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Short communication

Fungal contamination of bedding

Background: It is currently believed that most fungal exposure occurs external to the home.
Aims: To enumerate the fungal flora of used synthetic and feather pillows and the dust vacuumed from them, in the UK.
Methods: 10 pillows aged between 1.5 and >20 years in regular use were collected and quantitatively cultured for fungi. Swatches were taken from nine sections of the pillow and dust was also collected by vacuum from five pillows. Pillow vacuuming was carried out prior to pillow culture. All were cultured at room temperature, 30 and 37°C for 7 days in broth before plating, and a subset were also cultured for 24 h in broth and then plated. Fungi were identified by standard morphological methods.
Results: The commonest three species isolated were *Aspergillus fumigatus* ($n = 10$), *Aureobasidium pullulans* ($n = 6$) and *Rhodotorula mucilaginosa* ($n = 6$). Another 47 species were isolated from pillows and vacuum dust. The number of species isolated per pillow varied from 4 to 16, with a higher number from synthetic pillows. Compared with the nonallergenic *A. pullulans*, more *A. fumigatus* was found in synthetic than feather pillows.
Conclusions: We have examined pillows for fungal contamination, and show that the typical used pillow contains a substantial load of many species of fungi, particularly *A. fumigatus*. Given the time spent sleeping, and the proximity of the pillow to the airway, synthetic and feather pillows could be the primary source of fungi and fungal products. This has important implications for patients with respiratory disease, and especially asthma and sinusitis.

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Asthma prevalence has increased progressively over the past three decades in the UK. Numerous theories have been promulgated including the hygiene hypothesis, additional exposure to house dust mite etc., none of which have been substantiated on detailed study. An association with fungal exposure is less well studied. It is often assumed that such exposures generally occur outside, apart from mouldy buildings.

One substantial life-style change over the last 30 years has been altered bedding – from feather/flock pillows and sheets/blankets, to mainly polyester pillows and quilts. With no need for feather containment, the covers on pillows are more porous with pore size increasing from 2 to >10 μm. Synthetic pillows are a risk for both prevalence and for severity of asthma. Butland et al. estimated that the increase in synthetic bedding could explain 50% of the increase in prevalence in wheezing (1).

We have considered the hypothesis that fungi growing on bedding could be an environmental health risk. Adults may produce up to 100 l of sweat in bed every year, which for approximately 8 h/24 is at approximately 30°C and high humidity – an ideal fungal culture medium. Fungal contamination of kapok pillows was noted in 1936, and associated with wheezing (2).

Methods

Both synthetic and feather pillows that had been used for years in family homes, were collected and stored in sterile bags prior to culture. All pillows were cut into nine equal sections and then smaller samples (swatches) were prepared from each section of pillow; approximately 2 × 2 × 2 cm for synthetic pillows and 0.25 g for feather pillows. For five pillows, additional dust samples for culture were obtained by vacuuming the pillow through the cover for 2 min, prior to culture processing.

Pillow swatches and vacuum dust samples were transferred into 20 and 10 ml of Sabouraud glucose liquid medium containing antibiotics (ciprofloxacin 2 mg/l, gentamicin 16 mg/l and vancomycin 8 mg/l) respectively, vortexed for 20 s and then allowed to stand for 24 h or 7 days. The pillow swatch broths were vortexed and haemocytometer counts were performed to give an estimation of fungal load. Vacuum dust broths were vortexed, centrifuged (3000 rpm/10 min) and the supernatant was counted on a haemocytometer. Based on these counts, two selected 10-fold dilutions of each broth in phosphate-buffered saline were inoculated onto Sabouraud glucose agar in duplicate, and then the plates were incubated at room temperature, 30°C or 37°C for up to 1 week. Colony counts were performed and isolates identified using standard laboratory methods. Any isolate that could not be identified in our laboratory was sent to Centraalbureau voor Schimmelcultures (CBS), the Netherlands. Isolates were quantitated by colony-forming units (CFU) per gram pillow.

