C for 30 s, and at 72°C for 45 s. The reactions were terminated with a final incubation at 72°C for 5 min. Following the last cycle, 10 µl of the *benA* PCR amplicon was added to 1 × New England buffer 1 (New England Biolabs, UK),100 µg/ml bovine serum albumin (New England Biolabs), and 1.0 unit of BccI enzyme (New England Biolabs) and incubated at 37°C for 1 h. Banding patterns were examined after resolving on a 1% (w/v) agarose gel and staining with ethidium bromide.

Results

Monthly variation in total viable and *A. fumigatus* airborne spores during 2009–2010

Air samples were taken at monthly intervals in the city of Manchester, next to the Michael Smith Building, University of Manchester, over a 2-year period from 1 March 2009 through 28 February 2011. The total viable airborne fungal

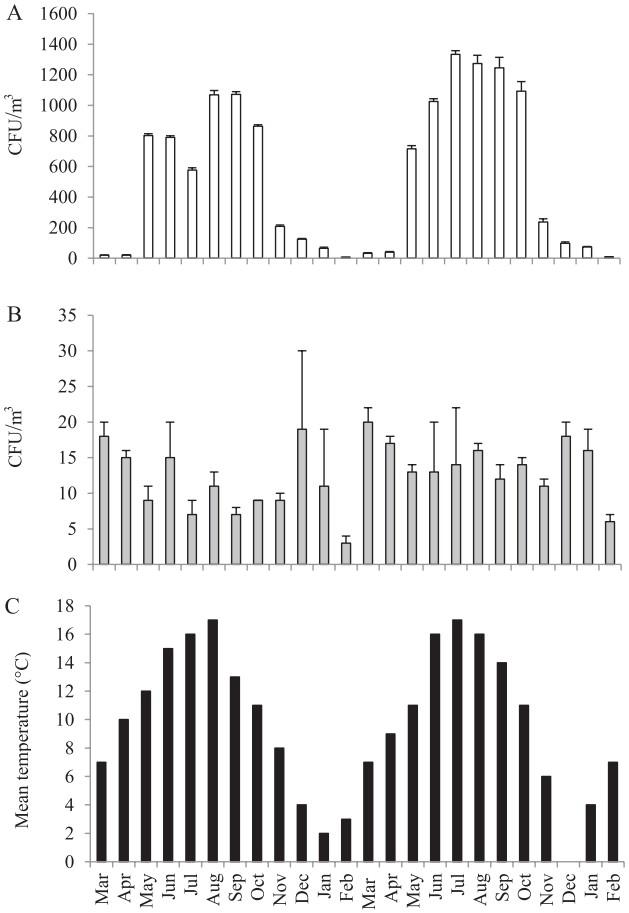

Figure 1. Monthly variation in (A) total viable airborne fungal count (colony-forming units [CFU]/m^3) determined on potato dextrose agar (PDA) media at 25°C, (B) viable *Aspergillus fumigatus* airborne count (CFU/m^3) determined on PDA at 45°C, and (C) mean temperature. Air samples were collected near the Michael Smith Building, University of Manchester, UK, between 2009 and 2011. Results represent the means of three replicates ± standard error of the mean. Monthly mean temperatures for Manchester (Manchester airport, UK) were obtained from the National Climate Data Centre, UK.

