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FILAMENTOUS FUNGI FROM AIDS PATIENT SECRETIONS AND FROM THE ENVIRONMENTAL AIR IN HOSPITAL

(Hongos filamentosos en secreciones de pacientes con SIDA y desde el aire del ambiente hospitalario)

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Palabras clave: Hongos filamentosos, AIDS, micota nosocomial
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RESUMEN

Mediante la toma de muestras de secreciones orofaringeas, nasales y del oido externo de 50 pacientes con Sindrome de Inmunodeficiencia Adquidida (SIDA-AIDS) y del aire de las salas de tratamiento de los mismos, se obtuvieron 238 muestras : 87 de las secreciones y 151 del aire. Se aislaron 29 spp. de hongos filamentosos de los especimenes clinicos y 38 del aire. En las secreciones de los pacientes, el grupo forma: ***Mycelia sterilia,*** *y* ***Fusarium oxysporum, F.lateritium, Aspergillus parasiticus,*** *fueron los Hyphomycetes más representativos y los géneros* ***Aspergillus*** *y* ***Fusarium*** *constituyeron el 47% delos aislamientos en proporciones semejantes*

En el ambiente hospitalario, el grupo forma ***Mycelia sterilia*** *y las especies* ***C. sphaerospermum, Aspergillus sydowii*** *y* ***C.cladosporioides*** *fueron los Hyphomycetes más representativos y los géneros* ***Aspergillus*** *y* ***Penicillium*** *constituyeron el 47,7% de los aislamientos. Los taxa más comunes que se presentaron al mismo tiempo en los pacientes como en su ambiente fueron:* ***Mycelia sterilia, C.sphaerospermum, A.sydowii, A.restrictus*** *y* ***P.implicatum.***

SUMMARY

Samples from oropharyngeal, nasal and from the external ear secretions were collected from 50 patients with the Acquired Immune Deficiency Syndrome (AIDS) and from the wards air in which they were begin treated. A total of 238 strains of filamentous fungi were obtained: 87 were clinical samples and 151 from the wards air. From clinical samples were isolated 29 species and 38 of the wards air. In the patient secretions, the form group ***Mycelia sterilia*** *and* ***Fusarium oxysporum, F.lateritium, Aspergillus parasiticus,*** *were the most representative Hyphomycetes, whereas genera* ***Aspergillus*** *and* ***Fusarium*** *represented 47% of isolations in similar proportions.*

In the environmental air of the hospital, the form group ***Mycelia sterilia*** *and* ***Cladosporium. sphaerospermum, Aspergillus sydowii, C.cladosporioides,*** *were the most representative Hyphomycetes, while the genera* ***Aspergillus*** *and* ***Penicillium,*** *represented 47,7% of isolations. Main taxa of fungi occurring at the same time both in patients and in the environmental were:* ***Mycelia sterilia, C.sphaerospermum, A.sydowii, A.restrictus*** *and* ***P.implicatum.***

INTRODUCTION

The immunological alterations exhibited by the AIDS infected patient, involve qualitative and quantitative defects in the T-lymphocytes with a consequent imbalance in the immunological system leading to suppressive stimuli and the occurrence of opportunistic infections (5, 10, 25, 36, 37, 40).

Factors whether linked or not to host explain the large incidence of fungal infections, mainly in hospital environments, where nosocomial mycota is rich in fungi

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called contaminating fungi (20, 21, 22).

Species of fungi denominated as anemophiles are responsible for allergic conditions or for opportunistic mycosis, the latter being the main cause of morbidity and mortality of HIV infected patients. These fungi have acquired in medical practice a remarkable increasing importance in hospital environments and in services where patients have been kept with various pathologies (4, 9, 10, 15, 16, 17, 22, 23, 24, 27, 30, 33, 35).

In the last decades the presence of fungi in the atmospheric air has been studied more strongly and is, at the moment, a theme of great importance since it is common both in allergic processes of the respiratory tracts and in infections by opportunistic agents in hospital environments (15, 18, 20, 21, 22, 28, 32).

In the atmospheric air of the Department of Infections Parasitical Disease, University Hospital, Center of Health Sciences, Federal University of Pernambuco-UFPE, there are wards in which AIDS patients were assisted for treatment.

