

# Thermotolerant filamentous fungi in belgian hospitals: 15 years of survey

Fungi in hospitals (species and amount)

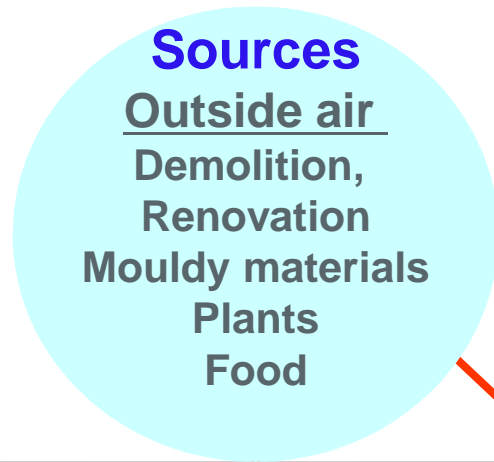
Different settings/context

Analyse particular situations (outbreak)

# Thermotolerant fungi

- Have an optimal growth between 30 and 40° C
- **potential** human pathogens
- **potential** agent of nosocomial infections in hospitals

# Filamentous fungi in hospital environment



vector  
= indoor air



# Indoor fungi

## ➤ From outdoor air

Most = mesophilic fungi

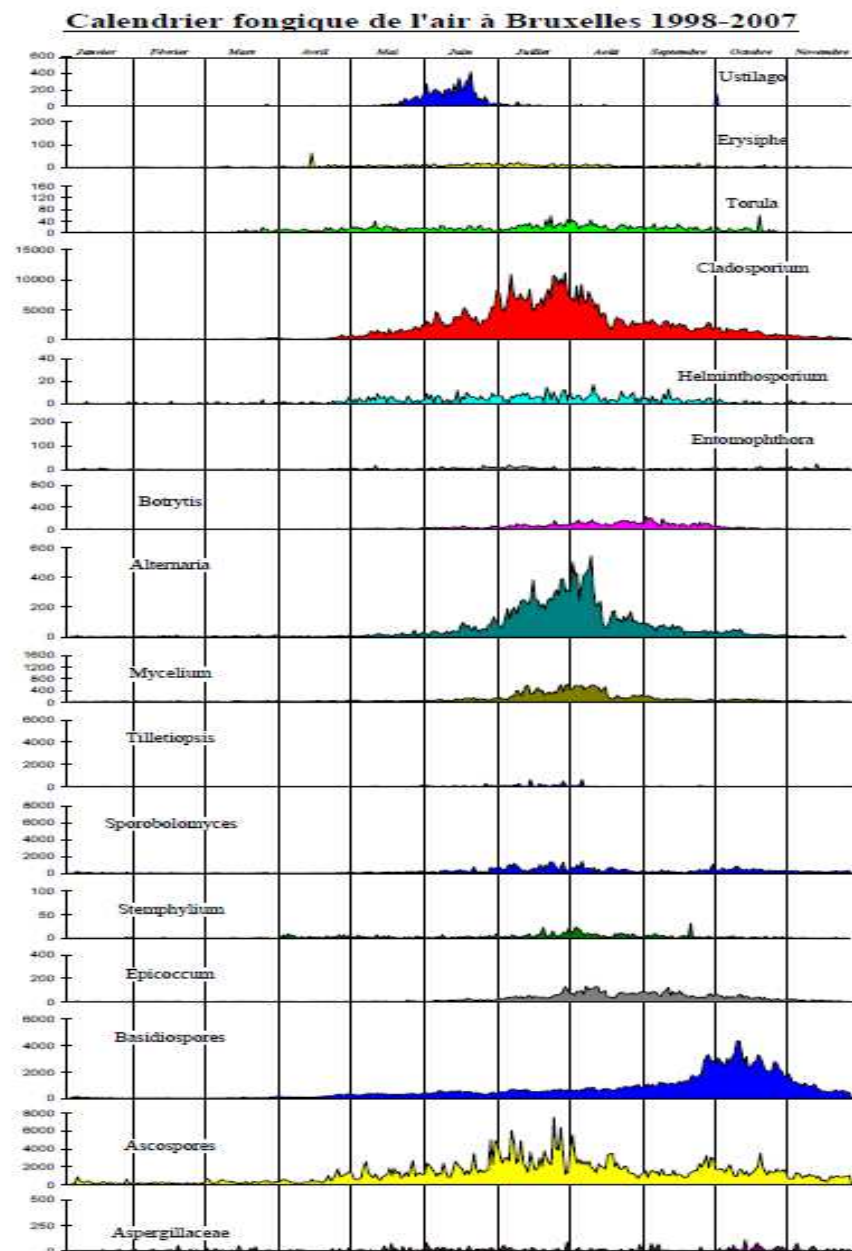
Have seasonal pattern

- *Cladosporium*, *Alternaria*, in summer period (June-> September)
- Aspergillaceae : *Aspergillus*, *Penicillium* are found all over the year.
- *Aspergillus fumigatus* more in winter.

