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Development of an innovative method for the evaluation of fungal contamination of surfaces

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Parole chiave: *Campionamenti di superfici,* Aspergillus sp*, metodo innovativo*

Riassunto

Messa a punto di un metodo per la valutazione della contaminazione fungina delle superfici

L'obiettivo della presente nota tecnica è stato quello di confrontare la capacità di catturare spore fungine mediante prelievi eseguiti con tre diverse metodiche: Piastre Rodac contact, tampone classico e tampone allestito con un panno catturapolvere selezionato tra altri presenti in commercio. Le prove sono state effettuate utilizzando una sospensione di conidi di A. niger *pari a 0.5 MacFarland diluita 1/30, 1/40, 1/50, 1/100. Con ciascuna di tali diluizioni sono state contaminate 3 lastre di acciaio inossidabile sterili, suddivise ciascuna in 16 piccoli quadrati, al centro dei quali veniva posto e lasciato essiccare 0,1 ml della diluizione prescelta (per un totale di 12 lastre). Inoltre, sono state utilizzate altre 6 lastre per ripetere l'esperienza con le diluizioni 1/40 ed 1/50. In totale sono state contaminate 288 superfici quadrate: 96 di queste sono state campionate con pastre Rodac contact, 96 con tamponi classici e 96 con tamponi allestiti mediante panno catturapolvere, con semina su piastre Petri. Per le prime 12 lastre è stato utilizzato, come terreno di coltura, il Sabouraud dextrose agar, mentre per le altre 6 lastre è stato utilizzato il Sabouraud dextrose agar addizionato di lecitina e polisorbato 80. Tutte le piastre sono state incubate a 37° per 18 ore. Per stimare le differenze tra le 3 metodiche di prelevamento e le 4 diluizioni saggiate è stata utilizzata la regressione lineare multipla. L'analisi ha dimostrato che, per quanto concerne la differenza tra le diluizioni, il numero di colonie rilevate per la diluizione 1/40 è più elevato del 13% (P = 0.09) rispetto alla diluizione 1/50 e che il numero di colonie rilevato per la diluizione 1/30 risulta più elevato del 6% rispetto alla diluizione 1/50 (P = 0.52). Per quanto riguarda il confronto tra il numero di colonie rilevato con le piastre Rodac contact, con i tamponi classici e con i tamponi allestiti con il panno catturapolvere, l'analisi matematica dimostra che i campioni classici rilevano una carica fungina 5 volte maggiore di quella rilevata con piastre Rodac, mentre i tamponi catturapolvere rilevano 6 volte più colonie fungine rispetto alle piastre Rodac (P<0.00005). Tali risultati, sebbene preliminari, mostrano che il metodo di prelievo con tampone catturapolvere rappresenta un sensibile e semplice approccio per il controllo ambientale della contaminazione fungina.*

Background

Health-care environments can be implicated in disease transmission in immunocompromised patients (2, 13, 15). Exposure of such patients to air moulds spores can result in fatal infections (9, 12, 14). Even concentrations of airborn aspergillus spores below 1 cfu/m^3 have been shown to be sufficient to cause outbreaks in immunocompromised patients (14).

The incidence of hospital-acquired aspergillosis can be minimized by adherence to ventilation standards for specialised care

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environments, appropriate maintenance and careful cleaning of the patient environment (3, 5, 8).

Different strategies are required for the surveillance of aspergillar biocontamination in hospital to prevent nosocomial aspergillosis outbreaks among high risk patients. The Centres for Disease Control and Prevention (CDC) consider the use of routine microbiological air sampling an unresolved issue and do not recommend this practice (3). Microbiological sampling is only recommended (category IB) if no epidemiological evidence exists of ongoing transmission of a fungal disease and it is preferable to collect the samples with a high-volume air sampler rather than settle plates, because single spores can remain suspended in air indefinitely (4). The opinions of other Authors (1, 7, 8, 11) are completely different. Since several studies show an association between spore density and outbreaks of invasive aspergillosis during hospital renovation or reconstruction and the inhalation of spores is the most common source of infection, they emphasize the utility of environmental surveillance and suggest mycological control in protective environments or operating rooms mainly during building renovation, even in a non-epidemic situation.