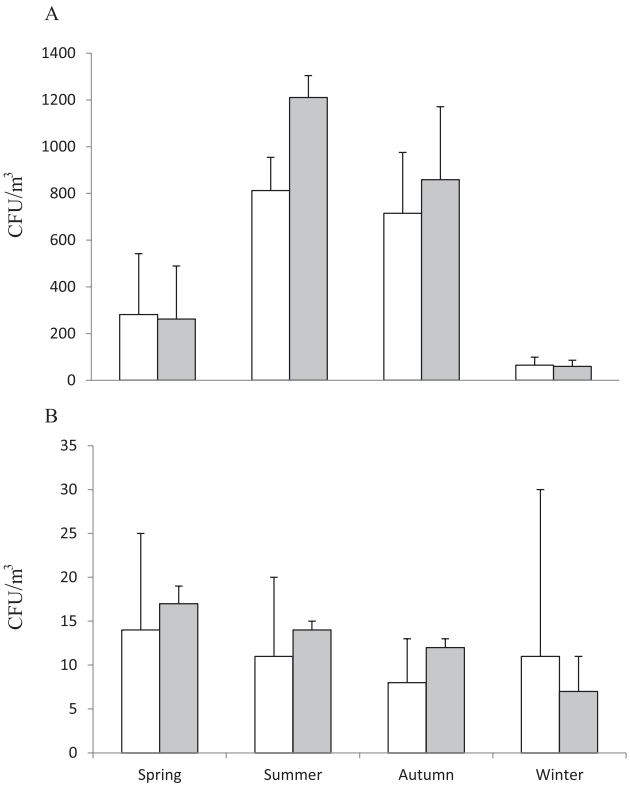

Figure 2. Seasonal variation in (A) total viable airborne fungal count (colony-forming units [CFU]/m^3) determined on potato dextrose agar (PDA) media at 25°C and (B) putative *Aspergillus fumigatus* airborne count (CFU/m^3) determined on PDA at 45°C. Air samples were collected near the Michael Smith Building, University of Manchester, UK, between 2009 and 2010 (□) and between 2010 and 2011(■). Results represent the means ± standard error of the mean.

counts ranged from 6 to 1333 CFU/m^3 over the sampling period. Total viable airborne spores showed a strong seasonal variation in both years, with the highest total counts found during August 2009 and September 2009 and July 2010 and August 2010. In both years, counts fell sharply in November and remained low during the colder winter period before increasing rapidly in May (Fig. 1a). The mean total airborne-viable spore counts were significantly higher in the summer (June through August) and autumn (September through November) compared with spring (March through May) and winter (December through February; $P < 0.05$; Fig. 2a). With the exception of summer, there was no significant difference ($P < 0.05$) between comparable seasons over the 2 years (Fig. 2a). When the mean monthly temperature and mean total viable airborne fungal counts were examined for the 2-year period, a significant ($P < 0.05$) positive correlation ($R^2 = 0.697$) was found such that higher total airborne fungal counts were associated with higher temperatures (Fig. 3).

By contrast, the putative airborne *A. fumigatus* counts fluctuated between 2 and 20 CFU/m^3 throughout the study period (Fig. 1b), and there was no significant ($P > 0.05$) seasonal variation (Fig. 2b) and no significant correlation ($R^2 = 0.018$) with temperature (data not shown). Consequently, airborne *A. fumigatus* spores as a percentage of the total airborne fungal spores varied from 80%–95% in the winter to <1% in the summer and autumn in both years (Fig. 1a, 1b).

Aspergillus fumigatus identification

A total of 220 putative *A. fumigatus* strains were isolated over the 2-year study period and were initially identified as *A. fumigatus* based on morphological characteristics and growth at 45°C. Thirty-four Manchester and Dublin environmental strains were randomly selected and subjected to ITS sequencing. Most isolates showed no differences in their

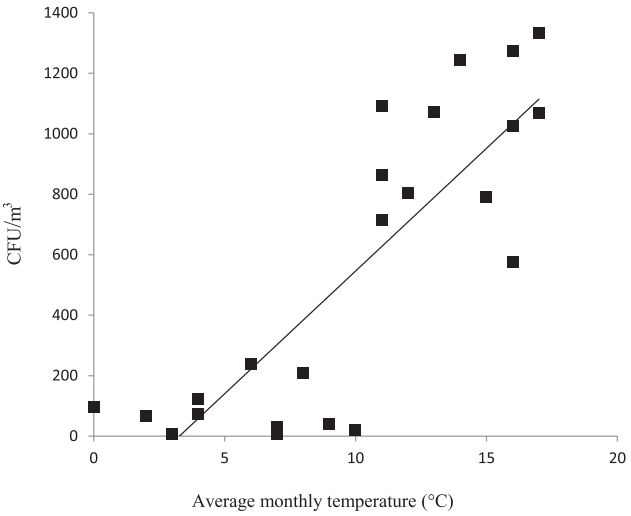

Figure 3. Correlation between total viable airborne fungal count and average monthly temperature. Air samples were collected near the Michael Smith Building, University of Manchester, UK, between 2009 and 2011. Monthly mean temperatures for Manchester (Manchester airport, UK) were obtained from the National Climate Data Centre, UK.

