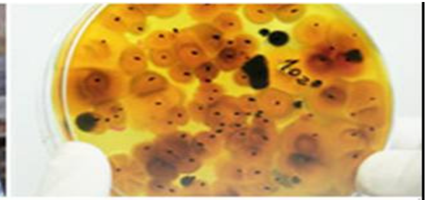


Occupational exposures and diseases

Raquel Sabino

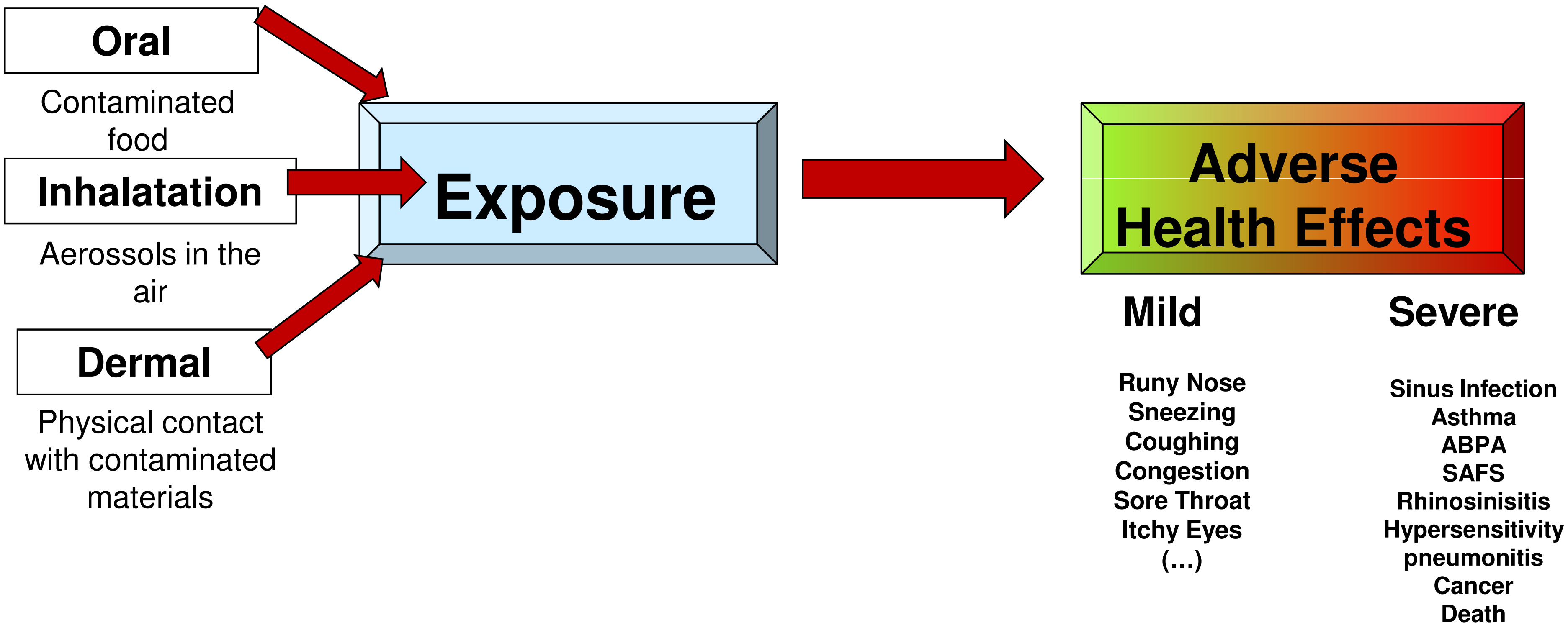
Department of Infectious Diseases

Reference Unit for Parasitic and Fungal Infections

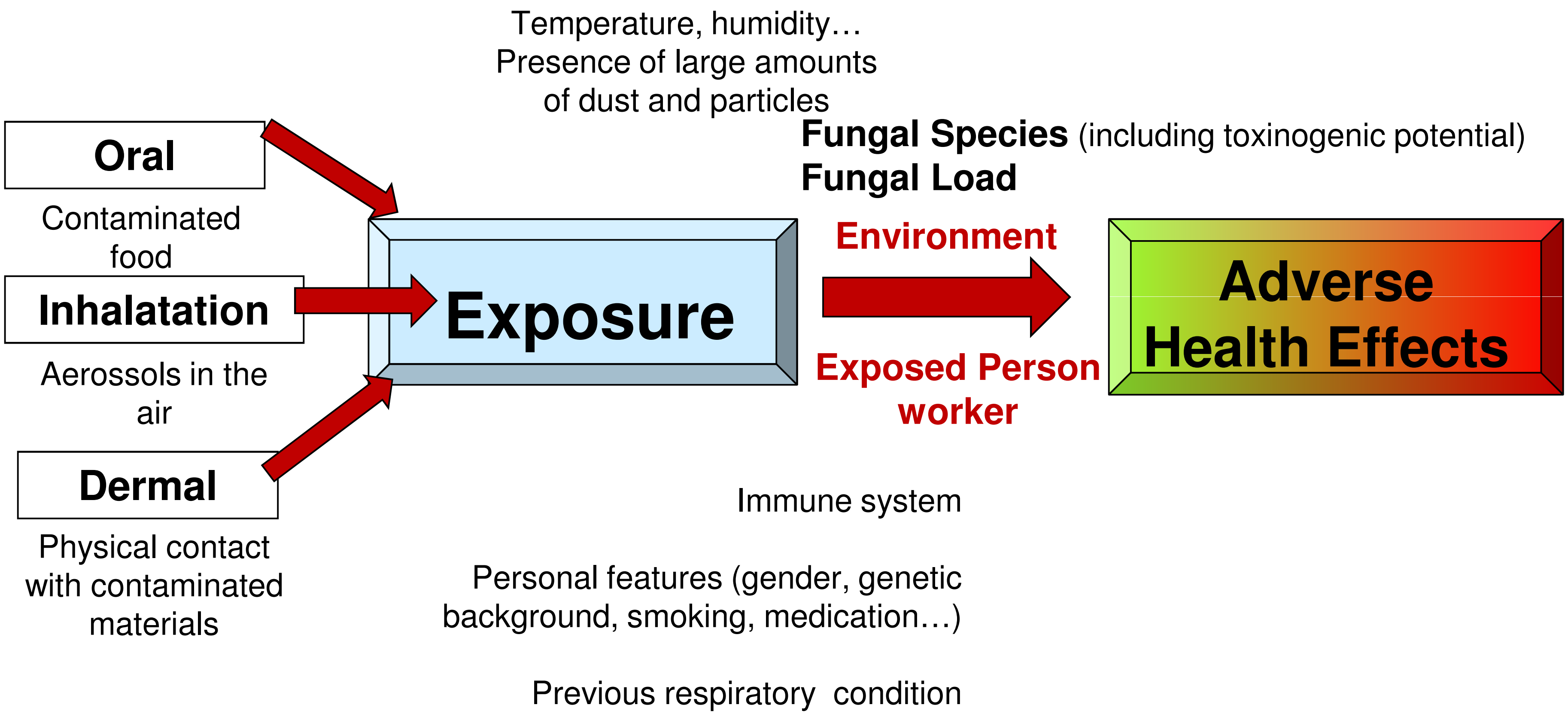
Summary

- Occupational exposure and sources of fungal exposure
- *Aspergillus* exposure
- Diseases associated with *Aspergillus* occupational /indoor exposures
- Case studies on *Aspergillus* occupational exposure