Results

Pillows ranged in age from 18 months to >20 years. Substantial quantities of numerous fungi were cultured from all pillows. The commonest three species in pillow swatches were *Aspergillus fumigatus* ($n = 10$), *Aureobasidium pullulans* ($n = 6$) and the yeast *Rhodotorula mucilaginosa* ($n = 6$) (Table 1). Other species cultured from pillows included *Aspergillus flavus* ($n = 5$), *A. niger* ($n = 1$), *A. sydowii* ($n = 1$), *A. glaucus* ($n = 1$) and another unidentified *Aspergillus* species, *Penicillium* spp. ($n = 6$), *Cladosporium herbarum* ($n = 6$, all only at room temperature), *C. cladosporioides* ($n = 2$) and *C. tenuissimum* ($n = 1$), *Epicoccum nigrum* ($n = 1$), *Botrytis cinerea* (grey mould of grape; $n = 1$), *Pithomyces chartarum* ($n = 1$), probable *Trametes* spp. (bracket fungus; $n = 1$), three different species of Agaricales (typical gilled mushrooms), *Stereum* cf. *sanguinolentum* (encrustation or bracket fungus on stumps; $n = 1$), *Arthrinium phaeospermum* ($n = 1$), *Pholiota* spp. (colourful inedible gilled mushrooms; $n = 1$) and 2 yeasts comprising *Candida parapsilosis* ($n = 1$) and *C. guilliermondii* ($n = 1$). Vacuum dust usually grew *A. pullulans*, but also *Pithomyces chartarum* ($n = 2$), *A. vitus* ($n = 2$), *Scopulariopsis brevicaulis* ($n = 1$), *R. mucilaginosa* ($n = 1$) and *Ar. phaeospermum* ($n = 1$). Some isolates (all filamentous fungi; $n = 16$) were not identifiable by us or at CBS.

The actual yield and quantitative culture results varied substantially by temperature of incubation and time (24 h or 7 days). Species yield was always higher at 7 days, and *A. fumigatus* CFU climbed by about 5 logs between 24 h and 7 days. There was a thick layer of matted hyphae and conidia on the surface of the culture broth after 7 days incubation, and so the increase in *A. fumigatus* CFU was probably artefactual. Incubation at 37°C inhibited the growth of *A. pullulans*, and slightly reduced the growth of *R. mucilaginosa*.

Table 1. Quantitative results from 24 h cultures of pillows ($n = 6$) and vacuum samples ($n = 4$)

Sample	Species	*N* (%)	Pillow (CFU/g), mean (range)
Pillow swatch	*Aspergillus fumigatus*	6 (100)	2130 (110–4500)
	Aspergillus pullulans	5 (83)	3571 (436–8530)
	*Rhodotorula mucilaginosa**	2 (33)	6500 (2500–10 500)
	Aspergillus flavus	2 (33)	236 (226–245)
	Cladosporium spp.	4 (67)	73 (28–133)
Vacuum from pillow surface	*Aureobasidium pullulans*	4 (100)	90 520 (34 800–2 910 000)
	Penicillium spp.	1 (25)	15 100
	Aspergillus vitus†	1 (25)	27 800
	Rhodotorula mucilaginosa†	1 (25)	69 400
	Aspergillus glaucus†	1 (25)	41 700
	Scopulariopsis brevicaulis†	1 (25)	13 900

*Previously known as *Rhodotorula rubrum*.
†Isolated from one feather pillow.

Table 2. Comparison of feather and synthetic pillows

Pillow type	*N*	Predominant species	Mean (CFU/g, 24 h)	Morphologically identifiable species cultured*, mean (range)
Synthetic	5	*Aspergillus fumigatus*	2745	10 (6–16)
		Aureobasidium pullulans	1926	
Feather	5	*Aspergillus fumigatus*	1863	7.8 (4–12)
		Aureobasidium pullulans	5110	

*Includes vacuum samples.

There was a poor relationship between the species cultured directly from pillows and the dust collected prior to pillow culture. In particular *A. fumigatus* was never cultured from vacuum samples, although it was the most prevalent fungus found in the pillow. The same was true of *A. flavus*, but not of *A. vitus*, which was isolated from the vacuum samples of two pillows. In contrast much higher CFU of *A. pullulans* were obtained from vacuum samples than in the pillow itself (Table 1).