## From indoor environment

Affected by

- Type of treatment of the air (filtration, HEPA filtration)
- Ventilation rates
- Rate of room air exchange
- Possible fungal colonization



# Methods for isolation of viable fungi from air and surfaces

Methods simple to highlight **viable** particles and allow identification of fungi at reasonable cost (no molecular biology, beta glucan...)

**Air sampling** : Biocollector MAS<sup>®</sup> (Merck) (volume 1000 liter)

Medium : Malt-Chloramphenicol -plate 9cm diameter

Incubation 37° C

Result = nb of CFU/m<sup>3</sup> and identification of fungi

**Surface sampling** : RODAC<sup>™</sup> contact plate (25cm<sup>2</sup>)

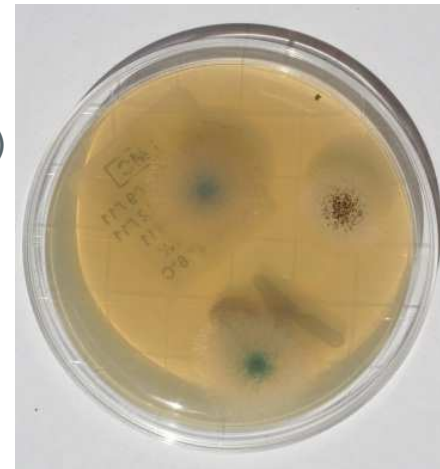
Medium : Malt-Chloramphenicol

Incubation 37° C

Result = nb of CFU/plate and identification of fungi

**Time from sampling to report**

- *Aspergillus fumigatus* : 2 days
- Other filamentous fungi: 2-5 days.



# Surveys in belgian hospital : Context



Period :1995-2012

**Belgian different hospital** : 43

**Surveys** : 246 (from 1 to 50 survey/hospital)

**Retrospective data**

- *Advantages* :

- *Cover various situations*

- Renovation works : 51%
    - Problem with a patient : 25%
    - Routine : 16%
    - Environmental alert : 7%
    - One situation of nosocomial outbreak (SSSI- *A. flavus*)

- *Cover various type of settings* (sterile unit, operating rooms, ICU, hematology, geriatrics, emergency unit ..).

- *Drawbacks*:

- no predefined and standardized sampling plan
    - no follow-up of the the same hospital, of the same units during the whole period

# Surveys in belgian hospital

## Nb of samples

- 11097 (4364 positive = 39.3%)
- air samples 2641 (845 positive = 32%)
- surface samples 8456 (3519 positive = 41.6%)

Nb samples/hospital : 17-3185 (average 338)

## Outbreak

- 5 surveys (772 samples - 273 air and 499 contact)

**% positive samples : thermotolerant species isolated air and surfaces :**  
**Mean values for all settings\* (air n=2368 – surfaces n=7957)(o)**

All type of settings	% air with + culture	% Surfaces with +culture		% air with + culture	% Surfaces with +culture
<b><i>Aspergillus</i></b>	24.7	30.0	<b>Mucorales</b>	2.0	3.8
<i>Aspergillus fumigatus</i> *	20.8	23.1	<i>Rhizopus (microsporus, oryzae)</i> *	0.68	1.9
<i>A. niger</i> *	3.7	10.4	<i>Rhizomucor (miehei , pusillus)</i> *	0.93	1.0
<i>A. flavus</i> *	1.1	3.5	<i>Absidia corymbifera</i> *	0.30	0.2
<i>A. ustus</i> *	1.8	3.1	<i>Mucor sp. (R)</i>	<0,1%	0.2
<i>A. nidulans</i> * (CGD)	2.3	2.7	<i>Mycotipha microspora (R)</i>	0	<0,1%
<i>A. terreus</i> *	0,13	0.5	<b>Other species</b>		
<i>A. fischeri</i> -	0.17	0.13	<i>Paecilomyces variotii (R)</i>	4.3	5.8
<i>A. versicolor</i> *	<0.1%	0.11	<i>Penicillium spp. (R)</i>	1.4	1.4
<i>A. glaucus (R)</i>	0	<0,1%	<i>Trichoderma sp. (R)</i>	1.6	3.3
<i>A sydowii (R)</i>	0	<0,1%	<i>Monilia sitophila (R)</i>	0.5	1.8
			Sterile mycelia (?)	2.7	4.8