Occupational exposure vs. development of fungal disease



Occupational exposure vs. development of fungal disease



Exposure to Fungal conidia

- Home
- Workplace
- During hospitalization (especially during construction or renovation work)



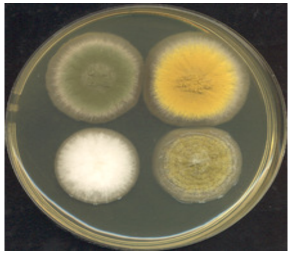


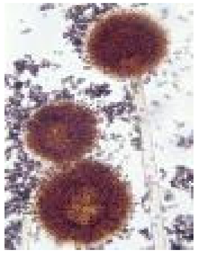
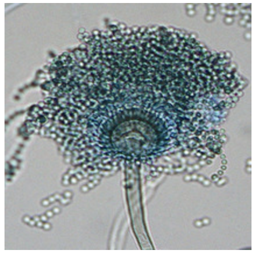
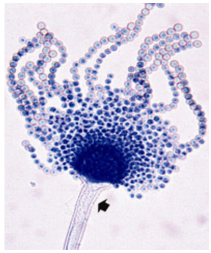



Occupational exposures to fungi

Most important fungi related with fungal exposure:

- *Cladosporium*
- *Alternaria*
- *Stachybotris*
- *Penicillium*
- *Aspergillus*




Aspergillus conidia

With dry wall spores, dessication-resistant

Very light

Produced in large amounts

Easy to dissociate

Very easy to disperse

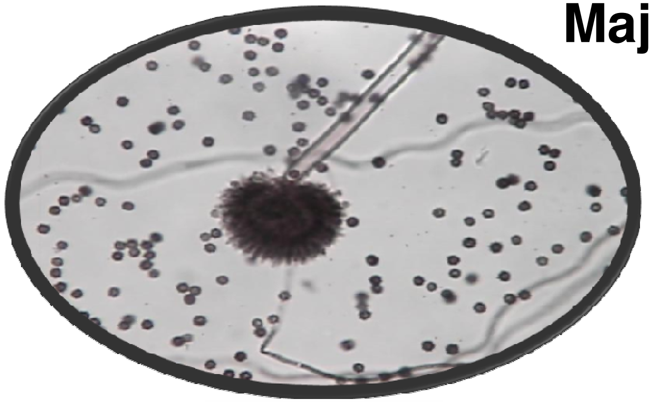
Long time in the air, associated to other particles

Easily airborne and inhaled

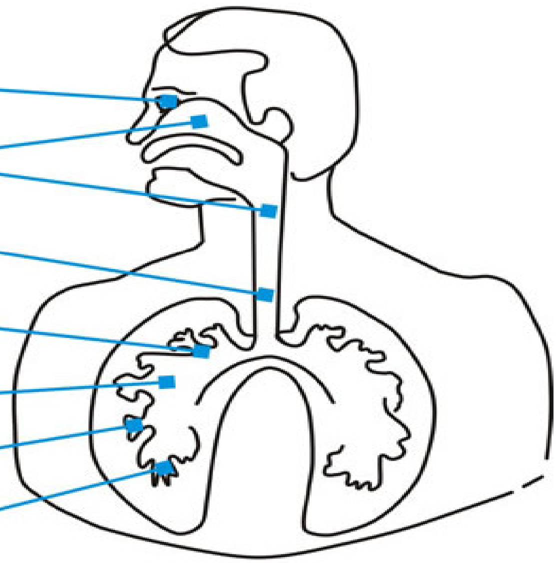
Why are *Aspergillus* so commonly found in occupational environments?

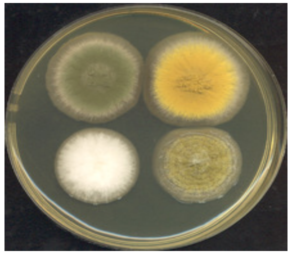
- Ability to grow at a high range of temperatures
- High nutritional versatility
- Moisture environments
- Good growth on a high variety of construction materials (concrete, acrylic paints, wood based and cellulose based materials)
- Associated with decomposing organic mater

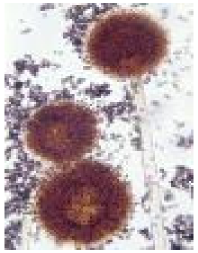
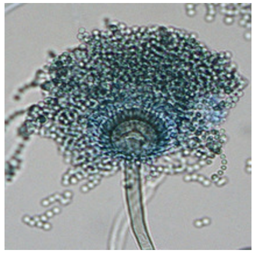
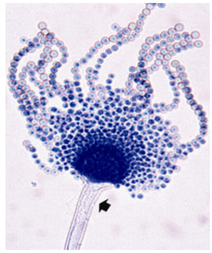
Major route of occupacional disease is inhalation



Particle size	Fate
9 - 30 µm	visual pollution
5.5 - 9 µm	settle in nose/throat
3.3 - 5.5 µm	lodge in main breathing passages
2 - 3.3 µm	lodge in small breathing passages
1 - 2 µm	lodge in bronchi
0.3 - 1 µm	penetrate to bronchioles and alveoli
0.1 - 0.3 µm	penetrate to bronchioles and alveoli



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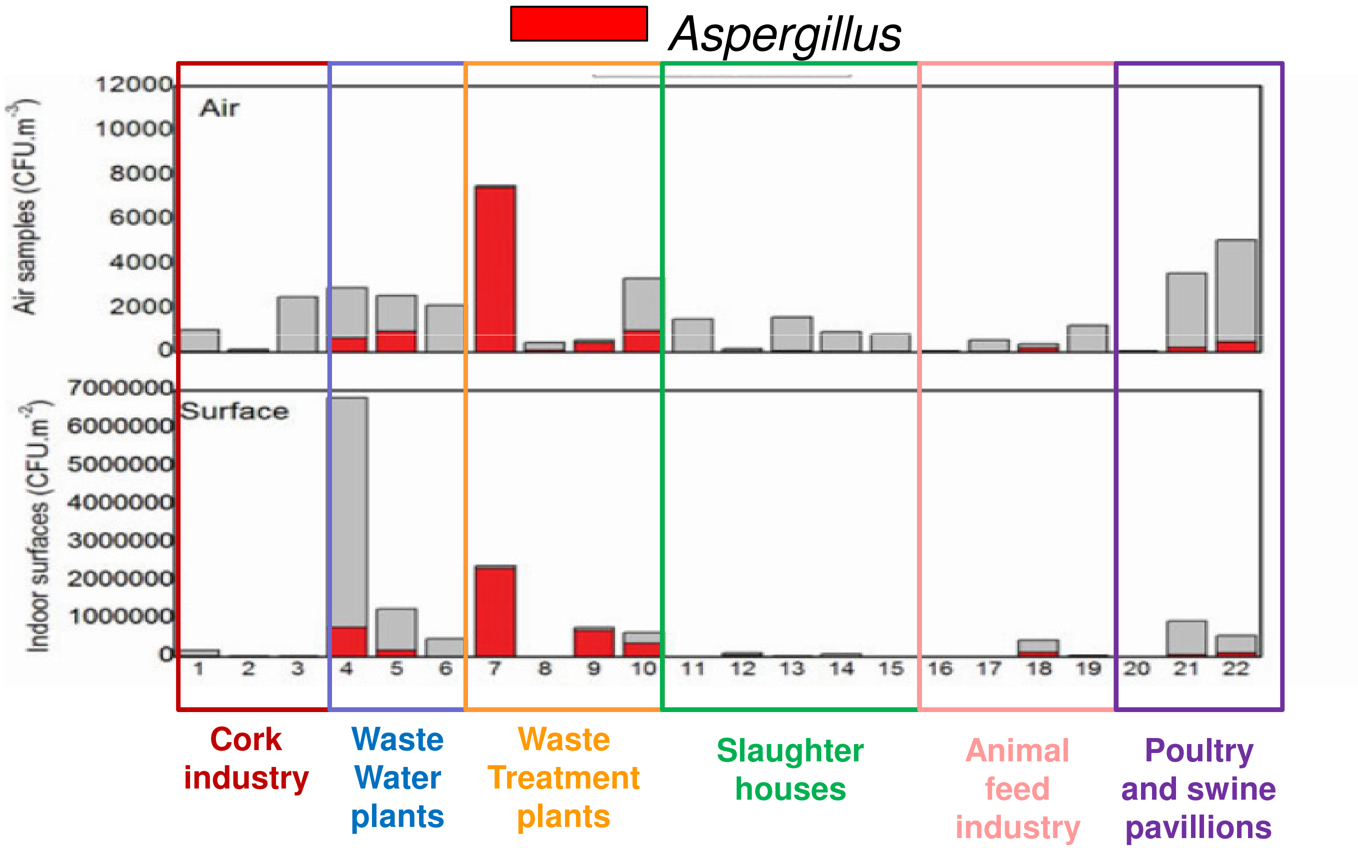
- Associated with decomposing organic mater