Some Authors (1, 6, 7, 10) disagree with CDC (4) about the sensitivity of surface sampling. The aerodynamic, physical and chemical characteristics of fungal spores lead to their sedimentation on surfaces, especially on electric devices (6). Air sampling can produce a false sense of security, because of the narrow range of the results (10), the inability to detect conidia observed in some studies (1, 6) and the possibility to miss the peak period of contamination (11). Conversely, results of surface sampling may be more sensitive, because spores may settle and remain for a long time (10).

Thus microbiological environmental sampling is an unresolved issue; the lack of standardized protocols and reference values for fungal environmental surveillance leave the choice to each hospital in terms of where, when, why and how to detect environmental fungi.

Some Authors (1, 5, 6, 7, 10, 11) suggest the use of surface sampling because it is cheap, easy to perform and efficient (1, 7). It can detect minor contamination, concomitant with or following air contamination.

Objective

To make surface sampling easier and to reduce surveillance costs, we developed a simple technique using a dusting cloth selected from those available on the market, after a comparative evaluation of their ability to capture spores of *A. niger* from the surfaces. The selected dusting cloth was used to set up a pad (DC pad). We used an experimental protocol to evaluate ability of this method to capture fungal conidia, in comparison with two other methods, Rodac Contact plates and cotton pads.

A summary of the research was presented at the 16th Congress of the International Society for Human and Animal Mycology (16).

Materials and Methods

A suspension of spores of reference *Aspergillus niger* was prepared in sterile distilled water, MacFarland optical density 0.5. From this suspension, dilutions of 1/30, 1/40, 1/50 1/100 were obtained.

Using a water resistant ink, 18 stainless steel tiles, 30x30 cm, were divided into sixteen small squares each (Figure 1). Then, the steel tiles were cleaned with water and chlorine, rinsed in distilled water and sterilized in autoclave. With each of the diluitions indicated above, three steel tiles were

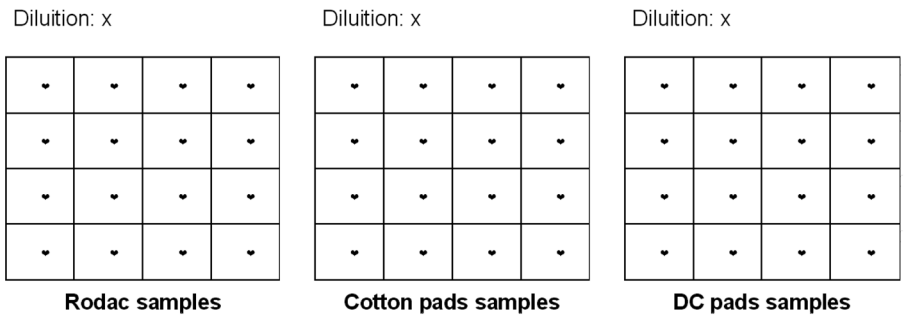

Figure 1 - Schematic representation of the modality of placement of A. niger suspensions on the stainless steel tiles

contaminated, for a total of 12 tiles. In the centre of each small square, 0,1 ml of the selected diluition was placed; all tiles were then allowed to dry. For each dilution, the first tile was harvested using Rodac contact plates prepared with Sabouraud agar, the second using specially prepared cotton pads and the third using DC pads.

The samples of *A. niger* obtained using the cotton pads and DC pads were inseminated in Petri dishes with Sabouraud dextrose agar. The Rodac plates and the Petri dishes were incubated at 37° for 16-18 hours before counting.

Another six stainless steel tiles were used to repeat the experiment for the diluitions of 1/40 and 1/50, but these samples were cultivated in Sabouraud dextrose agar neutralised with lecithin and polysorbate 80, in order to control the interference in the mould growth due to the possible residual presence of chlorine.

The Rodac contact plates and the Petri dishes were supplied - ready for use - by Beckton Dickinson®.

Thus, in all, 288 small square surfaces were contaminated. From 96 of these, Rodac plates were used to harvest the *A. niger*; from another 96, the cotton pads were used; and from the last 96, the DC pads were used.

The data of counts of colonies of *A. niger* were inspected and the distributions were found to be positively skewed. For the statistical analyses, the data were transformed using the natural logarithm. Multiple log-linear regression was used to estimate the differences between the three methods, between the four dilutions and the effect of neutralization.