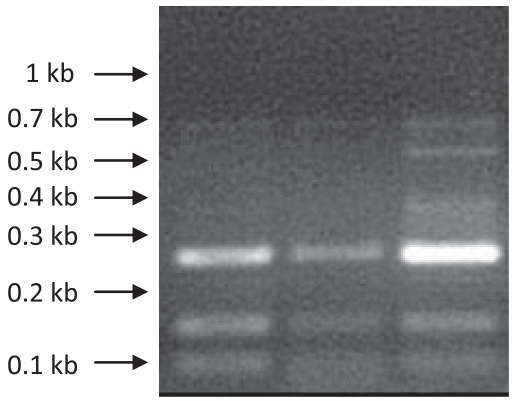

Figure 4. Representative banding pattern from three *Aspergillus fumigatus* isolates after restriction digestion of the 0.6 kb benA amplicon with the restriction enzyme BccI. Three fragments generated were ca. 0.25 kb, 0.15 kb, and 0.1 kb. Left hand arrows represent molecular weight marker bands using Hyperladder IV (Bioline, UK).

sequence; however, seven had between one and eight single-nucleotide polymorphisms (results not shown). However, *taq* polymerase, not proofreading polymerase, was used, and the difference may be due to the infidelity of the polymerase. Nonetheless, sequences were congruent with *A. fumigatus*. Recently, it has been shown that ITS sequencing alone is insufficient to discriminate between the closely related species *A. fumigatus*, *A. lentulus*, and *N. udagawae* within the section *Fumigati* and that these strains could be discriminated by comparing variable regions within the *benA* gene encoding β-tubulin by restriction digestion PCR amplification [36]. PCR *benA* amplicons of all 34 strains gave a single product of the predicted size that, after digestion with BccI, resolved as three distinct bands of 0.25, 0.15, and 0.1 kb (Fig. 4). The number and size of these fragments were in agreement with those published for *A. fumigatus* [36].

Colony Kr of environmental and clinical isolates

The relatively few studies that have compared the phenotypic variation in populations of *A. fumigatus* indicated, with small datasets, minor variations in growth rate, levels of pigmentation, and isoenzyme patterns [42–44]. Colony Kr is a convenient phenotypic parameter that is dependent on the specific growth rate, branching frequency, and peripheral growth zone width of the developing mycelium; changes in any of these are reflected in differences in the colony Kr [45]. The colony Kr of all isolates was determined on PDA and, for a subset of isolates, on Vogel's modified medium containing glucose or glucose supplemented with phosphatidylcholine (PC). Previously, we observed that PC stimulates the colony Kr of *A. fumigatus* due to a reduction in branching and an increase in the hyphal extension rate without affecting specific growth rate (Robson, unpublished observation). The mean colony Kr and the lowest and highest colony Kr for the Manchester environmental isolates, clinical isolates, and Dublin environmental isolates are summarized in Table 1. In all cases, the mean colony Kr of each group was significantly ($P < 0.05$) higher on PDA and Vogel's medium containing PC compared with the rate on Vogel's medium alone. The colony Kr of Manchester environmental isolates on PDA ranged from 426 to 550 μm/h. The Dublin environmental isolates ranged from about 452 to 496 μm/h, while the clinical isolates all had colony Kr in excess of 489 and up to 565 μm/h. A greater range of colony Kr was observed on both Vogel's medium alone and on Vogel's medium supplemented with PC. For the Manchester environmental isolates, the colony Kr ranged from approximately 235 to 397 μm/h on Vogel's medium alone and from 404 to 550 μm/h on Vogel's medium supplemented with PC. The colony Kr of the Dublin environmental isolates ranged from around 299 to 382 μm/h on Vogel's medium alone to roughly 428 to 454 μm/h on Vogel's medium supplemented with PC. The colony Kr of the clinical isolates ranged from around 260 to 397 μm/h on Vogel's medium alone to approximately 378 to 500 μm/h on Vogel's medium supplemented with PC. The data indicate that there is considerable variation in the colony Kr in the *A. fumigatus* population. When the mean colony Kr on each medium for the Manchester environmental, Dublin environmental, and clinical isolates were compared, there was no significant difference between the groups, with the exception of PDA where the mean colony Kr of the clinical isolates was significantly ($P < 0.05$) higher (515 μm/h

Table 1. Summary of the mean colony radial growth rate and lowest and highest colony growth rate for Manchester environmental, Dublin environmental, and clinical strains of *Aspergillus fumigatus* on potato dextrose agar and Vogel's medium in the presence and absence of phosphatidylcholine at 37°C. The number of isolates recorded is shown in brackets. Means are shown ± SEM.