Prevalence of *Aspergillus* spp. in highly contaminated occupational environments



125 air samples
125 surface samples

Viegas C, Faria T, Aranha Caetano L, Carolino E, Quintal Gomes A, Viegas S (2017) Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?, Journal of Occupational and Environmental Hygiene, 14:10, 771-785

OPEN ACCESS Freely available online PLOS | ONE

A Murine Inhalation Model to Characterize Pulmonary Exposure to Dry *Aspergillus fumigatus* Conidia

Amanda D. Buskirk[1], Brett J. Green[1], Angela R. Lemons[1], Ajay P. Nayak[1], W. Travis Goldsmith[2], Michael L. Kashon[3], Stacey E. Anderson[1], Justin M. Hettick[1], Steven P. Templeton[1], Dori R. Germolec[4], Donald H. Beezhold[1]*

1 Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, United States of America, **2** Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, United States of America, **3** Biostatistics and Epidemiology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, United States of America, **4** Toxicology Branch, National Toxicology Program Division, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, United States of America

Abstract

Most murine models of fungal exposure are based on the delivery of uncharacterized extracts or liquid conidia suspensions using aspiration or intranasal approaches. Studies that model exposure to dry fungal aerosols using whole body inhalation have only recently been described. In this study, we aimed to characterize pulmonary immune responses following repeated inhalation of conidia utilizing an acoustical generator to deliver dry fungal aerosols to mice housed in a nose only exposure chamber. Immunocompetent female BALB/cJ mice were exposed to conidia derived from *Aspergillus fumigatus* wild-type (WT) or a melanin-deficient (*Δalb1*) strain. Conidia were aerosolized and delivered to mice at an estimated deposition dose of 1×10^5 twice a week for 4 weeks (8 total). Histopathological and immunological endpoints were assessed 4, 24, 48, and 72 hours after the final exposure. Histopathological analysis showed that conidia derived from both strains induced lung inflammation, especially at 24 and 48 hour time points. Immunological endpoints evaluated in bronchoalveolar lavage fluid (BALF) and the mediastinal lymph nodes showed that exposure to WT conidia led to elevated numbers of macrophages, granulocytes, and lymphocytes. Importantly, $CD8^+$ $IL17^+$ (Tc17) cells were significantly higher in BALF and positively correlated with germination of *A. fumigatus* WT spores. Germination was associated with specific IgG to intracellular proteins while *Δalb1* spores elicited antibodies to cell wall hydrophobin. These data suggest that inhalation exposures may provide a more representative analysis of immune responses following exposures to environmentally and occupationally prevalent fungal contaminants.

Diseases associated with occupational/indoor exposures to *Aspergillus*

1) Allergic and other hypersensivity responses

2) Mycotoxicosis

3) Irritant effects caused by mold exposure

4) Opportunistic infection

Diseases associated with occupational/indoor exposures to *Aspergillus*

1) Allergic and other hypersensivity responses

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3) Irritant effects caused by mold exposure

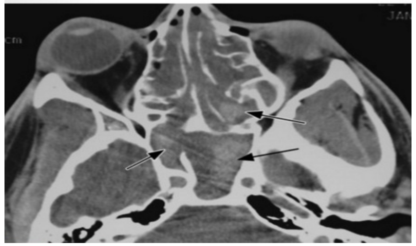
4) Opportunistic infection

- It is estimated 3 to 10% of the world's population is allergic to molds

Hamilos DL.Proc Am Thorac Soc. 2010 7(3):245-52.

- *Aspergillus* cause a large number of allergic diseases: allergic bronchopulmonary aspergillosis, rhinitis, allergic sinusitis and hypersensitivity pneumonitis.

RHINITIS AND ALLERGIC SINUSITIS



https://pt.slideshare.net/saritajie/fungalsinusitis?next_slideshow=2

Nasal congestion, sneezing, and runny or itchy nose.
Nasal obstruction with polyps

SAFS - SEVERE ASTHMA WITH FUNGAL SENSITIZATION

Occurs in patients with asthma
Fungal-associated (severe) asthma

Fungi responsible: Many, some alone, some collectively. *Aspergillus fumigatus, Penicillium chrysogenum, Cladosporium herbarum, Alternaria alternata, Candida albicans, Trichophyton* spp. and probably others.

ABPA –ALLERGIC BRONCOPULMONAR ASPERGILLOSIS

Occurs mainly in patients with asthma or cystic fibrosis

Hypersensivity reaction due to colonization of the bronchi with *Aspergillus*

Bronchial inflammation, obstruction, mucus accumulation, respiratory failure

http://www.aspergillus.org.uk

Am J Ind Med. 2000 Apr;37(4):438-42.

Two year follow-up of a garbage collector with allergic bronchopulmonary aspergillosis (ABPA).

Allmers H[1], Huber H, Baur X.

Author information

Abstract

BACKGROUND: Separate collection of biodegradable garbage and recyclable waste is expected to become mandatory in some western countries. A growing number of persons engaged in garbage collection and separation might become endangered by high loads of bacteria and fungi. Case history and examination A 29 year old garbage collector involved in emptying so-called biological garbage complained of dyspnea, fever, and flu-like symptoms during work beginning in the summer of 1992. Chest x-ray showed streaky shadows near both hili reaching into the upper regions. IgE- and IgG-antibodies (CAP, Pharmacia, Sweden) were strongly positive for Aspergillus fumigatus with 90.5 kU/L and 186%, respectively. Total-IgE was also strongly elevated with 5430 kU/L. Bronchial challenge testing with commercially available Aspergillus fumigatus extract resulted in an immediate-type asthmatic reactior Two years later he was still symptomatic and antibodies persisted at lower levels.

CONCLUSIONS: Our diagnosis was allergic bronchopulmonary aspergillosis (ABPA) including asthmatic responses as well as hypersensitivity pneumonitis (extrinsic allergic alveolitis) due to exposure to moldy household waste. A growing number of persons engaged in garbage collection and handling are exposed and at risk to develop sensitization to fungi due to exposure to dust of biodegradable waste. Further studies are necessary to show if separate collection of biodegradable waste increases the health risks due to exposure to bacteria and fungi in comparison to waste collection without separation.