Results

Table 1 shows, the arithmetic means and the standard deviations of the numbers of colonies of *A. niger* obtained according to the method used and the dilution.

Inspection of table 1 reveals that the mean number of colonies is approximately the same for each method at dilution 1/100. However, at dilutions 1/50, 1/40 and 1/30 the mean number of colonies is much higher for the cotton pads and DC pads than for Rodac plates. The mean number of colonies grown using neutralizing medium for dilution 1/40 and 1/50 are approximately the same of those obtained without neutralizing for the same dilution and sampling method.

The multiple regression of the natural logarithm of the number of colonies on method, dilution (excluding dilution 1/100) and neutralisation, gives the results in Table 2.

Table 1 - Average number of colonies of A. niger obtained according to the method used and diluition

Diluition	Rodac*		Cotton Pad*		DC Pad*		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Without Neutralization								
1/30	10.44	4.24	27.75	8.39	30.75	10.06	22.98	7.95
1/40	7.00	3.50	35.19	8.87	42.94	13.15	28.38	18.10
1/50	5.25	2.41	29.75	7.05	48.94	9.91	27.98	19.38
1/100	2.81	1.33	3.00	2.48	3.81	2.04	3.21	2.00
With Neutralization								
1/40	1.19	3.94	30.63	7.81	37.94	10.95	25.25	15.44
1/50	6.13	4.26	28.63	11.26	37.50	13.26	29.06	16.74

* The data refer to 16 samples for each diluition

These results show that the neutralization reduces the number of colonies on average by 12% (OR = 0.88) but this reduction is not statistically significant (p = 0.07). In comparison with dilution 1/50, the number of colonies recovered at dilutions 1/40 and 1/30 are slightly increased, by 13% and 6% respectively, but these increases are not statistically significant (p= 0.09 and p= 0.52, respectively). However, in comparison with Rodac plates, both cotton pads and DC pads identify many more colonies of *A. niger*, 4.92 times as many and 6.33 times as many respectively. These increases are both highly significant (p< 0.00005).

Conclusions

Dusting cloths are able to collect samples on every type of surface (smooth, mesh, flat, curved) and to provide information about contamination taking only one sample from the area (air ventilation outlets, filters, patient's beds, scialitic lamps, etc). Both cotton pads and DC pads recover more colonies of *A. niger* than Rodac plates (p<0.00005). It is estimated that cotton pads on average recover five times as many colonies of *A. niger* compared with Rodac plates and it is estimated that DC pads on average recover more than six times as many

Table 2 - Multiple regression of the natural logaritm of the number of colonies cultivated on method used, diluition (excluding diluition 1/100) and use of neutralization

Independent variabile	***Coefficient b***	**OR**	**95% CI**	**P**
Medium neutralised vs not neutralised	- 0.13	0.88	0.76 - 1.01	0.07
Cotton pad vs Rodac contact	1.59	4.92	4.22 - 5.75	<0.00005
BAMA pad vs Rodac contact	1.85	6.33	5.42 - 7.39	<0.00005
Dilution 1/40 vs dilution 1/50	0.12	1.13	0.98 - 1.30	0.09
Dilution 1/30 vs dilution 1/50	0.06	1.06	0.89 - 1.27	0.52
Intercept (coefficient a)	1.77			

as Rodac plates. With neutralisation, the number *A. niger* UFC is reduced on average by 12% but this change is not statistically significant at the 5% level. The number of colonies recovered at dilutions 1/40 and 1/30 is higher than the numbers recovered at dilution 1/50, but these increases are not statistically significant.

These results imply that this technique improves the quantitative recovery of *A. niger* from stainless steel tiles. Although these results are preliminary, we believe that this sampling method could provide an easy, sensitive and inexpensive approach to environmental control.

Summary

The objective of this technical report is to compare the ability to capture fungal spores through samples performed with three different methods: Rodac contact plates, cotton pad and a pad prepared with a dusting cloth (DC pads) selected from those available on the market.