	Colony radial growth rate (μm/h^1)					
	Potato dextrose agar		Vogel's medium		Vogel's medium + phosphatidylcholine	
Isolate group	Mean	Range	Mean	Range	Mean	Range
Manchester environmental isolates	465 ± 3 (70)	426–550	346 ± 9 (25)	235–397	467 ± 8 (25)	404–550
Dublin environmental isolates	481 ± 4 (10)	452–496	339 ± 7 (10)	299–382	442 ± 3 (10)	428–454
Clinical isolates	515 ± 12[a] (25)	489–565	354 ± 8 (25)	260–397	462 ± 5 (25)	378–500

The number of isolates recorded is shown in brackets. Means are shown ± standard error of the mean. [a]significantly different ($P < 0.05$).

compared with 465 and 481 μm/h for the Manchester and Dublin environmental strains, respectively; Table 1).

Discussion

This study provides information on the concentration of viable *A. fumigatus* airborne spores in the outdoor environment in Manchester, UK, compared with the total viable airborne fungal spores over a 2-year period. Determining the airborne spore level over a prolonged period of time is important since it would reflect the potential risk of pulmonary infections in immunocompromised individuals [2,5]. However, it should be noted that in this study sampling was restricted to 1 day per month and variation in numbers between monthly readings was not measured. Despite the importance of *A. fumigatus* as an opportunistic pathogen and its ubiquitous occurrence in the environment, there have been relatively few studies on the annual levels of airborne *A. fumigatus* spores in outdoor environments [2]. More studies are needed to gain a comprehensive view of their frequency and seasonal variation in outdoor environments.

In this study, we recovered 220 *A. fumigatus* isolates that were putatively identified on the basis of their morphological characteristics and growth at 45°C. Sequencing of the ITS1 and ITS4 regions of the rDNA of a number of randomly selected isolates indicated that they belonged to section *Fumigati*. Recently, it has become apparent that a number of isolates phenotypically highly similar to *A. fumigatus* are, in fact, closely related species within *Fumigati* and that ITS sequencing alone is insufficient to differentiate them [37]. However, it has been shown that there is interspecies variability at intronic regions in the sequence of the gene encoding β-tubulin (*benA*) sufficient to distinguish three of the major species within the section *Fumigati*, namely, *A. fumigatus*, *A. lentulus*, and *N. udagawae* [36]. Using a PCR-restriction fragment polymorphism of the *benA* amplicon of the strains subjected to ITS sequencing, all were confirmed to be *A. fumigatus* (Fig. 4). Differentiating *A. fumigatus* from closely related species in the section *Fumigati* is clinically important as the related species have been shown to exhibit variable levels of azole resistance [33]. In addition, it also confirms that at least the majority of airborne spores thought to be *A. fumigatus* based on morphology in this study were, in fact, *A. fumigatus*.

The annual total airborne fungal count in Manchester varied widely from >10 to 1333 CFU/m^3 and was strongly associated with the seasons, with higher concentrations recovered in the warmer summer and autumn and lower concentrations in the cooler winter and spring over the 2-year study (Fig. 2a.). Such peaks in the total airborne fungal counts over the summer and autumn have been observed in temperate countries where warmer summer weather and autumnal leaf fall are thought to play important roles in increasing fungal growth, sporulation, and subsequent airborne spore numbers [6,9,12,46–50]. In our study, this seasonal variation in total counts was also strongly positively associated with the average monthly temperature (Fig. 3) but not with humidity or rainfall (data not shown). While some researchers have found a correlation with other meteorological factors, including humidity, rainfall, and wind speed, others have found little or no such associations [10–12,50]. Also, many have determined that various genera, such as *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*, are largely responsible for these seasonal fluctuations in airborne spore levels, with the dominant genera varying depending on geographical location [6–14,46–50]; however, few studies have focused on specific species and, in particular, on *A. fumigatus* [2]. The majority of earlier studies report a small rise in airborne *A. fumigatus* counts during the winter. In a study of the outdoor air in Cambridge, UK [51], elevated *A. fumigatus* airborne counts were reported during the winter (approximately 6–14 CFU/m^3); the highest levels of airborne *A. fumigatus* spores were also