Aspergillus fumigatus

Chest. 1987 Feb;91(2):285-6.

Allergic bronchopulmonary aspergillosis due to Aspergillus oryzae.

Akiyama K, Takizawa H, Suzuki M, Miyachi S, Ichinohe M, Yanagihara Y.

Abstract

A 19-year-old female student with allergic bronchopulmonary aspergillosis (ABPA) due to Aspergillus oryzae is reported. This organism was used for fermentation starter to make soybean paste in her father's workshop adjacent to the home where she lived. ABPA might be considered an occupational disease in certain situations.

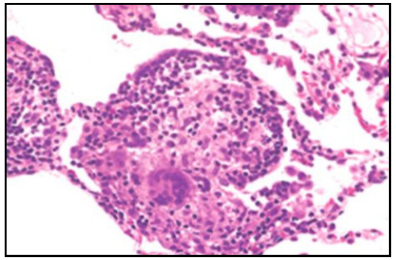
PMID: 2433099

Soy sauce brewer lung

Aspergillus oryzae

HYPERSENSITIVITY PNEUMONITIS

- Inflammation of the alveoli within the lung caused by hypersensitivity to inhaled organic dusts with a large variety of antigens.



- Sufferers are commonly exposed to the dust by their occupation or hobbies.
- Hypersensitivity pneumonitis associated with *Aspergillus*:

Tobacco worker's lung
Aspergillus spp.

Compost lung
Aspergillus spp.

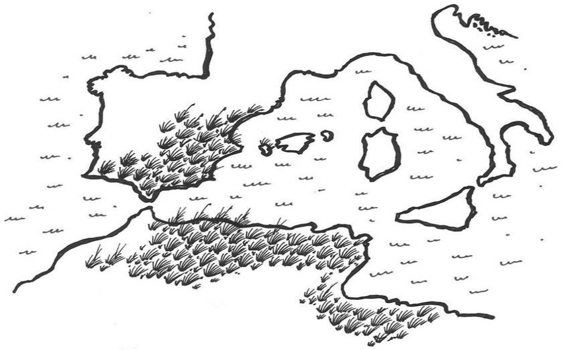
Farmer's lung
Aspergillus spp.

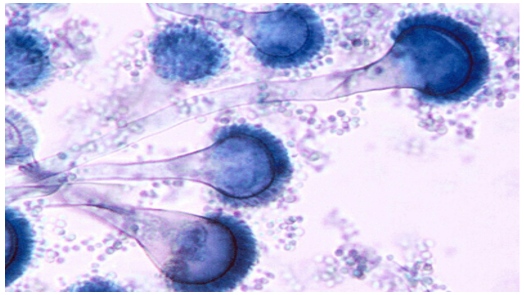
Malt worker's lung
A. clavatus

A. fumigatus **associated to 80% of the cases**

Esparto grass lung

Stipa tenacissima





Familiar presentation of occupational hypersensitivity pneumonitis caused by aspergillus-contaminated esparto dust

A. Moreno-Ancillo[a], C. Domínguez-Noche[a], A. Carmen Gil-Adrados[b] and P.M. Cosmes[a]

[a]Allergy Unit. Hospital Virgen del Puerto. Plasencia (Cáceres). Spain. [b]Centro de Salud. Castillo de Bayuela (Toledo). Spain.

Hypersensitivity pneumonitis in workers exposed to esparto grass *(Stipa tenacissima)* fibers

Miguel Hinojosa, MD,[a] Juan Fraj, MD,[a] Belen De La Hoz, MD,[a] Raimundo Alcazar, MD,[b] and Antonio Sueiro, MD[c] *Madrid and Jaen, Spain*

Instituto_Nacional de Saúde 1899 www.insa.pt

Journal of Toxicology and Environmental Health, Part A, 75:1381–1391, 2012

ISSN: 1528-7394 print / 1087-2620 online
DOI: 10.1080/15287394.2012.721170

Taylor & Francis
Taylor & Francis Group

OCCUPATIONAL EXPOSURE TO *ASPERGILLUS* BY SWINE AND POULTRY FARM WORKERS IN PORTUGAL

R. Sabino[1*], V. M. Faísca[3], E. Carolino[4], C. Veríssimo[1], C. Viegas[2]

TABLE 1. Quantitative Results Obtained From *Aspergillus* Isolation From the Air, Surfaces and Coverage of the Floor in the Analyzed Settings

	Poultry farms			Swine		
	Air (CFU/m^3) (range)	Surfaces (CFU/m^2) (range)	Coverage of the floor (CFU/g) (range)	Air (CFU/m^3) (range)	Surfaces (CFU/m^2) (range)	Coverage of the floor (CFU/g) (range)
A. candidus	0–40	$0–1 \times 10^{-4}$	$0–5 \times 10^{4}$	0–60	$0–1 \times 10^{-4}$	0
A. clavatus	0	$0–2 \times 10^{-4}$	$0–1 \times 10^{4}$	0–20	$0–2 \times 10^{-4}$	0
A. clavatus-nanicus	0	0	$0–1 \times 10^{3}$	0	0	0
A. flavus	40–>2000	$0–>1 \times 10^{-2}$	$0–12.5 \times 10^{3}$	0–20	$0–1 \times 10^{-4}$	0
A. fumigatus	0–80	$0–1.1 \times 10^{-3}$	$0–1 \times 10^{4}$	0–100	$0–1 \times 10^{-4}$	0
A. glaucus	0–40	$0–1 \times 10^{-4}$	0	0–40	$0–6 \times 10^{-4}$	0
A. niger	0–80	$0–1 \times 10^{-4}$	0	0–20	$0–2 \times 10^{-4}$	0
A. ochraceus	0	$0–2 \times 10^{-4}$	0	0–20	$0–6 \times 10^{-4}$	0
A. terreus	0	$0–1 \times 10^{-4}$	0	0–100	$0–1 \times 10^{-4}$	0
A. ustus	0	0	0	0–240	$0–1 \times 10^{-4}$	0
A. versicolor	40–960	$0–>3 \times 10^{-2}$	0	0–>2000	$0–3 \times 10^{-2}$	$0–5 \times 10^{3}$
Aspergillus spp.	0–220	0	$0–1 \times 10^{4}$	0–40	$0–6 \times 10^{-4}$	0

Note. Aspergillus spp. refers to not identified species or species with lower representativeness, such as *A. sidowii* and *A. niveus*.

