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Brief Report

A 2-year comparative study of mold and bacterial counts in air samples from neutral and positive pressure rooms in 2 tertiary care hospitals

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Immunocompromised patients are at risk of invasive fungal infection. These high-risk patients are nursed in protective isolation to reduce the risk of nosocomial aspergillosis while in hospital—ideally in a positive pressure single room with high-efficiency particulate air filtration. However, neutral pressure rooms are a potential alternative, especially for patients requiring both protective and source isolation. This study examined mold and bacterial concentrations in air samples from positive and neutral pressure rooms to assess whether neutral pressure rooms offer a similar environment to that of positive pressure rooms in terms of mold concentrations in the air. Mold concentrations were found to be similar in the positive and neutral pressure room types examined in this study. These results add to the paucity of literature in this area.

Aspergillus spp are ubiquitous fungi, many of which cause human infections, particularly in immunocompromised patients.[1] Many guidelines recommend that patients at high risk of infection with *Aspergillus* spp be nursed in a positive pressure high-efficiency particulate air (HEPA)–filtered room for the duration of their neutropenic period, as one of the measures to try to reduce the risk.[2-5] Recommended threshold levels of *Aspergillus* spp are <1 and <5 colony forming units (CFU)/m^3 in HEPA-filtered air and in a ward with no filtration, respectively.[3] Positive pressure ventilated lobby (neutral pressure) rooms are specifically designed to keep the room at neutral pressure relative to the corridor outside. HEPA-filtered air is supplied in the lobby at positive pressure to both the room and the corridor and air extraction is via an en suite toilet. This type of room has many other specific design parameters which must be met for it to function correctly, including a pressure stabilizer above the door between the patient room and the lobby, and a transfer grille on the lower section of the en suite door.[5] The room has been validated from an engineering perspective only as being appropriate for source isolation and protective isolation.[6] This room type is potentially suitable for an immunocompromised patient with an airborne infection, but it has not yet been clinically validated as providing both source and protective isolation.[3] There are very little data available to document the relative performance of any neutral pressure room (positive pressure ventilated lobby design or otherwise) in reducing exposure to fungal elements and specifically *Aspergillus* spores; therefore, the aim of this study was to compare concentrations of mold and bacteria in air sampled from positive and neutral pressure rooms.

METHODS

Air samples of defined volume were collected on a monthly basis over consecutive 1-year periods at each of 2 hospitals, at times when there was construction work on-site. In total, 216 air samples were collected over 24 months (Table 1). At the first hospital, samples were collected from 12 areas, including 2 positive pressure rooms, 2 neutral pressure rooms, 2 unventilated rooms, and the corridor directly outside each of the 6 rooms (2 samples per month, 144 in total). Samples were collected once per month from October 2014-September 2015. At the second hospital, samples were collected from one of each room type and the corridor directly outside, once per

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Table 1
Technical specifications for each room type examined

Hospital	Room type	Lobby	HEPA-filtered air supply	Filter types (efficiency)	Air changes per hour	Pressure differential	Negative pressure extract
1	Positive pressure (ward Z)	Yes	Yes	G4 (85.9%) F8 (95%) H13 (99.95%)	10	10 Pa (room relative to lobby)	Yes
1	Neutral pressure 1 (ward A)	No	Yes	G4 (85.9%) F8 (95%) H13 (99.95%)	12-14	Neutral	Yes
1	Neutral pressure 2 (high dependancy ward)	Room located off of a HEPA-filtered room with nurse's station and 3 bays	Yes	G4 (85.9%) F8 (95%) H13 (99.95%)	10	Patient room neutral to the open area, which was 5 Pa positive to the corridor	Yes
1	Unventilated single room	No	No	—	—	—	No
2	Positive pressure	Yes	Yes	G4 (85.9%) F7 (80-90%) H14 (99.995%)	10	10 Pa (room relative to lobby)	Yes
2	Neutral pressure (PPVL)	Yes	Yes	G4 (85.9%) F8 (95%) H14 (99.995%)	10 in patient room and in en suite toilet	Lobby 8-12 Pa relative to corridor	Yes
2	Unventilated single room	No	No	–	—	—	No

NOTE. Ward Z in hospital 1 and the positive pressure room in hospital 2 accommodated immunocompromised patients with hematologic or oncologic malignancies. Neutral pressure rooms in both hospitals were used for general patients or those considered to be infectious. All rooms had an en suite toilet (accessed only from the patient room). The negative pressure air extract grilles were located in the en suite toilets of the ventilated rooms. The 3 neutral pressure rooms were all of different design.
HEPA, high-efficiency particulate air; *PPVL*, positive pressure ventilated lobby.