reported to be present in the winter in a study in Cardiff, UK [52]. A later study in Cardiff that compared airborne spore counts for *A. fumigatus* with those obtained in St. Louis, Missouri, USA, also confirmed higher counts in the winter [53]. A study in Spain, which compared airborne *A. fumigatus* counts from several locations throughout the province of Madrid, including both urban and rural sites, found peak counts in the autumn [16]. By contrast, a study in Michigan, USA, indicated a weak seasonal trend that peaked slightly in summer [54], while a study at four sites in Dublin, Ireland, showed no seasonal trend [6]. In the present study, no seasonal variation was observed in the airborne counts of *A. fumigatus* over the 2-year period, with numbers falling within the range of 3 to 20 CFU/m^3 (Fig. 1b, 2b.). The persistence of low levels of *A. fumigatus* throughout the year is consistent with many other studies in which *A. fumigatus* was monitored specifically. In an earlier study in Cambridge, UK, levels of 0.13–13 conidia/m^3 were reported, while in a year-long study in Cardiff, UK, and St. Louis, Missouri, USA, average spore concentrations were found to be 11.3 and 13.5 CFU/m^3, respectively [51,53]. In another study in Michigan, USA, mean *A. fumigatus* airborne counts were 6.25 CFU/m^3 [54], while Shelton et al. [7] reported a median *A. fumigatus* count of 20 CFU/m^3, and a study in Austria recorded a mean count of 20.3 CFU/m^3 [55]. In a study at sites throughout Dublin, Ireland, normal counts were found to be <10 CFU/m^3, with occasional counts of up to 400 CFU/m^3, which was attributed to occasional capture of conidiophores [6]. The only exceptions to these low spore levels were those found in investigations conducted close to composting facilities, where airborne counts as high as 104 CFU/m^3 have been reported. Note that *A. fumigatus* is a thermophilic mould and high levels of growth are associated with the composting process due to the high temperatures generated by this process [3,17,18]. In urban areas, the lack of seasonality and persistence throughout the year independent of temperature may suggest that the source of the spores is buildings and ventilation systems rather than decomposing vegetation and therefore not affected by seasonal fluctuations in temperature. Many studies have found a causative link between local building renovation and demolition work and an increase in the number of airborne *A. fumigatus* fungal spores [19–21]. The present study falls broadly in line with the majority of previous investigations that suggest *A. fumigatus* is present at low but persistent levels in the outdoor environment despite differences in geographical location, sampling methods, and culture media.

To examine variation in phenotypic characteristics within the Manchester environmental isolates and between these isolates and environmental isolates from Dublin and clinical isolates, colony Kr were assessed on three media (Table 1). This is a convenient phenotypic parameter that is dependent on the specific growth rate, branching frequency, and peripheral growth zone width of the developing mycelium; changes in any of these are reflected in differences in the colony Kr [45]. In addition, several studies have suggested a positive correlation between growth rate and pathogenicity [56,57]. Colony Kr of all isolates showed a wide variation on all media; however, the growth of every isolate was consistently and significantly ($P < 0.05$) higher on PDA and on Vogel's medium supplemented with PC. When the mean colony Kr of Manchester environmental, Dublin environmental, and clinical environmental isolates were compared, there was no significant difference when the isolate was grown on Vogel's medium or on Vogel's medium supplemented with PC. However, the group mean colony Kr of the clinical isolates on PDA media was significantly higher than the group means for the Manchester and Dublin environmental isolates. Overall, the range in colony Kr of the clinical strains was higher than the environmental strains. However, the highest colony Kr in the environmental strains overlapped the lower end of the clinical strains (Table 1). The environmental *A. fumigatus* population is known to be genetically highly diverse [58], and the higher colony Kr found in clinical isolates may therefore reflect selection of the environment of strains with the highest growth rate, which may aid in establishment of infection and evasion of macrophage engulfment [59]. The effect of PC is potentially highly significant as the surfactant coating the lung surface is made up of about 90% phospholipid of which PC is the most abundant [60,61]. Apparently, this increase in the hyphal extension rate is not due to a change in the specific growth rate (Robson, unpublished observation) but rather to a change in morphology, with higher hyphal extension rates being achieved with a reduction in branching frequency. In the lung of immunocompetent hosts, conidia are rapidly removed by phagocytosis before they achieve a minimum germ tube length to escape this immune response [5,59]. Hence, a faster extension rate in response to PC may be an adaptive response that enables germinating conidia to achieve a germ tube length of sufficient size to evade phagocytosis more quickly. A morphologically similar response has been reported for the cereal plant pathogen *Fusarium graminearum* and the related nonpathogenic mycoprotein fungus *F. venenatum* in response to choline and the related compound betaine [40,62]. Both choline and betaine levels peak during anther emergence in wheat, which is the primary infection site of *F. graminearum* and has been postulated to aid in the infection process by stimulating rapid extension across the host [63–65]. Moreover, mutants in impaired choline transport in *F. venenatum* were no longer responsive to choline, suggesting uptake was essential and an internal site of action [66]; a similar loss of response to PC has been shown

in choline transport mutants in *A. fumigatus* (Robson, unpublished observation). This strongly suggests that choline or phosphorylcholine liberated from PC as the result of extracellular phospholipase activity [67,68] is likely to be the active compound that elicits the observed morphological effect. The morphological response of *A. fumigatus* to PC, correlation between pathogenicity, and the extent of the morphological effect and its possible role in lung infection all warrant further investigation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper. This work was supported by a scholarship from the Saudi Arabian ministry.