80 workers analyzed
47 (58.8%) workers in poultry
33 (41.2%) in swine farms

Incidence of diagnosed asthma was 8.8%

5% occupational asthma

High prevalence of respiratory symptoms in professionals without asthma

- wheezing associated with dyspnea (24%)
- dyspnea after strenuous activities (12%)
- sneezing, runny nose, or nasal stuffiness (19%)

Diseases associated with occupational exposures/indoor to *Aspergillus*

1) Allergic and other hypersensivity responses

2) Mycotoxicosis

3) Irritant effects caused by mold exposure

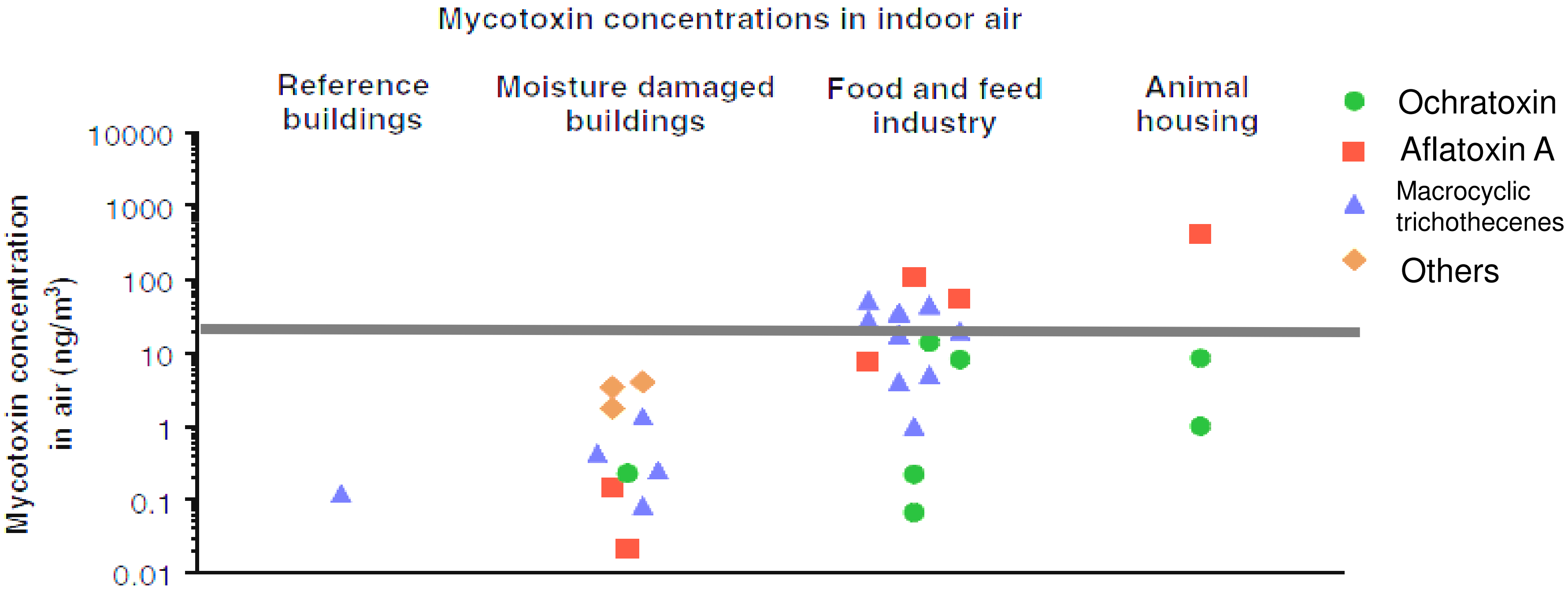
4) Opportunistic infection

Most important mycotoxins produced by *Aspergillus*

Mycotoxin	*Aspergillus* Species	Target organ	Acute toxicity	Chronic Toxicity
Aflatoxins (AFB1)	*A. flavus* *A. parasiticus*	Liver	Gastrointestinal disturbance, jaundice, photosensitivity, hepatosplenomegaly, ascites, coma, death	Chronic liver injury, cirrhosis, ascites, liver carcinoma
Ochratoxins (OTA)	*A. ochraceus* *A. alliaceus* *A. auricomus* *A. carbonarius* *A. melleus* *A. niger* (…)	Kidney	Acute renal failure	Chronic renal injury in Balkan endemic nephropathy
Sterigmatocystin	*A. versicolor*	Liver	hepatosplenomegaly	liver carcinoma

Adapted from: Peraica M, Mycotoxicosis. InViegas C, Viegas S, Sabino R, Pinheiro C, Brandão J, Veríssimo C (ed) Environmental Mycology in Public Health: An overview on Fungi and Mycotoxins Risk Assessment and Management. Elsevier, Oxford (2015). ISBN: 978-0-12-411471-5

Airborne concentrations of mycotoxins found in different occupational settings



Adapted from: K. Huttunen and M. Korkalainen. Microbial Secondary Metabolites and Knowledge on Inhalation Effects. In: Viegas C, Viegas S, Quintal-Gomes A, Taubel M, Sabino R (Eds), Exposure to Microbiological Agents in Indoor and Occupational Environments. Springer (2017). ISBN: 978-3-319-61688-9

Detection of AFB1 in the serum of poultry and swinne workers

Group	N				Range level of AFB1 (ng/ml)	Mean
Swinne workers	34	Positive to AFB1	18	53%	≥1 - 4,23	1,36
		Negative to AFB1	16			
Poultry workers	11	Positive to AFB1	6	55%	≥1 - 8,94	1,05
		Negative to AFB1	7			
Control	30	Positive to AFB1	0			
		Negative to AFB1	30		<1	

Occupational exposure to AFB1 by inhalation

Viegas S, Veiga L, Figueiredo P, Almeida A, Carolino E, Sabino R, Veríssimo C, Viegas C (2013). Occupational exposure to Aflatoxin B1: the case of poultry and swine production. World Mycotoxin Journal; 6(3): 309-315,

Diseases associated with occupational/indoor exposures to *Aspergillus*

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Molds produce a number of potentially irritating substances that can be divided into microbial volatile organic compounds (**MVOCs**) and particulates (eg, **spores, hyphae fragments, and their components like glucans, chitins …**).

TABLE 1 Commonly Detected MVOC

Alcohols	**Esters**
1-Butanol	Isobutyl acetate
2-Butanol	Ethyl isobutyrate
2-Methyl-1-butanol	Ethyl-2-methylbutyrate
2-Methyl-1-propanol	**Ketones**
3-Methyl-1-butanol	2-Heptanone
3-Methyl-2-butanol	2-Hexanone
3-Octanol	3-Octanone
1-Octen-3-ol	**Terpenoids**
2-Octen-1-ol	Geosmin
2-Pentanol	2-Methylisoborneol
Ethers	**Sulfur and nitrogen compounds**
3-Methylfuran	Dimethyl disulfide
2-Pentylfuran	2-Isopropyl-3-methoxy pyrazine

Nasal congestion

Airway hyperreactivity

Upper and lower respiratory symptoms

Eye irritations

Headache

Fatigue/tiredness

Joint pains

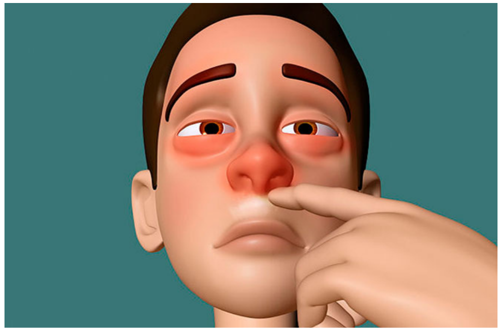
Skin symptoms

Flu-like symptoms

Nausea

Gastro-intestinal symptoms

Contradictory studies showing (or not) this possible relashionship



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Nosocomial vs. Community-acquired aspergillosis

Occupational and indoor exposure

Nosocomial	Community acquired
• Due to a break in or contamination of hospital water system • Due to a break in HEPA filtration system • Due to construction or demolition work in the hospital or near by • Other sources of *Aspergillus* found in hospital environment: Foods, Plants, Fabrics, Mattresses and pillows, Computers	• Due to the air quality/ *Aspergillus* burden in the patients' houses • Due to occupational or leisure activities developed leading to direct exposure to high amount of conidia

Adapted from: Praz-Christinaz *et al.* (2007). *Transplant Infect Dis*; 9: 175-181

Transpl Infect Dis. 2007 Sep;9(3):175-81. Epub 2007 May 19.