Having in mind the occurrence of fungal infections in AIDS patients and the viable fungal propagules in hospital environments, the objectives of this paper are to isolate and identify fungi in AIDS patient secretions and from the enveronmental air in which they are being treated.

MATERIAL AND METHODS

Clinical samples were collected simultaneously of oropharyngeal, nasal and from the external ear secretions of 50 AIDS patients; at the same time air samples from 7 wards in which these patients were being treated, were collected. The collections were developed during 4 months from february to may 1996, at a 28°C ± 1°C room temperature, in the Infectious Parasitical Diseases Department - DIP, University Hospital, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

The clinical samples were collected from patients that had been in Hospital at least for one week on fast with a sterilised swab which was transferred to a tube containing sterilised water which 50 mg of chlororamphenicol/l. The wards air samples were collected at three equidistant points by the aspirating hand vacuum type microsyringe system, with a 13mm Millipore membrane and 8 µm pore.

After collection both the clinical samples and the air samples were duly processed for direct examination (clinical samples) and culture (clinical and air samples).

The direct examination of the clinical samples was carried out at room temperature, clarified with aqueous solution at 20% KOH and coloured by the Gram and Giemsa methods.

To obtain culture from the clinical and air samples, Sabouraud agar was used to which 50 mg of chloramphenicol /l and 5% of yeast extract (YE) was added.

The clinical samples were sown in duplicate on the surface of the medium contained on Petri dishes and maintained at room temperature (RT= 28° C +/- 1° C).

To obtain cultures from the air collections, the Millipore membranes were placed in 1 ml of sterilised distilled water that was added 50 mg of chloramphenicol contained in test tubes and beaten in vortex. Both, suspensions and the membranes were separately sown in duplicate on the surface of the medium and maintained at RT.

The development of the colonies was mantained for up to 20 days. After purification, the colonies were transferred into Sabouraud agar additioned with 0,5% YE contained in tube.

Identification and classification of the fungi were made by consulting Domsch et al. (11), Ellis (13, 14), Nelson *et al.* (26), Pitt (29), Raper & Fennel (31).

RESULTS AND DISCUSSION

A total of 238 strains of filamentous fungi: 87 clinical and 151 from the wards air samples were obtained.

From clinical samples collected of 50 AIDS patients; 87 strains of filamentous fungi were obtained, of which 69 (77,53%) were from nasal, 17 (19,19%) from the external ear and 3 (3,39%) from oropharyngeal secretions (Table 1).

In the patient secretions the form group: ***Mycelia sterilia*** and ***Fusarium oxysporum, F.lateritium, Aspergillus parasiticus,*** were the most representative ***Hyphomycetes***, whereas genera ***Aspergillus*** and ***Fusarium*** represented 47% of isolations in similar proportions.

Species of ***Absidia, Aspergillus, Cladosporium, Coprinus, Eupenicillium, Eurotium, Fusarium, Paecilomyces, Penicillium, Pestalotiopsis, Phialophora, Trichoderma, Verticillium, Volutella, Xylaria,*** were isolated as well as representatives of the order **Aphyllophorales** and from the form group ***Mycelia sterilia*** **(Agonomycetales)**. Representatives of the order **Aphyllophorales**, ***A. sydowii*** and the form group ***Mycelia sterilia***, occurred in the three types of clinical samples; ***Penicillium implicatum*** occurred in nasal and external ear secretions; ***Phialophora richardsiae*** in oropharyngeal secretion; ***Aspergillus niger, A. parasiticus, A. restrictus, Volutella tristis*** and ***A. versicolor*** occurred in the external ear secretion; the other species occurred only in nasal secretion from which the greatest number of fungi were isolated. ***Mycelia sterilia*** and ***F. oxysporum*** predominated among the remaining fungi (Table 1).

Although the recognition of mycosis infections is of the greatest importance, not only from the epidemio-

logical but also by the therapeutic point of view, it is not always frequent that contact with the infecting agent of mycotic origin leads to the appearance of mycosis disease (25).

On the other hand, allergic processes arise from inhalation and ingestion of spores which induce various manifestations such as a leaking nose, rhinitis, bronchial asthma or conjunctivitis (22).

