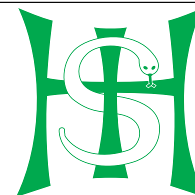
Journal of Hospital Infection 83 (2013) 122–126

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin



Rapid quantification of viable fungi in hospital environments: analysis of air and surface samples using solid-phase cytometry

D. Méheust [a,b,*], P. Le Cann [a,b], J.-P. Gangneux [b,c,d]

[a] EHESP, Sorbonne Paris Cité, F-35043 Rennes, France
[b] Inserm, U1085-IRSET, F-35043 Rennes, France
[c] Université de Rennes 1, Faculté de Médecine, F-35043 Rennes, France
[d] Centre Hospitalier Universitaire de Rennes (CHU), Service de Parasitologie-Mycologie, F-35033 Rennes, France

A R T I C L E I N F O

Article history:
Received 10 April 2012
Accepted 14 October 2012
Available online 11 January 2013

Keywords:
Environmental surveillance
Hospital
Solid-phase cytometry
Viable fungi

S U M M A R Y

Background: Environmental surveillance is important in high-risk areas of hospitals to prevent fungal infections in immunosuppressed patients. Conventional culture methods for enumerating environmental fungi are time-consuming.
Aim: In this field study, a solid-phase cytometry technique (SPC) and a more conventional culture-based method to quantify fungal contamination of hospital air and surface samples were compared.
Methods: For the air sampling, a liquid cyclone air sampler was used with a flow rate of 300 L/min for 10 min in each of four hospital locations. Surface swabbing was done in two locations, with two different swab types. Samples from all areas were processed by SPC and by culture on malt extract agar.
Findings: The mean airborne concentrations of viable fungi determined by SPC were about 1.5-fold higher than the mean concentrations obtained with the culture-based method. These differences for air samples were significant in three hospital environments. No significant difference was observed for surface samples between the two swab types and between the two analytical methods. One of the prominent advantages of SPC was its rapidity in comparison with the culture-based method (5 h versus 5 days).
Conclusion: In this study, we showed that SPC allows for rapid monitoring of viable fungi in hospital environments. SPC can thus be used to provide an early warning and a rapid implementation of corrective measures. Viable fungi detection may be an important tool to assess infectious risk in wards with immunosuppressed patients.
© 2012 The Healthcare Infection Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Over the past 20 years, the number of invasive fungal infections has continued to persist, due primarily to the increased numbers of patients subjected to severe immunosuppression.[1,2] Monitoring of environmental fungal contamination is strongly recommended, especially for outbreak investigation in epidemic situations and during building

* Corresponding author. Address: EHESP, Avenue du Professeur Léon Bernard CS74312 – 35043 Rennes Cedex, France. Tel.: +33 (0) 2 99 02 29 22; fax: +33 (0) 2 99 02 29 29.
E-mail address: Delphine.Meheust@ehesp.fr (D. Méheust).

0195-6701/$ – see front matter © 2012 The Healthcare Infection Society. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jhin.2012.10.004

construction and renovation work.[3,4] Moreover, environmental surveillance programmes are also useful to assess air control efficiency in wards with high-risk patients.[5,6]

Culture-based analysis is the conventional approach used for fungal quantification in air and surface samples. However, this method often results in an underestimation of the number of micro-organisms, as it detects only culturable micro-organisms and is dependent on culture conditions.[7] Furthermore, it usually requires at least a five-day incubation period to enumerate most of the environmental fungi. In contrast to traditional methods, quantitative polymerase chain reaction (qPCR) has been developed for specific quantification of fungi and is being used more frequently owing to its low detection limit and high accuracy.[8,9] It nevertheless does not differentiate between viable and non-viable cells; the viability of a micro-organism is still a critical factor during hospital environmental surveillance since it determines infectivity.[10] Moreover, this molecular-based method is nowadays widely used to detect *Aspergillus* species, but other airborne fungi may cause respiratory infections including *Fusarium* and *Zygomycetes* species.[11]