References

1. Pringle A, Baker DM, Platt JL et al. Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. *Evolution* 2005; **59**: 1886–1899.
2. O´Gorman CM. Airborne *Aspergillus fumigatus* conidia: a risk factor for aspergillosis. *Fungal Biol Rev* 2011; **25**: 151–157.
3. Tekaia F, Latgé JP. *Aspergillus fumigatus*: saprophyte or pathogen? *Curr Opin Microbiol* 2005; **8**: 385–392.
4. Gregory PH, *The Microbiology of the Atmosphere*, 2nd edn, London: Leonard Hill, 1973.
5. Latgé JP. The pathobiology of *Aspergillus fumigatus*. *Trends Microbiol* 2001; **9**: 382–389.
6. O'Gorman CM, Fuller HT. Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. *Atmos Environ* 2008; **42**: 4355–4368.
7. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Environ Microbiol* 2002; **68**: 1743–1753.
8. Frey D, Durie EB. The incidence of airborne fungus in Sydney. *Mycopath Mycol Appl* 1960; **13**: 93–99.
9. Gonçalves FL, Bauer H, Cardoso MR et al. Indoor and outdoor atmospheric fungal spores in the São Paulo metropolitan area (Brazil): species and numeric concetration. *Int J Biometeorol* 2010; **54**: 347–355.
10. Grinn-Gofroń A. Airborne *Aspergillus* and *Penicillium* in the atmosphere of Szczecin (Poland) (2004–2009). *Aerobiologia* 2011; **27**: 67–76.
11. Kasprzyk I. Aeromycology—Main research fields of interest during the last 25 years. *Ann Agr Env Med* 2008; **15**: 1–7.
12. Beaumont F, Kauffman HF, Vandermar THEA. Volumetric aerobiological survey of conidial fungi in the northeast Netherlands. 1. Seasonal patterns and the influence of metrological variables. *Allergy* 1985; **40**: 173–180.
13. Oliveira M, Ribeiro H, Delgado JL, Abreu I. The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level. *Int J Biometeorol* 2009; **53**: 61–73.
14. Sabariego S, Bouso V, Pérez-Badia R. Comparative study of airborne *Alternaria* conidia levels in two cities in Castilla-La Mancha (central Spain), and correlations with weather-related variable. *Ann Agric Environ Med* 2012; **19**: 227–232.
15. Guinea J, Pelaez T, Alcala L, Bouza E. Outdoor environmental levels of *Aspergillus spp*. conidia over a wide geographical area. *Med Mycol* 2006; **44**: 349–356.
16. García-Pantaleón FI, Romero JA, Vilches ED et al. Presence of *Aspergillus* Mich. Ex Fr. in several different biotopes in Córdoba (Spain). *Aerobiologia* 1995; **11**: 137–143.
17. Deacon LJ, Pankhurst LJ, Drew GH et al. Particle size distribution of airborne *Aspergillus fumigatus* spores emitted from compost using membrane filtration. *Atmos Environ* 2009; **43**: 5698–5701.
18. Fischer JL, Beffa T, Lyon P-F, Aragno M. *Aspergillus fumigatus* in windrow composting: effect of turning frequency. *Waste Manage Res* 1998; **16**: 320–329.
19. Bouza E, Peláez T, Pérez-Molina J et al. Demolition of a hospital building by controlled explosion: the impact on filamentous fungal load in internal and external air. *J Hosp Infec* 2002; **52**: 234–242.
20. Hansen D, Blahout B, Benner D. Environmental sampling of particulate matter and fungal spores during demolition of a building on a hospital area *J Hosp Infec* 2008; **70**: 259–264.
21. Streifel AJ, Lauer JL. *Aspergillus fumigatus* and other thermotolerant fungi generated by hospital building demolition. *Appl Environ Microbiol* 1983; **46**: 375–378.
22. Rintala H, Pitkäranta M, Täubel M. Microbial communities associated with house dust. *Adv Appl Microbiol* 2012; **78**: 75–120.
23. Anaissie EJ, Kuchar RT, Rex JH et al. Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clin Infect Dis* 2001; **33**: 1871–1878.
24. Cho SH, Reponen T, Bernstein DI et al. The effect of home characteristics on dust antigen concentrations and loads in homes. *Sci Total Environ* 2006; **371**: 31–43.
25. Dassonville C, Demattei C, Detaint B et al. Assessment and predictors determination of indoor airborne fungal concentrations in Paris newborn babies' homes. *Environ Res* 2008; **108**: 80–85.
26. Dharmage S, Bailey M, Raven J et al. Prevalence and residential determinants of fungi within homes in Melbourne, Australia. *Clin Exp Allergy* 1999; **29**: 1481–1489.
27. Hamada N, Abe N. Comparison of fungi found in bathrooms and sinks. *Biocontrol Sci* 2010; **15**: 51–56.
28. Kemp PC, Neumeister-Kemp HG, Murray F, Lysek G. Airborne fungi in non-problem buildings in a Southern-Hemisphere Mediterranean climate: Preliminary study of natural and mechanical ventilation. *Indoor Built Environ* 2002; **11**: 1–44.
29. Arnow PM, Sadigh M, Costas C, Weil D, Chudy R. Endemic and epidemic aspergillosis associated with in-hospital replication of *Aspergillus* organisms. *J Infect Dis* 1991; **164**: 998–1002.
30. Engelhart S, Hanfland J, Glasmacher A et al. Impact of portable air filtration units on exposure of haematology–oncology patients to airborne *Aspergillus fumigatus* spores under field conditions. *J Hosp Infect* 2003; **54**: 300–304.
31. Srinivasan A, Beck C, Buckley T et al. The ability of hospital ventilation systems to filter *Aspergillus* and other fungi following a building implosion. *Infect Control Hosp Epidemiol* 2002; **23**: 520–524.