Occupational risk assessment of aspergillosis after renal transplantation.

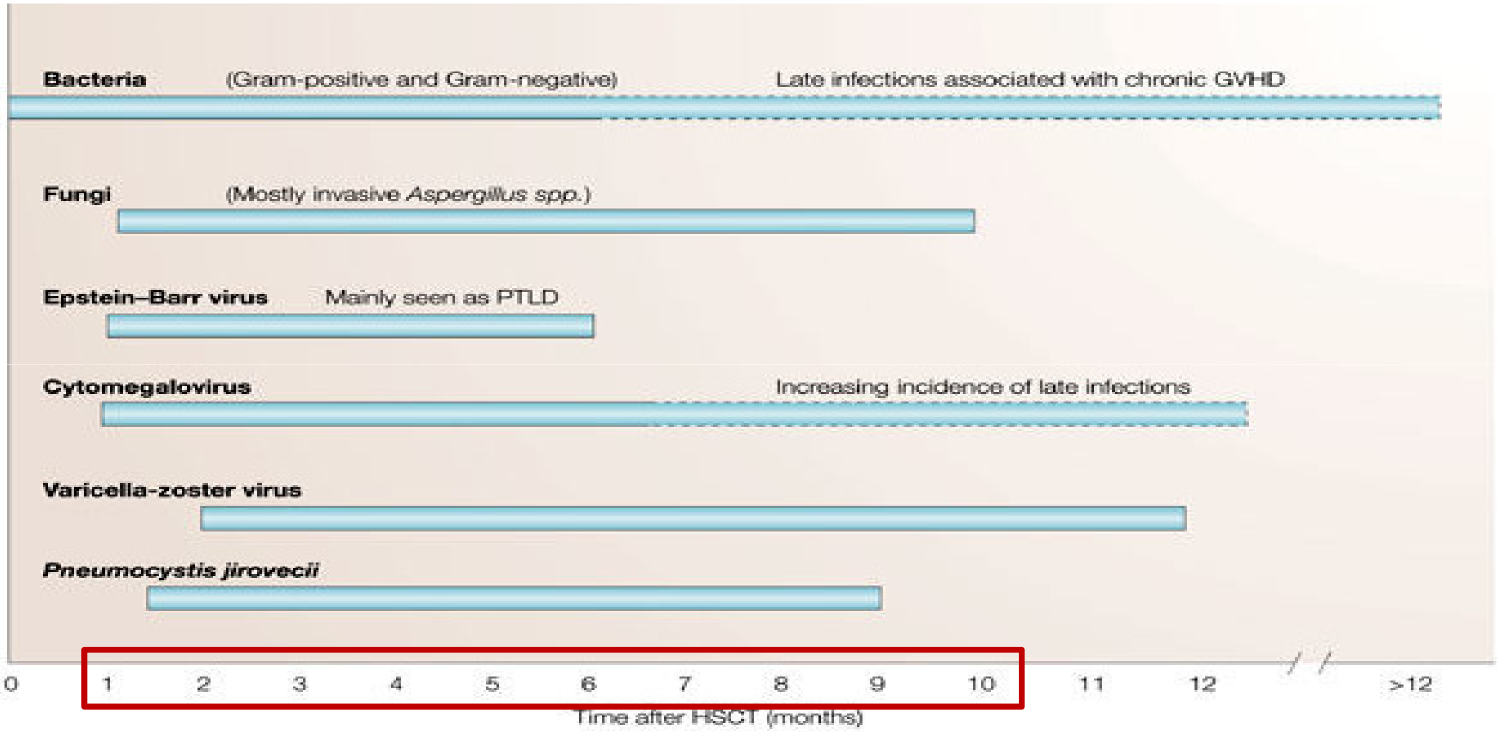
Praz-Christinaz SM[1], Lazor-Blanchet C, Binet I, Boillat MA, Danuser B.

Author information

Abstract

Returning to work after transplantation is a much-discussed topic today, especially as a measure to avoid permanent work disability. Many transplant patients regain their ability to work 2-6 months after transplantation. However, returning to work should not endanger their health. This means that occupational risks such as occupational exposure to Aspergillus spores must be evaluated. We evaluated the community-acquired aspergillosis risk and in particularly the occupational aspergillosis risk, using the example of a 39-year-old construction worker immunosuppressed after renal transplantation. On one hand the risk is linked to the exposure to microorganisms that the individual is likely to be subjected to, and on the other hand to the factors that modify his state of susceptibility or resistance to these infectious agents. The necessity of immunosuppressive therapy after transplantation elevates the aspergillosis risk, especially 1-6 months after transplantation. There are many professions in which exposure to Aspergillus spores can occur. The risk of acquiring aspergillosis at work exists, but is not quantifiable today. Nevertheless, the risk should be minimized during the period of vulnerability by preventive measures such as restriction of certain activities, changing work methods and reorganizing the work day to adapt to the risk, and wearing personal protective equipment, as well as attention to information about aspergillosis risk and about the likelihood of exposure in the patient's professional and leisure activities.

PMID: 17511825 DOI: 10.1111/j.1399-3062.2007.00223.x



Nature Reviews | Immunology

House inspection: Avoiding moldy and dusty houses

Indoor exposure

Avoiding gardening and composting

Usage of protective equipment

4) Opportunistic infection (**ONLY in the immunocompromised host?**)

The Telegraph

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Gardener killed by fungus in his compost

A keen gardener died after contracting a rare lung infection from the compost he spread on his garden.

By Kate Devlin, Medical Correspondent
6:21PM BST 12 Jun 2008

Just a day after he raked the rotten leaves and plants over his garden the 47-year old man was admitted to hospital complaining of chest pains and a shortness of breath. Less than four days later he was dead.

Doctors believe that he contracted the infection by inhaling fungus spores which had grown among the dead leaves during storage.

His death has prompted a warning to other gardeners about the dangers of compost.

Writing in the Lancet medical journal, Dr David Waghorn, who treated the man at Wycombe Hospital in Buckinghamshire in May last year, warns that the condition is rare "but may be considered an occupational hazard for gardeners".

The patient died from complications related to aspergillosis, a disease caused by spores of aspergillus, a fungus which grows on dead plant or animal material.

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Celebrity Sightings: 25 March

Up Helly Aa Festival

The Telegraph
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Acute invasive pulmonary aspergillosis, shortly after occupational exposure to polluted muddy water, in a previously healthy subject

Vikas Pilaniya[1], Kamal Gera[1], Rajesh Gothi[2], Ashok Shah[1]

1. Department of Respiratory Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India.
2. Department of Radiology and Imaging, Saket City Hospital, Saket, New Delhi, India.

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Study carried out in the Department of Respiratory Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India.

ABSTRACT

Invasive pulmonary aspergillosis (IPA) predominantly occurs in severely neutropenic immunocompromised subjects. The occurrence of acute IPA after brief but massive exposure to *Aspergillus* conidia in previously healthy subjects has been documented, although only six such cases have been reported. The diagnosis was delayed in all six of the affected patients, five of whom died. We report the case of a 50-year-old HIV-negative male, a water pipeline maintenance worker, who presented with acute-onset dyspnea and fever one day after working for 2 h in a deep pit containing polluted, muddy water. Over a one-month period, his general condition deteriorated markedly, despite antibiotic therapy. Imaging showed bilateral diffuse nodules with cavitation, some of which were surrounded by ground-glass opacity suggestive of a halo sign (a hallmark of IPA). Cultures (of sputum/bronchial aspirate samples) and serology were positive for *Aspergillus fumigatus*. After being started on itraconazole, the patient improved. We conclude that massive exposure to *Aspergillus* conidia can lead to acute IPA in immunocompetent subjects.