The tests were conducted using a suspension of *Aspergillus niger* conidia equal to 0.5 MacFarland diluted 1/30, 1/40, 1/50, 1/100. With each of these dilutions 3 sterile tiles of stainless steel were contaminated, each divided into 16 small squares, in the center of which 0.1 ml of the dilution chosen was placed and left to dry (for a total of 12 sheets). In addition, we have used other 6 tiles to repeat the experience with dilutions 1/40 and 1/50. A total of 288 squared surfaces were contaminated: 96 of these were sampled with Rodac contact plates, 96 with cotton pads and 96 with DC and then inseminated in Petri plates. Sabouraud dextrose agar was used as culture medium for the first 12 plates, while, for the other 6 plates Sabouraud dextrose agar added with lecithin and polysorbate 80 was used. All plates were incubated at 37° for 18 hours. To estimate the differences among the sampling methods and the dilutions tested, multiple linear regression was used. The analysis showed that the number of colonies harvested at dilution 1/40 is 13% higher (P = 0.09) than the number harvested at dilution 1/50 and the number of colonies harvested at dilution 1/30 is 6% higher than dilution 1/50 (P = 0.52).

With regard to the comparison between the number of colonies harvested with Rodac contact plates, with cotton pads and DC pads, regression analysis shows that cotton pads harvest a number of fungal cfu 5 times higher than those detected with Rodac plates, while DC pads harvest a number of fungal ufc 6 times higher than those detected with Rodac plates (P <0.00005).

These results, although preliminary, indicate that DC pads are a sensitive and simple approach for the environmental control of fungal contamination.

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References

1. Alberti C, Bouakline A, Ribaud P, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. J Hosp Infect 2001; **48**: 198-206.
2. Carter CD, Barr BA. Infection control issues in construction and renovation. Infect Control Hosp Epidemiol 1997; **18**: 587-96.
3. CDC. Guidelines for preventing Health-Care-Associated Pneumonia, 2003. MMWR 2004; **53** (No. RR-3).
4. CDC. Guidelines for environmental control in healthcare facilities. MMWR 2003; **52**: (No. RR-10).
5. Conférence de consensus. 2000. Prévention du risque aspergillaire chez les patients immunodéprimés (Hématologie, Transplantation). Institut Pasteur-Paris, 21 mars.
6. Diaz-Guerra TM, Mellado E, Cuenca-Estrella M, et al. Genetic similarity among One Apsergillus flavus strani isolated from a patient who underwent heart surgery and two environmental strains obtained from the operatine room. J Clin Microbiol 2000; **38**: 2419-22.
7. Faure O, Friker-Hidalgo H, Lebeau B, et al. Eight-year surveillance of environmental fungal contamination in hospital operatine rooms and haematological units. J Hosp Infect 2002; **50**: 155-60.
8. Gangneux JP, Bretagne S, Cordonnier C, et al. Prevention of Nosocomial Fungal Infection: The French Approach. Clin Infect Dis 2002; **35**: 343-6.
9. Gerson SL, Talbot GH, Hurwitz S, et al. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. Ann Intern Med 1984; **100**: 3451.
10. Heinemann S, Symoens F, Gordts B, Jannes H, Nolard N. Environmental investigations and molecular typing of Aspergillus flavus during an outbreak of postoperative infections. J Hosp Infect 2004; **57**: 149-55.

11. Mahieu LM, De Dooy JJ, Van Lear FA, Jansens H, Leven MM. A prospective study on factors influencing aspergillus spore load in the air during renovation works in a neonatal intensive care unit. J Hosp Infect 2000; **45**: 191-7.
12. Marr KA, Bowden RA. Fungal infections in patients undergoing blood and marrow transplantation. Transpl Infect Dis 1999; **1**: 237-46.
13. Rhame FS. Prevention of nosocomiasl aspergillosis. J Hosp Infect 1991; **18** (Suppl A): 466-72.
14. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak setting. J Hosp Infect 2006; **63**: 246-54.
15. Warnock DW, Hajjeh RA, Lasker BA. Epidemiology and prevention of Invasive Aspergillosis. Curr Infect Dis Rep 2001; **3**: 507-16.
16. Vescia N, Osborn JF, D'Alessandro D, Grillot R. Detection of fungal contamination: surface sampling with a dusting cloth improves quantitative recovery of aspergilli. Proceedings of ISHAM 2006. The 16th Congress of the International Society for Human and Animal Mycology. Paris, 25-29 June 2006.

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