32. Samson RA, Hong SB, Frisvad JC. Old and new concepts of species differentiation in *Aspergillus*. *Med Mycol* 2006; **44**: S133–S148.
33. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A et al.. *Aspergillus* section *Fumigati*: Antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Ch* 2008; **4**: 1244–1251.
34. Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* 2005; **4**: 625–632.
35. Serrano R, Gusmão L, Amorim A, Araujo R. Rapid identification of *Aspergillus fumigatus* within the section *Fumigati*. *BMC Microbiol* 2011; **11**: 82.
36. Staab JF, Balajee SA, Marr KA. *Aspergillus* section *Fumigati* typing by PCR-restriction fragment polymorphism. *J Clin Microbiol* 2009; **47**: 2079–2083.
37. Balajee SA, Nickle D, Varga J, Marr K. Molecular studies reveal frequent misidentification of *Aspergillus fumigatus* by morphotyping. *Eukaryot Cell* 2006; **5**: 1705–1712.
38. Hong SB, Shin HD, Hong J et al. New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. *Anton Leeuw* 2008; **93**: 87–98.
39. Webb JS, Nixon M, Eastwood IM et al. Fungal colonization and biodeterioration of plasticized polyvinyl chloride. *Appl Environ Microbiol* 2000; **66**: 3194–3200.
40. Robson GD, Wiebe MG, Trinci APJ. Choline- and acetylcholine-induced changes in the morphology of *Fusarium graminearum*: evidence for the involvement of the choline transport system and acetylcholinesterase. *Microbiology* 1995; **141**: 1309–1314.
41. Vogel HJ. A convenient growth medium for *Neurospora* (Medium N). *Microb Genet Bull* 1956; **13**: 42–43.
42. Leslie CE, Flannigan B, Milne LJR. Morphological studies on clinical isolates of *Aspergillus fumigatus*. *J Med Vet Mycol* 1988; **26**: 335–341.
43. Rinyu JA, Varga J, Ferenczy L. Phenotypic and genotypic analysis of variability in *Aspergillus fumigatus*. *J Clin Microbiol* 1995; **33**: 2567–2575.
44. Schmidt A, Wolff MH. Morphological characteristics of *Aspergillus fumigatus* strains isolated from patient samples. *Mycoses* 1997; **40**: 347–351.
45. Trinci APJ, Wiebe MG, Robson GD. The mycelium as an integrated entity. In: Wessels JGH, Meinhardt F, eds. *The Mycota, vol I. Growth, Differentiation and Sexuality*. Berlin: Springer, 1994.
46. Fang Z, Ouyang Z, Zheng H, Wang X. Concentration and size distribution of culturable airborne microorganisms in outdoor environments in Beijing, China. *Aerosol Sci Tech* 2008; **42**: 325–334.
47. Larscn LS. A three-year-survey of microfungi in the air of Copenhagen. *Allergy* 1981; **36**: 15–22.
48. Millington WM, Corden JM. Long term trends in outdoor *Aspergillus/Penicillium* spore concentrations in Derby, UK from 1970 to 2003 and a comparative study in 1994 and 1996 with the indoor air of two local houses. *Aerobiologia* 2005; **21**: 105–113.
49. Li D-W, Kendrick B. A year-round study on functional relationships of airborne fungi with meteorological factors. *Int J Biometeorol* 1995; **39**: 74–80.