(o) Without outbreak surveys

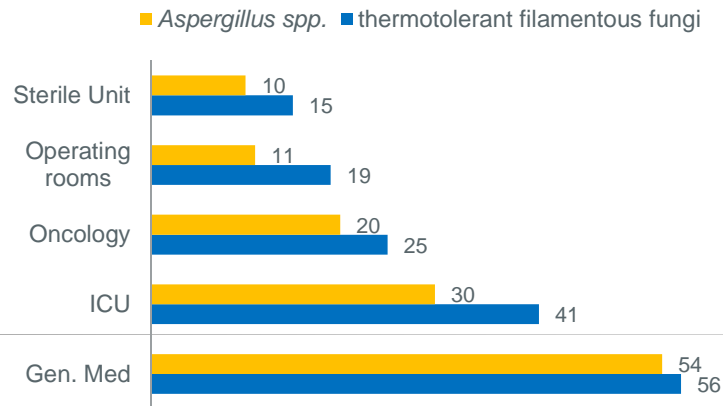
\*important Nb cases; R=very rare cases;



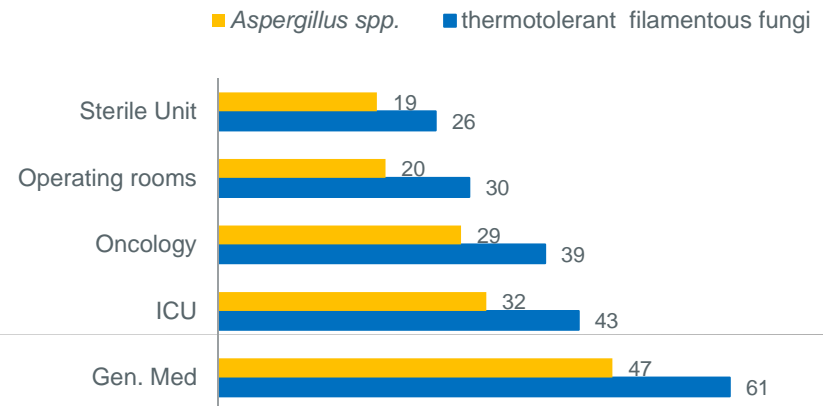
# % of positive samples and mean value of CFU

Thermotolerant filamentous fungi and *Aspergillus* spp. in air (n= 2641) and surfaces (n=8456) from different hospital settings