Keywords: Environmental exposure; Azoles; Water pollution; Immunocompetence; Invasive pulmonary aspergillosis.

Nosocomial vs. Community-acquired aspergillosis

Occupational and indoor exposure

Nosocomial

- Due to a break in or contami... of hospital water syste...
- Due to a break in ... system
- Due to ... demolition w... or near by
- ...s of *Aspergillus* found ...al environment: Foods, ...ts, Fabrics, Mattresses and pillows, Computers

Important to understand the epidemiology *Aspergillus* in the different hospital wards housing high risk patients.

Community acquired

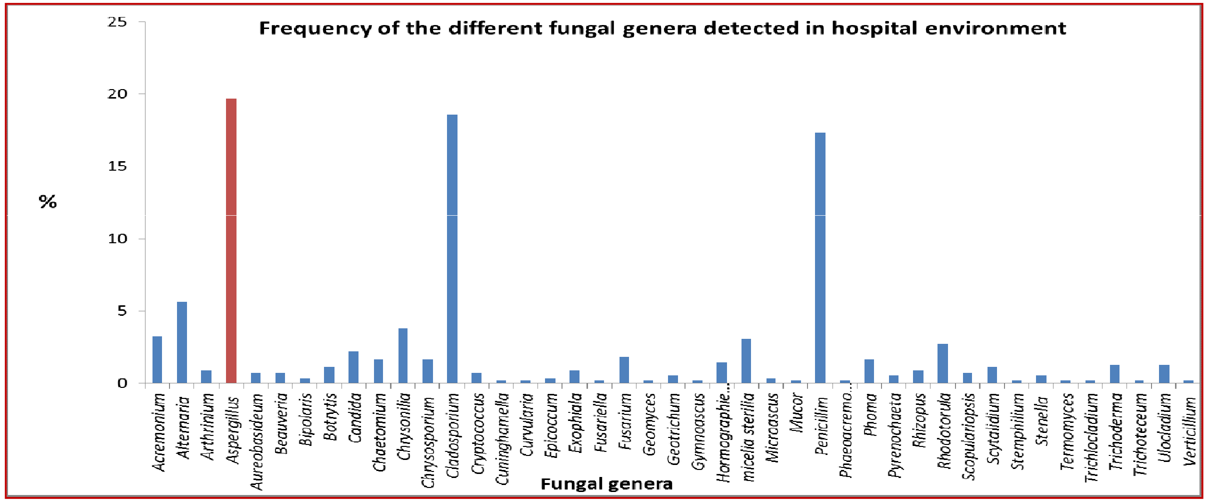
- Due to the air quality/ *Aspergillus* burden in the patients' houses
- Due to occupational or leisure activities developed leading to direct exposure to high amount of conidia

Adapted from: Praz-Christinaz *et al.* (2007). *Transplant Infect Dis*; 9: 175-181

Aspergillus in hospital environment

1 year study: 101 air and 99 surface samples (impaction method and swabbing)

- From all samples, **548** fungal isolates were obtained
- ***Aspergillus*** was the most frequently fungal genera found (**19.7%**)



- **25 different species** of *Aspergillus* were identified by β-tubulin and calmodulin sequencing, and a high percentage of **cryptic species** (i.e., not sensu stricto) was found **(59%)**

Aspergillus section	*Aspergillus* species	No. of isolates
Versicolores (N=20)	*A. versicolor sensu stricto*	1
	A. protuberus	8
	A. sidowii	6
	A. tabacinus	1
	A. tenneensis	1
	A. venenatus	1
	A. creber	2
Fumigati (N=8)	*A. fumigatus sensu stricto*	7
	Neosartorya hiratsukae	1
Flavi (N=10)	*A. flavus sensu stricto*	10
Nigri (N=11)	*A. niger sensu stricto*	5
	A. phoenicis	2
	A. tubigensis	4
Terrei (N=4)	*A. terreus sensu stricto*	4
Nidulantes (N=4)	*Emmericella nidulans*	3
	Emmericella quadrilatera	1
Usti (N=4)	*A. minutus*	3
	A. insuetus	1
Circumdati (N=10)	*A. ochraceus sensu stricto*	1
	A. sclerotium	4
	A. westerdjikae	3
	A. persii	2
Aspergilli (N=3)	*Eurotium repens*	2
	Eurotium rubrum	1

Sabino R, Veríssimo C, Parada H, Brandão J, Viegas C, Carolino E, Clemons KV, Stevens DA (2014). Molecular screening of 246 Portuguese *Aspergillus* isolates among different clinical and environmental sources. Medical Mycology; 52(5):519-29.

Aspergillus in hospital environment

- Among the tested isolates, sections ***Circundati***, ***Versicolores*** showed isolates – namely **cryptic species-** with reduced susceptibility to some of the antifungals used as clinical therapeutics.

Source	Ward	Species	MIC AMB (µg/mL)	MIC ICZ (µg/mL)	MIC VCZ (µg/mL)	MIC PCZ (µg/mL)
air	Hematology	*A. sidowii*	2	4	1	2
surface	Hematology	*A. sidowii*	4	>8	4	2
air	Hematology	*A. sidowii*	4	4	0.5	2
surface	Hematology	*A. versicolor sensu stricto*	2	4	0.5	1
air	Hematology	*A. venenatus*	2	4	0.5	1
air	Hematology	*A. tubigensis*	1	4	≤0.25	1
surface	ICU	*A. phoenicis*	1	4	≤0.25	1
air	Hematology	*A. niger sensu stricto*	1	4	≤0.25	1
air	Hematology	*A. niger sensu stricto*	1	4	0.5	1
surface	ICU	*A. persii*	2	2	0.5	1
air	ICU	*A. persii*	4	4	0.5	0.5
air	Hematology	*A. slerotium*	2	4	0.5	1
air	Hematology	*A. sclerotium*	2	4	≤0.25	1
air	Hematology	*A. sclerotium*	4	8	0.5	1
air	ICU	*A. westerdjikae*	>8	4	≤0.25	1
air	Hematology	*A. westerdjikae*	>8	4	≤0.25	1
air	Hematology	*A. westerdjikae*	>8	4	≤0.25	1
air	ICU	*A. flavus sensu stricto*	2	2	0.5	0.5
surface	ICU	*A. flavus sensu stricto*	2	2	0.5	1
surface	ICU	*A. flavus sensu stricto*	2	2	0.5	1
air	Hematology	*A. flavus sensu stricto*	2	2	≤0.25	0.5
air	Hematology	*Emmericella nidulans*	2	2	≤0.25	0.5

Sabino R, Viegas C, Francisco M, Martins C, Veríssimo C, Clemons KV, Stevens DA (2016). Aspergillus nosocomial infections - Do cryptic species found in hospital environment matter? Proceedings from Advances Against Aspergilosis 2016: P24, pg 99