50. Mediavilla A, Angulo J, Dominguez E, Castro A, Infante F. Annual and diurnal incidence of *Cladosporium* conidia in the atmosphere of Córdoba, Spain. *J Investig Allergol Clin Immunol* 1997; **7**: 179–182.
51. Hudson HJ. Thermophilous and thermotolerant fungi in the airspora at Cambridge. *Trans Brit Mycol Soc* 1973; **60**: 596–598.
52. Mullins J, Harvey R, Seaton A. Sources and incidence of airborne *Aspergillus fumigatus* (Fres). *Clin Allergy* 1976; **6**: 209–217.
53. Mullins J, Hutcheson PS, Slavin RG. *Aspergillus fumigatus* spore concentration in outside air: Cardiff and St Louis compared. *Allergol Clin Immunol* 1984; **14**: 351–354.
54. Solomon WR, Burge HP, Boise JR. Airborne *Aspergillus fumigatus* levels outside and within a large clinical center. *J Allergy Clin Immunol* 1978; **62**: 56–60.
55. Blum G, Perkhofer S, Grif K et al. A 1-year *Aspergillus terreus* surveillance study at the University Hospital of Innsbruck: molecular typing of environmental and clinical isolates. *Clin Microbiol Infec* 2008; **14**: 1146–1151.
56. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009; **22**: 447–465.
57. Rhodes JC. *Aspergillus fumigatus*: Growth and virulence. *Med Mycol* 2006; **44**: S77–S81.
58. Chazalet V, Debeaupuis JP, Sarfati J et al. Molecular typing of environmental and patient isolates of *Aspergillus fumigatus* from various hospital settings. *J Clin Microbiol* 1998; **36**: 1494–500.
59. Waldorf AR. Pulmonary defense mechanisms against opportunistic fungal pathogens. *Immunol Ser* 1989; **47**: 243–271.
60. Bernhard W, Hoffmann S, Dombrowsky H et al. Phosphatidylcholine molecular species in lung surfactant: composition in relation to respiratory rate and lung development. *Am J Resp Cell Mol Biol* 2001; **25**: 725–731.
61. Morgenroth K, Newhouse M. *The Surfactant System of the Lungs*. Berlin: Walter de Gruyter, 1988.
62. Wiebe MG, Robson GD, Trinci APJ. Effect of choline on the morphology, growth and phospholipid composition of *Fusarium graminearum*. *J Gen Microbiol* 1989; **135**: 2155–2162.
63. Strange R, Smith H. A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum in vitro*. *Physiol Plant Pathol* 1971; **1**: 141–150.
64. Strange R, Smith H. Effect of choline, betaine and wheat-germ extract on growth of cereal pathogens. *Trans Br Mycol Soc* 1978; **70**: 193–199.
65. Strange RS, Majer JR, Smith H. The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate *Fusarium graminearum in vitro*. *Physiol Plant Pathol* 1974; **4**: 277–290.
66. Robson GD, Wiebe MG, Cunliffe B, Trinci APJ. Choline- and acetylcholine-induced changes in the morphology of *Fusarium graminearum*: evidence for the involvement of the choline transport system and acetylcholinesterase. *Microbiology* 1995; **141**: 1309–1314.
67. Birch M, Robson G, Law D, Denning DW. Evidence of multiple extracellular phospholipase activities of *Aspergillus fumigatus*. *Infect Immun* 1996; **64**: 751–755.
68. Robson GD. Phospholipases of *Aspergillus fumigatus*. In Latgé JP, Steinbach WJ, eds. *Aspergilus fumigatus and Aspergillosis*. Washington: ASM Press, 2009.