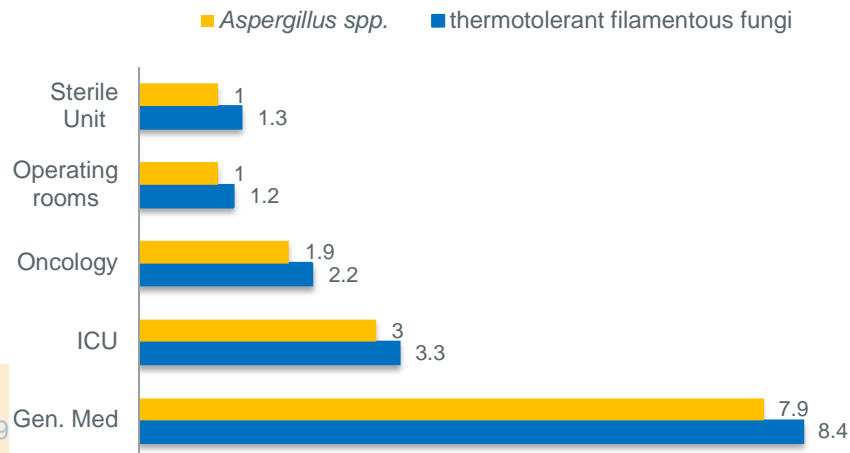
## % positive air samples



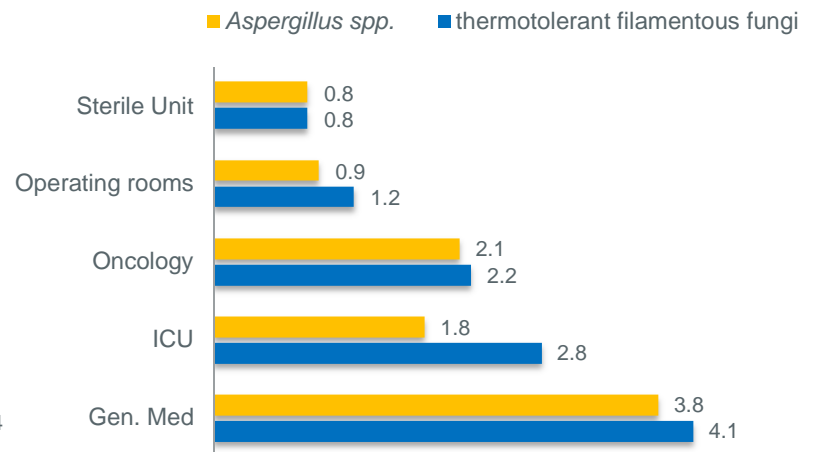
## % positive surface samples



## Mean values CFU/m3 air



## Mean values CFU/plate



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## Follow-up of the different settings

1. The fungal flora vary from one hospital to another
2. Useful to follow the different settings regularly to have qualitative and quantitative baseline values
3. Important to regularly follow air and surfaces contamination
4. Cutoff / threshold values depend of the context type of settings

## Particular situations

1. Nosocomial outbreak *A. flavus*
2. Contamination of sterile material stockroom/OR

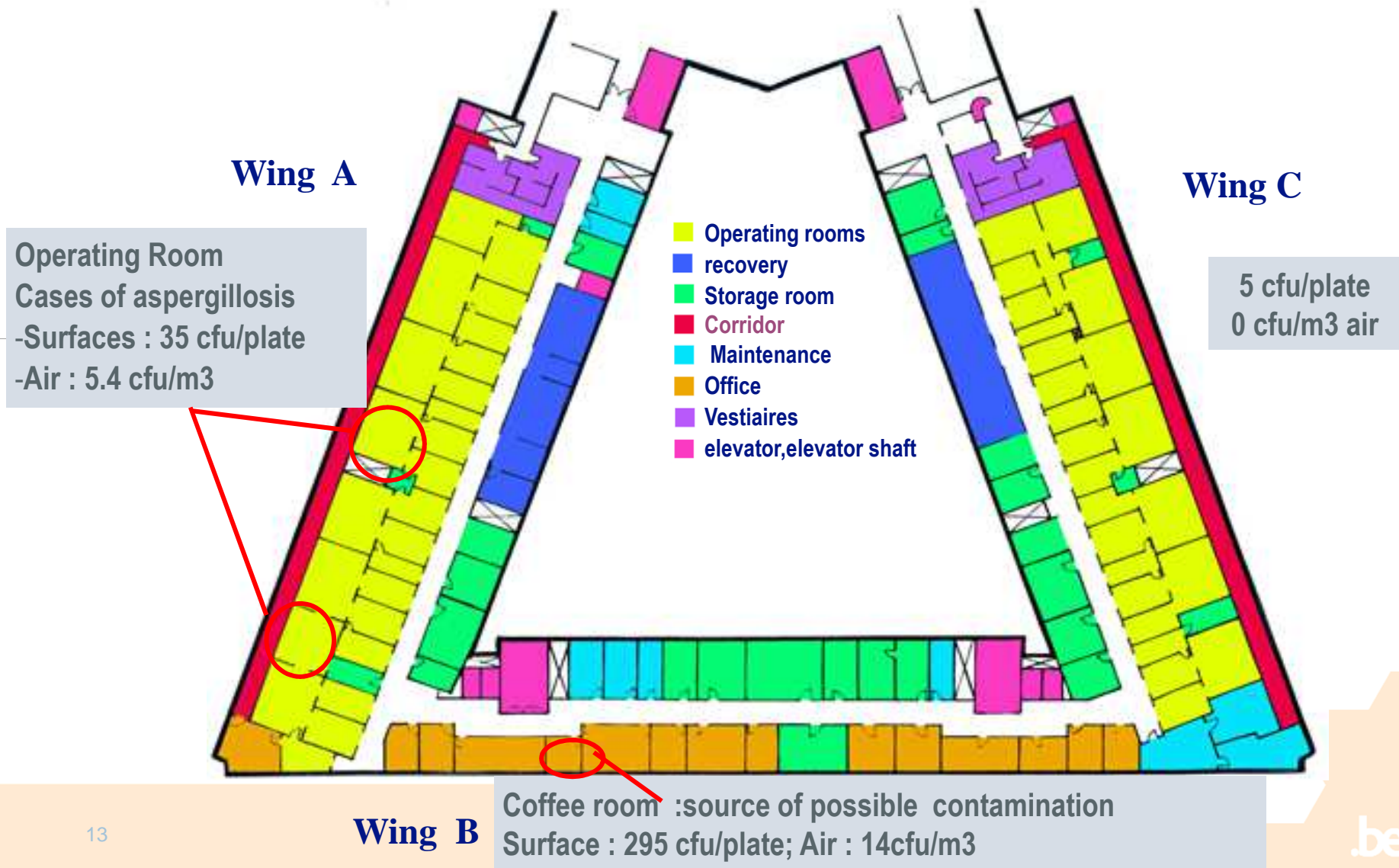
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# 1. Postoperative infections outbreak and environmental surveys