To reduce the time for the microbiological controls in pharmaceutical and drinking water industries, a rapid assay with high-level sensitivity was developed a few years ago: a solid-phase cytometry (SPC) system, using the ChemScan RDI system (AES Chemunex, Bruz, France), is based on direct fluorescent labelling of viable micro-organisms, coupled with an ultrasensitive laser scanning and counting system.[12] It allows for direct detection of a single cell and eliminates the need for cell growth. Fluorescent viability staining is based on the cleavage of carboxyfluorescein diacetate (ChemChrome V6) by esterases, resulting in the formation of fluorescent carboxyfluorescein in intact and metabolically active cells only.[13] A specific protocol was recently developed to detect fungi in environmental samples. To our knowledge, this protocol was only tested to analyse air samples from various outdoor and indoor locations.[14,15]

Thus, in the present study, we investigated the benefits of this system in hospital environmental surveillance. After microbial samplings in different areas, viable fungi in air and surface samples were quantified by SPC. This surveillance strategy was compared with common sampling techniques and conventional culture-based methods.

Methods

This study was performed in field conditions in the Teaching Hospital of Rennes (Brittany, France).

Air sampling protocol

Four sites expected to have different amounts of airborne fungi were selected: an office (high level), a conventional room (medium level), a corridor in haematology unit (low level), and a room with laminar air flow (very low level). A liquid cyclone air sampler, Coriolis collector (Bertin Technologies, Montigny-le-Bretonneux, France), was used with a flow rate of 300 L/min. Airborne micro-organisms were sampled in Collection liquid (AES Chemunex) with an initial volume of 15 mL (the liquid evaporated a little during air sampling). The sampler was placed in the middle of the room at a height of 1 m above the floor, and 10 air samples of 10 min were collected for each site.

Surface sampling protocol

Twenty-five surface samplings per swab type were performed in different departments of the Teaching Hospital of Rennes: the haematology unit ($N = 15$) and the parasitology–mycology laboratory ($N = 10$). The ChemSwab (AES Chemunex), a flocked swab, was moistened by the ChemSwab SRK solution, and the excess solution was removed by pressing gently against the wall of the tube. The swabs were turned during sampling on a 100 cm^2 stainless steel disinfected template. The ChemSwab was transferred into the SRK tube containing 1.25 mL of protective solution, and rotated 10 times in the solution before pressing and removing the swab. According to the same protocol, 100 cm^2 were then sampled with a conventional cotton swab (Dutscher, Brumath, France) moistened with 1.25 mL of sterile saline solution. This sampling was performed at a distance of 5 cm from the ChemSwab sampling.

Culture-based method

One-third of the Coriolis liquid volume (equivalent to 1 m^3 of air) and 500 μL of the suspension for the swabs were spread on malt extract agar plates (MEA, Merck, Germany) from the same batch. All MEA plates were incubated at 25 °C and fungal colonies were counted on days 3 and 5 (Figure 1). Results for air and surface samples were expressed as cfu per m^3, and cfu per 100 cm^2, respectively.

Solid-phase cytometry

One-third of the Coriolis liquid samples and 500 μL of the ChemSwab suspensions were analysed by solid-phase cytometry (Figure 1). All the following materials and reagents were obtained from AES Chemunex.

Fungal labelling protocol

The liquid sample was filtered through a 2.0 μm Cycloblack-coated polyester membrane filter. Interfering particles were counterstained by filtration of 1 mL CSE/5 to reduce non-specific fluorescence.[16] The filter was transferred to a cellulose pad soaked with 600 μL of the activation medium ChemSol A6 and incubated at 30 °C for 3 h. This pre-labelling step is required to activate the fungal metabolism and to optimize the cells for the following labelling step. Viable micro-organisms were fluorescently labelled by incubating the filters at 37 °C for 1 h on a cellulose pad saturated with 600 μL of viability staining reagent (ChemChrome V6 diluted 1:100 in the labelling buffer ChemSol B2).

Laser scanning and microscopic validation

After labelling of the micro-organisms, the 25 mm diameter membrane filter was placed in a holder, on top of a support pad moistened with 600 μL of labelling buffer. The filters were subsequently scanned by the ChemScan RDI. This solid-phase cytometer consists of an argon laser, emitting light of 488 nm, and two photomultiplier tubes, which detect the fluorescent light emitted by the labelled cells. The signals produced were processed by a computer, applying a series of software discriminants to differentiate valid signals (labelled

Download English Version:

https://daneshyari.com/en/article/6122354

Download Persian Version:

https://daneshyari.com/article/6122354

Daneshyari.com