Comparison of synthetic pillows with feather pillows showed a larger number of species cultured from synthetic pillows (Table 2), although there was substantial differences between individual pillows. In addition, the predominant species in synthetic pillows was *A. fumigatus*, whereas it was *A. pullulans* in feather pillows.

Discussion

We have shown high levels of fungi in pillows with substantial interpillow variation in flora and some differences between feather and synthetic pillows.

Of the three most abundant fungi found, *A. fumigatus* is a well recognized allergenic fungus. Indeed more allergens have been identified in *A. fumigatus* than any other fungus to date. In addition to the approved 18 allergens (3), another 60+ immunoglobulin E (IgE)-binding proteins have been identified (4). In contrast, *A. pullulans* is also common in the environment but without any allergens described, although it has been associated with an outbreak of extrinsic allergic alveolitis, when found in profusion in an air conditioning system (5). *Rhodotorula mucilaginosa* has been found to be allergenic in skin prick testing (6), and a single enolase antigen identified (7). Some of the other fungi found in the pillows are allergenic including *C. herbarum*, *A. flavus*, *A. niger* and *Penicillium* spp. Of note, we did not grow *Alternaria* from any pillow, a common allergenic fungus in outdoor air closely associated with so-called 'thunderstorm asthma' (8).

Synthetic pillows are made of inert hollow fibrils of polyester, with a variety of coatings, e.g. oleic acid, for ease of spinning. It might be expected that the closer weave of feather pillow covers might prevent larger spores from entering or exiting feather pillows, but we grew *E. nigrum* which has spores of 15–30 μm from a

feather pillow. Both colonisation and exit of larger fungal spores (*Cladosporium* etc.) will be greater although the coarser weave cover on synthetic pillows, consistent with Butland et al.'s (1) data, although some escape of the small 2–3 μm spores of *A. fumigatus* is likely through the finer weave covers.

Human respiratory tract exposure could be to the spores themselves, to hyphae, to volatile fungal secondary metabolites or to fungal degradation products. Only a few recognized antigens are found on the spore surface (9), most being expressed once spore swelling and germination have taken place, although often very early after germination (10). Thus, direct mucosal exposure to spores may not induce a typical allergic response, if the respiratory tract is normal and spore germination does not take place. Sinus or airways blockage from respiratory infection, with excess mucous and local epithelial damage, may however provide a germination medium, allowing antigenic exposure. This might explain why adults are so much more frequently sensitized to fungi than children. Asthma severity in adults is related to fungal sensitization in long-standing asthmatics (11, 12) in which varying degrees of bronchiectasis is common. In these patients, fungal colonisation and germination may be a semipermanent fixture of the airways, and so providing continuous antigenic exposure. Exposure during sleep because of fungal contamination of bedding could initiate and drive the process.

The majority of asthma commences in childhood. Evidence of abnormal lung function is present in a substantial proportion of 3-year-old children with a family history of allergy (13). This implies that lung damage sustained in early life may lead to the later development of asthma, when permanent airway remodelling is present. Fungal products in pillows/bedding could damage the airways at a sensitive time in their development. For example, β-(1,3)-glucan, an important constituent of many fungal cell walls, is proinflammatory (14).

Further work is required on the ecology of fungi in bedding, including the environmental factors which are important and the relative contribution of duvets and pillows. Measures of individual fungal exposure need development. There is little correlation between reservoir and airborne fungal levels (15), and it may be that direct exposure from bedding is more important. The use of a tight woven or other protective cover such as Goretex^{TM} (W. L. Gore, Livingston, UK) cover might be protective, and needs investigating. It is extraordinary that such a major unidentified source of fungal exposure has literally been staring us in the face.

Author contributions

AA Woodcock conceived the idea, and wrote the primary draft of the paper. N Steel, SJ Howard and CB Moore undertook the cultures, quantitation and identification of the fungi. A Custovic and DW Denning contributed to the research plan and writing the paper.

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