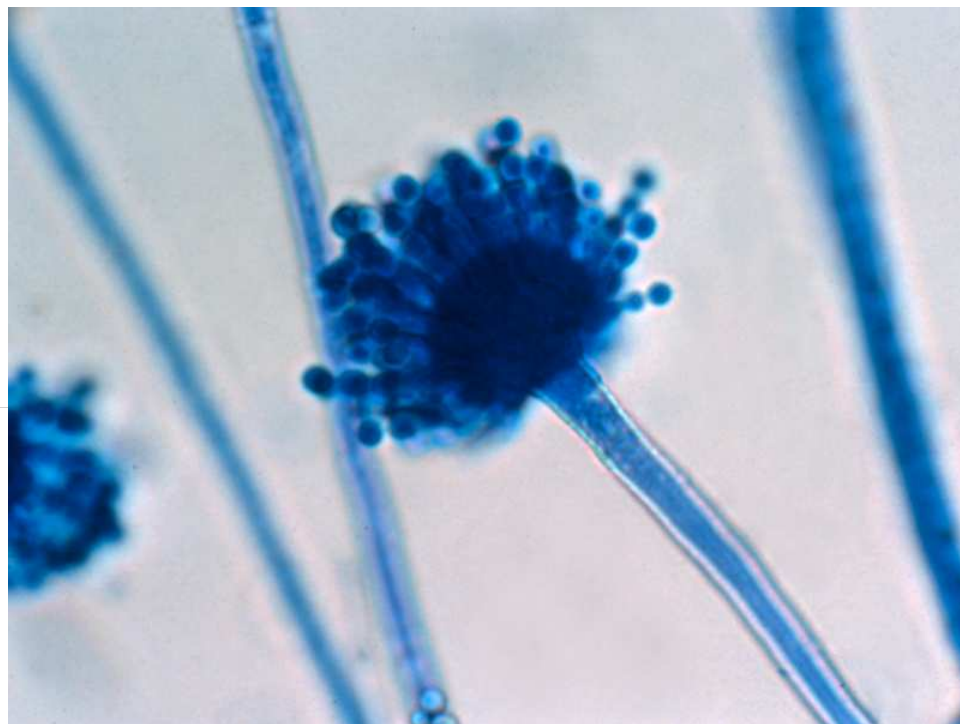
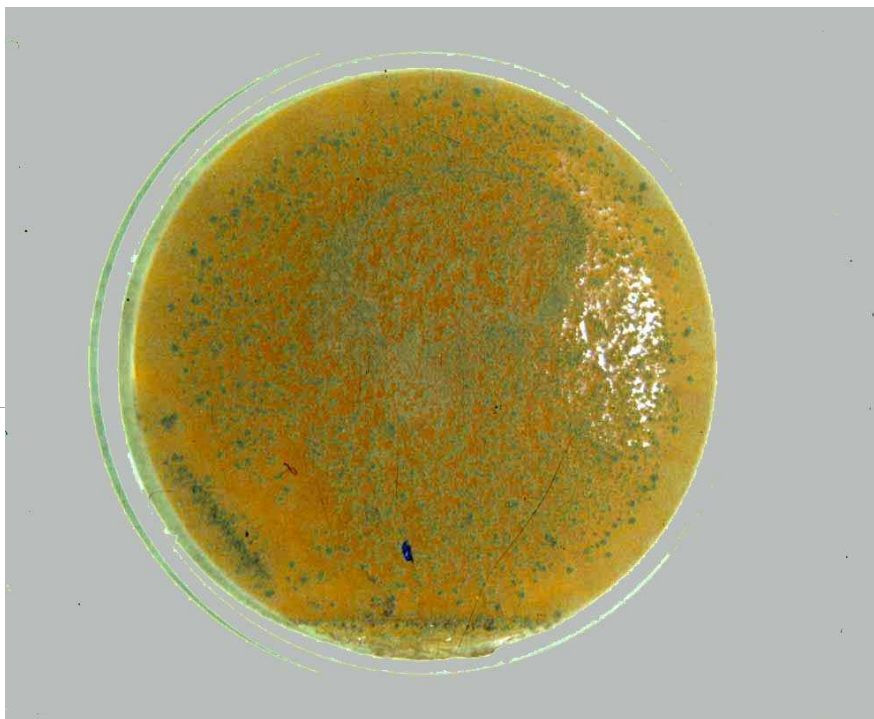
-April 1998 IPH was involved in environmental surveys in the framework of SSSI= sternal surgical site infection : agent *Aspergillus flavus* (AZ-SJan Hospital, Brugge)