Occupational environments

	Clinical (N = 41)	Hospital (N = 23)	Poultries (N = 39)	Swineries (N = 29)	Beach sand (N = 35)	Other environments (N = 8)
MEC[¥] or MIC parameters			CAS MEC (μg/ml)			
Median	25	0.39	12.50	1.60	1.19	18.75
Geometric mean	6.2	1.2	4.4	2.9	2.2	17.7
Range	≤0.39–>50	≤0.39–>50	≤0.39–>50	≤0.39–>50	≤0.39–>50	12.5–25
No.isolates(%) with MIC > 6.3 μg/ml	27 (65.8)	4 (17.4)	20 (51.3)	13 (44.8)	13 (36.1)	4(100.0)
P-value clinical vs. environmental isolates			0.047*			
			AMB MIC (μg/ml)			
Median	2	2	2	2	2	2
Geometric mean	1.5	2.4	2.0	2.1	2.4	2.0
Range	1–2	1–>8	1–8	≤0.5–>8	1–>8	2
No.isolates(%) with MIC > 2 μg/ml	0 (0.0)	7 (30.4)	6 (15.4)	6 (20.4)	12 (33.3)	0 (0.0)
P-value clinical vs. environmental isolates			<0.001*			
			ITC MIC (μg/ml)			
Median	4	4	2	4	4	2
Geometric mean	2.8	3.5	2.6	3.1	3.0	2.0
Range	1–8	2–>8	≤0.5–>8	1–>8	1–>8	2
No.isolates(%) with MIC > 2 μg/ml	21 (51.2)	17 (73.9)	13 (33.3)	16 (55.2)	23 (63.9)	0 (0.0)
P-value clinical vs. environmental isolates			.4			
			VRC MIC (μg/ml)			
Median	0.3	0.5	0.25	0.25	0.25	0.25
Geometric mean	0.3	0.4	0.3	0.3	0.3	0.3
Range	≤0.25–1	≤0.25–4	≤0.25–4	≤0.25–4	≤0.25–1	≤0.25–0.5
No.isolates(%) with MIC > 2 μg/ml	0 (0.0)	1 (4.3)	1 (2.6)	1 (3.4)	0 (0.0)	0 (0.0)
P-value clinical vs. environmental isolates			.8			
			PCZ MIC (μg/ml)			
Median	0.5	1	0.5	1	1	0.25
Geometric mean	0.5	0.9	0.7	0.9	0.8	0.2
Range	≤0.25–2	0.5–2	≤0.25–>8	≤0.25–>8	≤0.25–8	0.25
No.isolates(%) with MIC > 2 μg/ml	0 (0.0)	0 (0.0)	1 (2.6)	2 (6.9)	1 (2.8)	0 (0.0)
P-value clinical vs. environmental isolates			<0.001*			

*Statistically significant differences at 5% significance level using the Mann–Whitney U test.
¥ MEC applies only to caspofungin.

Sabino R, Carolino E, Verissimo C, Martinez M, Clemons KV, Stevens DA (2016). Antifungal susceptibility of 175 *Aspergillus* isolates from various clinical and environmental sources. Medical Mycology 1;54(7):740-756

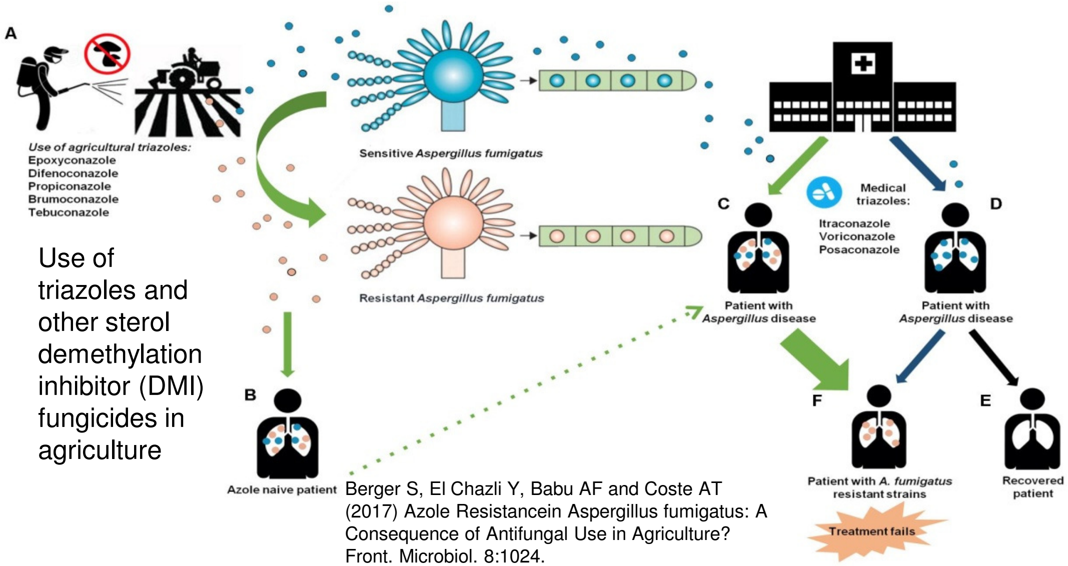
Clear differences were found between the susceptibility of clinical and environmental isolates

For AMB and PCZ significant higher values in environmental isolates

4 environmental isolates with MICs ≥ 8 μg/ml to PCZ + MICs ≥8 μg/ml to ITC:
- 1 isolate from beach sand,
- 1 isolate from poultry
- 2 isolates from swineries

Other than *A. Fumigatus: Versicolores, Nidulantes and Aspergilli sections)*

The problem of antifungal resistances in *Aspergillus* related to occupational exposure

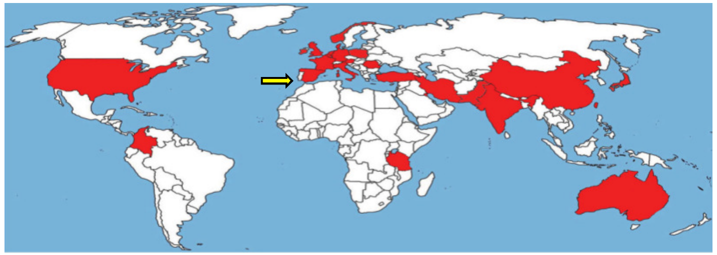


Use of triazoles and other sterol demethylation inhibitor (DMI) fungicides in agriculture

Berger S, El Chazli Y, Babu AF and Coste AT (2017) Azole Resistancein Aspergillus fumigatus: A Consequence of Antifungal Use in Agriculture? Front. Microbiol. 8:1024.

EXPOSURE to high levels of environmental isolates with intrinsic / less susceptibility to antifungals

EXPOSURE to environmental isolates with induced azole-resistance (secondary resistance): in agricultural settings– PESTICIDES/ OTHER FUNGICIDES????



Meis JF, Chowdhary A, Rhodes JL, Fisher MC, Verweij PE. 2016 Clinical implications of globally emerging azole resistance in Aspergillus fumigatus. Phil. Trans. R. Soc. B 371: 20150460.

Final considerations…

✓ **Strategies to avoid *Aspergillus* exposure** of the worker in contaminated occupational settings and these include: engineering, sampling and control, as well usage of personal protective equipment.

✓ *Aspergillus* occupational exposure is a **public health problem** that shoud be **managed** properly

Hazard Identification

- Fungal contamination
- *Aspergillus* spp. contamination
- *Aspergillus* sections / species
- Detection of *Aspergillus* resistant isolates

Air Samples | **Surface Samples**

Cultural methods

Molecular methods

Risk Characterization

Adapted from: Viegas C, Faria T, Aranha Caetano L, Carolino E, Quintal Gomes A, Viegas S (2017) Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?, Journal of Occupational and Environmental Hygiene, 14:10, 771-785

✓ The **molecular study** of *Aspergillus* epidemiology (including the identification of cryptic species) and determination of the **susceptibility profile** of environmental isolates may contribute to the prevention of diseases associated to occupational exposure.

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Thank you!!!

Hope to receive you again in Lisbon!!!