In this paper the predominance of filamentous fungi isolated from nasal secretion is supported by the results obtained by Choi (8) with the same types of clinical samples. The quantity and variety of filamentous fungi isolated from nasal secretion may be a result of the function of nostrils, acting as a natural filter of the respiratory tracts.

Fusarium solani, F. oxysporum, F. lateritium, Aspergillus niger, A. versicolor, A. ochraceus, Absidia corymbifera, Paecilomyces variotii, Coprinus sp. have been reported as a species of fungi which easily causes infirmity in the weakened patients (2, 7, 12, 22, 26, 31, 33, 34, 38, 39).

One hundred and twenty nine collections from the air of seven wards denominated by the number 5 to 11 were taken , and 954 ufc/ ml, were counted out of which 151 filamentous fungi strains were obtained, being 4 (2,65%) from ward 5; 60 (39,73%) from ward 6; 17 (11,26%) from ward 7; 18 (11,92%) from ward 8; 19 (12,58%) from ward 9; 7 (4,64%) from ward 10 and 26 (17,22%) from ward 11 (Table 2). In the hospital environment, the form group *Mycelia sterilia* and species *C. sphaerospermum, Aspergillus sydowii* and *C.cladosporioides*, were the most representative **Hyphomycetes**, whereas genera *Aspergillus* and *Penicillium* representes 47,7% of isolations. Strains of species of the genera *Aspergillus, Cephaliophora, Cladosporium, Eurotium, Penicillium, Phialophora, Rhizopus, Trichoderma* and *Tritirachium*, were obtained, as well as representatives of the order **Aphyllophorales** and from the form group *Mycelia sterilia* (Table 2).

A.sydowii and *C.sphaerospermum* occurred in six wards; *Mycelia sterilia* and *Penicillium fellutanum* in five wards; *A. flavipes, A. terreus, C.cladosporioides, P. aurantiogriseum complex, P. janthinellum, P. implicatum* and *Tritirachium dependens*, occurred in 3 wards; *A. caespitosus, A. restrictus, A. tamarii, Eurotium chevalieri, P. decumbens, P. expansum, P. lividum, P. melinii, P. restrictus* and *Phialophora richardsiae* ocurred in 2 wards; strains of order **Aphyllophorales** and the other species occurred in one ward (Table 2).

Cladosporium tenuissimum and *Rhizopus oryzae* ,were isolated from the air and from the floor of enclosed hospital environments (3); *C. cladosporioides* and *P. richardisiae* are cited as ethiological agent of mycosis (7, 22, 32, 34, 39). The species *C. cucumerinum* (13, 14), *T. dependens* (19) and *Eurotium chevalieri* (1) are related as phytopathogens; the species *P. fellutanum, P. janthinellum, P. aurantiogriseum* complex, *P. lividum, P. decumbens, P.melinii, P. restrictum, P.expansum, P. citreonigrum, P. citrinum, P.corylophylum, P.funiculosum, P.janczeweskii, P. solitum* and *P. thomii* obtained in this work, have also been isolated from the air of enclosed hospital environments by Barros et al. (3), from the soil and dry foodstuffs (29); *Trichoderma pseudokoningii* is a saprobes found in the soil and recognised as an antagonist of great potencial (1, 6); among the species of the order **Aphyllophorales** that are saprobes in your majority, some species are found parasiting plants (2).

In the 113 strains of filamentous fungi occurred in the clinical and the wards air samples (Table 3), *A. sydowii* ocurred in all clinical samples and in 6 wards; *Mycelia sterilia* ocurred in all clinical samples and 5 wards; *C. sphaerospermum* in one clinical samples and 6 wards; *P. implicatum* in 2 clinical samples and 3 wards; *A. restrictus, Eurotium chevalieri* and *Phialophora richardsiae* in one clinical sample and 2 wards; **Aphyllophorales** and others species in one clinical sample and in one ward (Table 3).

These results suggest that propagules of fungi present in the wards air vary qualitatively and quantitatively.

The species *A. flavus,A. restrictus,* and *A. sydowii* are related as pathogenic to man and animals (22). *A. flavus* and *A.sydowii* there were isolated of the air closed environments of hospitals (3). According to Raper & Fennel (31), *A. flavus* is found in the soil and vegetable remains and *A. sydowii* in the soil and humid air.