- Period January to April : 9 patients affected after cardiac surgery of which 2 died – 3 had multiple relapses (Vandecasteele et al., CID, 2002)
- 5 extensive environmental survey April 15, May 5, May 18, May 28, August 18 (Heinemann et al., JHI, 2004)
  - 5 surveys (772 samples - 273 air and 499 contact)

# Localisation of *Aspergillus flavus* source outbreak SSSI (cardiac surgery) – Survey May 5



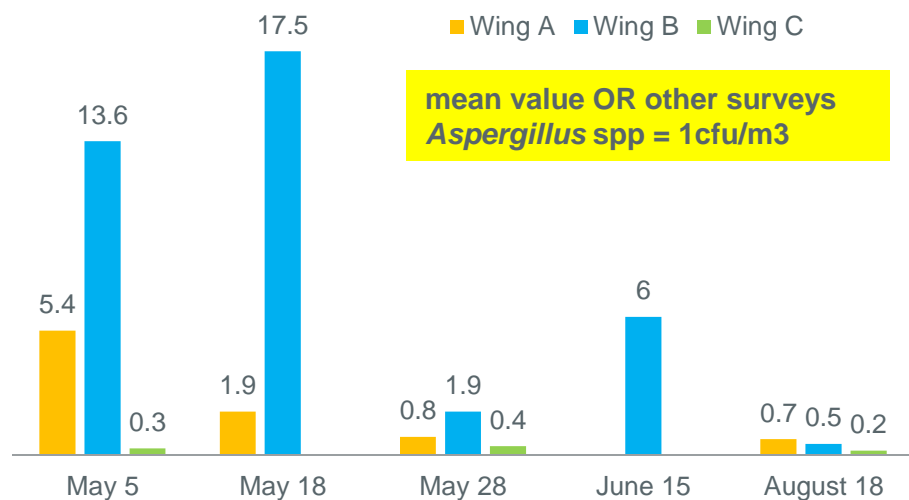
## Controles of surfaces – Wing B



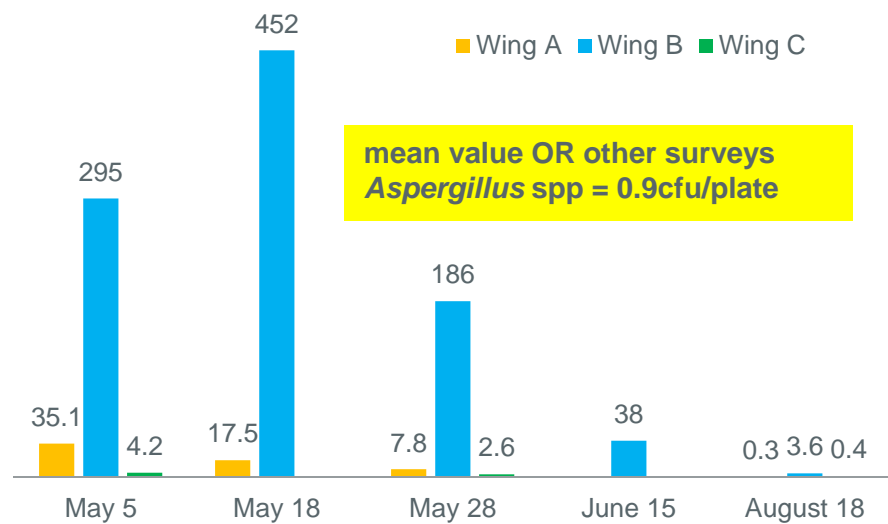
***Aspergillus flavus* > 1000 cfu (estimation)**