month from August 2015-July 2016 (6 samples per month, 72 in total). The rooms were occupied on most air sampling occasions (and rarely with 1 visitor). If the doors were open on arrival, they were then closed for approximately 5 minutes prior to the air samples being taken. This time frame was chosen arbitrarily, while also trying to take samples that reflect actual air concentrations of mold in the patient's rooms. Windows were sealed in the rooms and the corridors were closed. Technical validation of the ventilated rooms was performed yearly by hospital engineers, with no further verification (eg, smoke test) performed at the time of sampling. Access to the rooms during the air sampling period could not be restricted; therefore, on some occasions, the doors were opened during the procedure to allow doctors or nurses to enter. The person collecting the samples remained inside the room to operate the air sampler. They did not wear protective clothing, but stood away from the sampler (SAS Air Sampler M079; International pbi Spa, Milan Italy), which was situated approximately 1 m above floor level. This directs 1,000 L of air toward an agar surface of a 55-mm-diameter tryptone soy agar contact plate. Before and after sampling, the head was cleaned with 70% alcohol. The plates were incubated at 22°C for 72 hours, and then 30°C for 48 hours per the International Organization for Standardization guidelines.[7] Total colonies and mold colonies were counted after the total 5 days incubation and after conversion using the positive-hole conversion table, expressed as cfu per cubic meter of air. Molds were identified by characteristic colonial morphology and on microscopy using lactophenol blue preparations. Representatives of distinct bacterial colonial morphologies were identified by matrix assisted laser desorption ionisation-time of flight mass spectrometry from selected samples. Statistical analyses were performed using SPSS for Windows (version 21.0; IBM, Armonk, NY).

RESULTS

Aspergillus spp was above the recommended threshold (<1 and <5 cfu/m^3 in HEPA-filtered air and in a ward with no filtration, respectively) in 1 out of 36 samples from neutral pressure rooms and in 1 out of 36 samples from positive pressure rooms. These samples were collected at different dates: 1 in the neutral pressure room in ward A (2 cfu/m^3) and 1 in a positive pressure room in ward Z (1 cfu/m^3) in hospital 1 (Table 1). If applying the previously mentioned thresholds to total mold concentrations, the mold concentrations were above the thresholds in 15 out of 36 samples from the positive pressure rooms and 14 out of 36 samples from the neutral pressure rooms (>1 cfu/m^3). However, 23 out of 31 samples from unventilated rooms were above the threshold (>5 cfu/m^3). There was no statistically significant difference in the number of occasions that overall mold concentrations were above the threshold level in the neutral pressure rooms compared with the positive pressure rooms ($P = .81$), but the unventilated rooms had mold concentrations above the threshold level more frequently than the ventilated rooms ($P = .002$).

There was no significant difference between mold concentrations found in the positive pressure rooms compared with the neutral pressure rooms ($P = .460$), whereas the unventilated rooms had significantly higher mold counts than either ventilated room ($P < .001$) (Table 2). The mold concentrations were also significantly higher in the corridors outside the unventilated rooms compared with the corridors outside the ventilated rooms ($P < .001$). The most frequently isolated molds include *Penicillium* spp (59.5%) and *Cladosporium* spp (30.4%); the much less frequent molds included *Paecilomyces* spp (4.3%), *Fusarium* spp (2.6%), *Aspergillus* spp (0.15%), *Mucor* spp (0.1%), and *Alternaria* spp (0.07%). Similarly, the unventilated rooms had significantly higher bacterial counts than the ventilated rooms ($P < .001$), with no statistically significant difference between the neutral and positive pressure rooms ($P = .197$). Bacteria present were mostly coagulase-negative *Staphylococcus* spp, *Micrococcus* spp, *Pseudomonas* spp, *Pantoea agglomerans*, and *Acinetobacter lwoffii*. Between-hospital comparison for similar categories of rooms showed no statistically significant difference in total bacterial counts ($P = .402$).

There were sporadic incidents of high mold counts observed in each of the 2 hospitals. At the first hospital in July (month 10 of sampling), there was a higher than average mold count in one neutral pressure room and the corresponding corridor, and in both the unventilated rooms and corridors. At the second hospital, high mold counts occurred in neutral, positive, and unventilated rooms and corresponding corridors in May (month 10 of sampling) (Table 2). There was no discernible reason for these sporadic increases in mold counts.