C. sphaerospermum occurs occasionally in man, in animals (14, 15), in several species of plants and in the soil (1).

Mycelia sterilia is a group of fungi which do not express the capacity to form spores but have been isolated from vegetables and soil. There have been no accounts of the isolation of human substracts (2).

P. implicatum was isolated from the air and the floor of enclosed hospital environments (3), and they have also been isolated from the soil, and dry foodstuffs and as a result of this is considered a biodeteriogenic (29); *Eurotium chevalieri* is a phytopathogen (1); *Phialophora richardsiae* is an ethiological agent of mycosis (7, 33, 35, 40).

Allergy causing fungi belong to various genera and species of which almost all are contaminating and easily isolated from the atmospheric air (2). According to Faria (15), Heiman (18), Osvath *et al.*, (28) and Rippon, (32), there is a correlation of fungi with allergic process because

there is a big amount of allergenic fungi in the air. Allergic processes arose from inhalation of spores, ingestion or through circulating allergens which cause various clinical manifestations (22).

It was not possible to trace a profile of fungi that occurred in the clinical and ward air samples, because the number of patients as well as the time of hospitalization of the latter in the wards varied a great deal. The number of patients changed due to their recovery. In some wards it was not possible to collect air at a single time, because there were no patients in the marked days for two follwing collect. Most of the patients in the marked days for two following collections. Most of patiens were not in the same ward, periods of time ranging from 24 h and up more than 30 days, depending on clinical state. Consequently, work could not be accomplished in a sequential order.

Therefore the lesser or the major number of isolated fungi from the ward air, did not represent a lesser or a major frequency of occurrence of the species in the wards.

Table 1.- Filamentous fungi isolated from AIDS patients (University Hospital).

Fungi	Clinical samples			Total of samples	%
	OFS	NS	EES		
Absidia corymbifera	-	02	-	02	2,30
Aphyllophorales	01	03	02	06	6,90
Aspergillus aculeatum	-	01	-	01	1,15
A. flavus	-	01	-	01	1,15
A. japonicus	-	02	-	02	2,30
A. melleus	-	01	-	01	1,15
A. niger	-	-	02	02	2,30
A. ochraceus	-	01	-	01	1,15
A. parasiticus	-	-	04	04	4,60
A. restrictus	-	-	03	03	3,45
A. sclerotiorum	-	01	-	01	1,15
A. sydowii	01	01	01	03	3,45
A. versicolor	-	-	01	01	1,15
Cladosporium sphaerospermum	-	02	-	02	2,30
Coprinus sp.	-	01	-	01	1,15
Eupenicillium javanicum	-	01	-	01	1,15
Eurotium chevalieri	-	01	-	01	1,15
Fusarium lateritium	-	05	-	05	5,75
F. oxysporum	-	11	-	11	12,65
F. solani	-	04	-	04	4,60
Mycelia sterilia	01	17	02	20	23,00
Paecilomyces variotii	-	01	-	01	1,15
Penicillium implicatum	-	01	02	03	3,45
Pestalotiopsis guepini	-	01	-	01	1,15
Phialophora richardsiae	01	-	-	01	1,15
Trichoderma harzianum	-	01	-	01	1,15
T. koningii	-	01	-	01	1,15
T. pseudokoningii	-	01	-	01	1,15
Verticillium albo-atrum	-	01	-	01	1,15
Volutella tristis	-	-	02	02	2,30
Xylaria sp.	-	02	-	02	2,30
Total	04	64	19	87	100

OFS = oropharyngeal secretion; NS = nasal secretion; EES = external ear secretion.

Table. 2.- Filamentous fungi isolated from the ward air with AIDS patients in the University Hospital.