# Evolution of contamination in the air and surfaces (Wing A,B,C)

Mean values *A. flavus* cfu/m<sup>3</sup>  
Survey outbreak SSSI



Mean value of *A. flavus* cfu/plate  
Survey outbreak SSSI



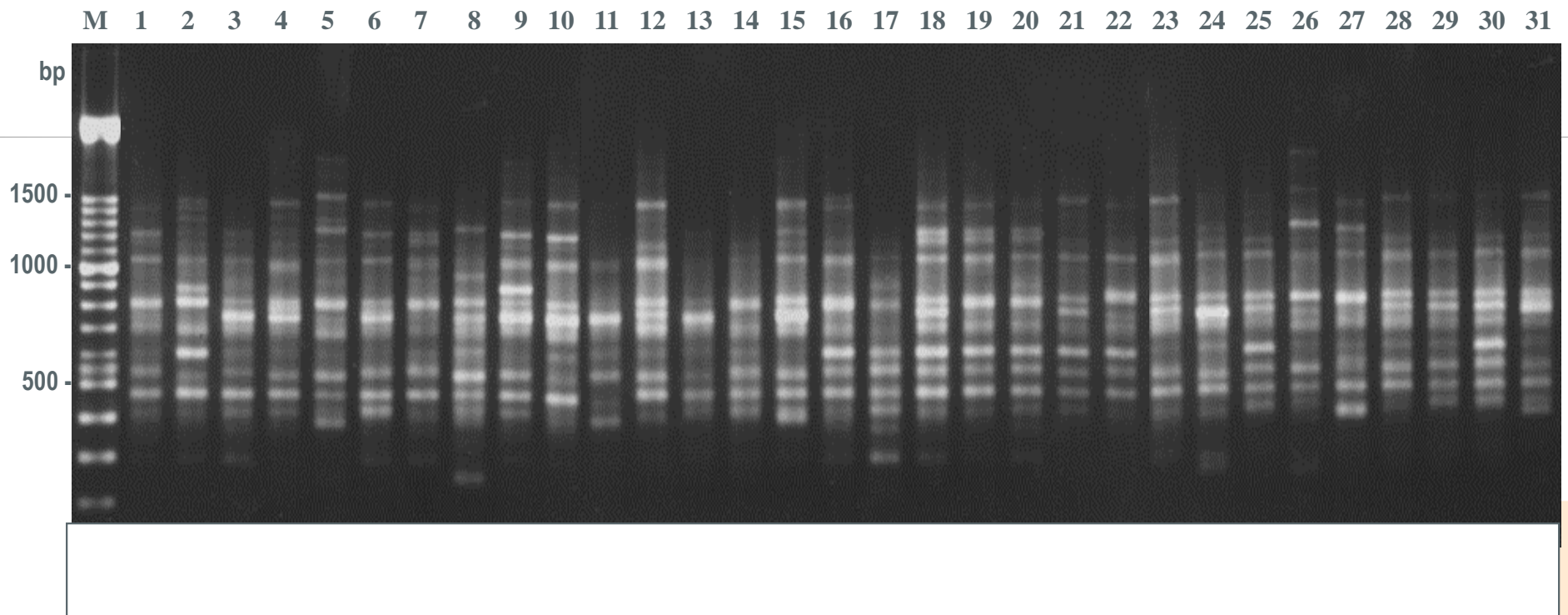
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## Nosocomial origin ?