FUNGI	WARDS 5	6	7	8	9	10	11	TOTAL OF STRAINS	%
Aphyllophorales	-	-	01	-	-	-	-	01	0,66
Aspergillus asperescens	-	01	-	-	-	-	-	01	0,66
A. caespitosus	-	01	01	-	-	-	-	02	1,32
A. carbonarius	-	01	-	-	-	-	-	01	0,66
A. carneus	-	01	-	-	-	-	-	01	0,66
A. duricaulis	-	-	-	-	01	-	-	01	0,66
A. flavipes	-	05	-	01	-	-	01	07	4,63
A. flavus	-	-	-	-	01	-	-	01	0,66
A. niveus	-	-	01	-	-	-	-	01	0,66
A. restrictus	-	03	-	02	-	-	-	05	3,31
A. sydowii	-	03	01	02	03	01	03	13	8,61
A. tamarii	-	-	02	-	01	-	-	03	1,99
A. terreus	-	02	-	01	01	-	-	04	2,65
Cephaliophora tropica	-	01	-	-	-	-	-	01	0,66
Cladosporium cladosporioides	-	07	01	-	-	-	02	10	6,62
C. cucumerinum	04	-	-	-	-	-	-	04	2,65
C. sphaerospermum	-	10	02	03	02	01	02	20	13,24
C. tenuissimum	-	-	-	01	-	-	-	01	0,66
Eurotium chevalieri	-	01	-	-	-	-	01	02	1,32
Mycelia sterilia	-	07	06	01	-	01	08	23	15,23
Penicillium aurantiogriseum comp.	-	01	-	01	01	-	-	03	1,99
P. citreonigrum	-	-	-	-	01	-	-	01	0,66
P. citrinum	-	01	-	-	-	-	-	01	0,66
P. corylophylum	-	-	-	-	-	-	01	01	0,66
P. decumbens	-	-	-	01	-	-	01	02	1,32
P. expansum	-	01	-	-	01	-	-	02	1,32
P. fellutanum	-	02	-	01	02	02	01	08	5,30
P. funiculosum		01	-	-	-	-	-	01	0,66
P. janczeweskii	-	-	-	-	-	01	-	01	0,66
P. janthinellum	-	06	-	-	01	-	01	08	5,30
P. implicatum	-	-	-	01	-	01	01	03	1,99
P. lividum	-	02	-	-	01	-	-	03	1,99
P. melinii	-	01	-	-	01	-	-	02	1,32
P. restrictus	-	-	01	-	01	-	-	02	1,32
P. solitum	-	01	-	-	-	-	-	01	0,66
P. thomii	-	-	-	-	-	-	01	01	0,66
Phialophora richardsiae	-	01	-	01	-	-	-	02	1,32
Rhizopus oryzae	-	-	-	-	-	-	01	01	0,66
Tritirachium dependens	-	-	01	02	01	-	-	04	2,65
Trichoderma pseudokoningii	-	-	-	-		-	02	02	1,32
Total	**04**	**60**	**17**	**18**	**19**	**07**	**26**	**151**	**100%**

Table. 3.- Filamentous fungi isolated from AIDS patients and from the ward air in which they were treated (University Hospital)

FUNGI	SAMPLES CLINICAL			NS	WARDS							NS	TS	%
	OFS	NS	EES		5	6	7	8	9	10	11			
Aphyllophorales	-	06	-	06	-	-	01	-	-	-	-	01	07	6,78
Aspergillus flavus	-	01	-	01	-	-	-	-	01	-	-	01	02	1,69
A. restrictus	-	-	03	03	-	03	-	02	-	-	-	05	08	7,63
A. sydowii	01	01	01	03	-	03	01	02	03	01	03	13	16	13,56
Cladosporium sphaerospermum	-	02	-	02	-	10	02	03	02	01	02	20	22	21,19
Eurotium chevalieri	-	01	-	01	-	01	-	-	-	-	01	02	03	2,54
Mycelia sterilia	01	17	02	20	-	07	06	01	-	01	08	23	43	36,44
Penicillium implicatum	-	01	02	03	-	-	-	01	-	01	01	03	06	5,09
Phialophora richardsiae	01	-	-	01	-	01	-	01	-	-	-	02	03	2,54
Trichoderma pseudokoningii	-	01	-	01	-	-	-	-	-	-	02	02	03	2,54
Total	**03**	**30**	**08**	**41**	**-**	**25**	**10**	**10**	**06**	**04**	**17**	**72**	**113**	**100**

OFS = oropharyngeal secretion; NS = nasal secretion; EES = external ear secretion;
NS = Number of samples; TS = Total of samples.

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