- Not questionable
  - Typing to assess identity of the genotypes from environment and patients.
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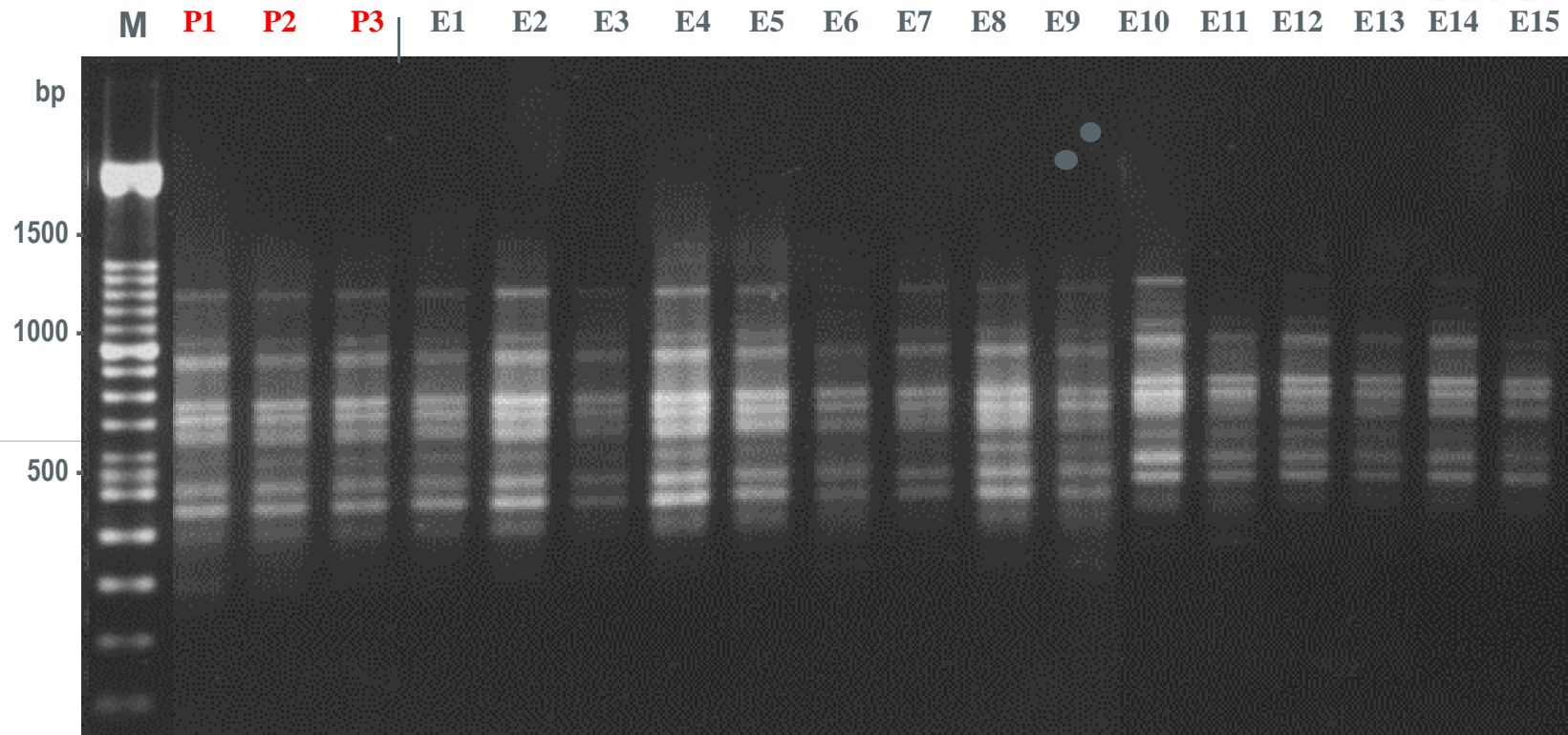


# Molecular typing RAPD (primer ERIC1) unrelated *Aspergillus flavus* strains



# Molecular typing RAPD (primer ERIC1)

## Strains from outbreak - patients and environment



### Patients

**P1 Patient 1**

**P2 Patient 2**

**P3 Patient 3**

### Environnement

E1 Room 4 (air)

E2 Room 6 (air)

E3 Room 4 (surface)

E4 Room 6 (surface)

E5 Room 10 (surface)

E6 Room 13 (surface)

E7 Corridor (surface)

E8 Recovery (surface)

E9 Storage (surface)

E10 Office (surface)

E11 Office (air)

E12 Coffee room (surface)

E13 Storage (air)

E14 Corridor (surface)

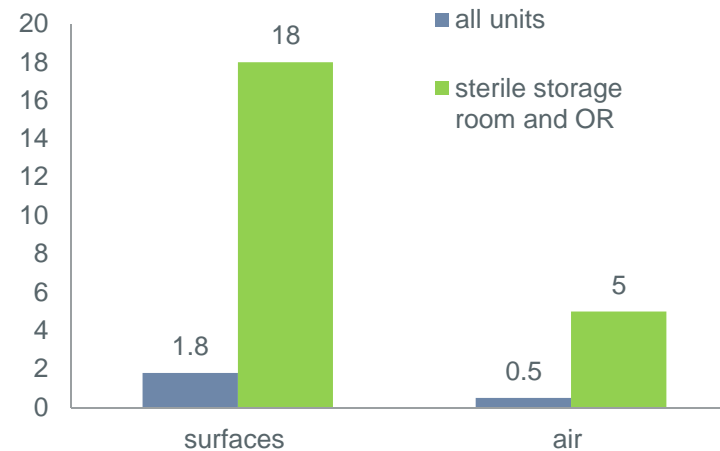
E15 Storage sterile

## 2. Contamination of sterile stock room and adjacent operating rooms



Air sample plate

% samples containing *Monilia sitophila*

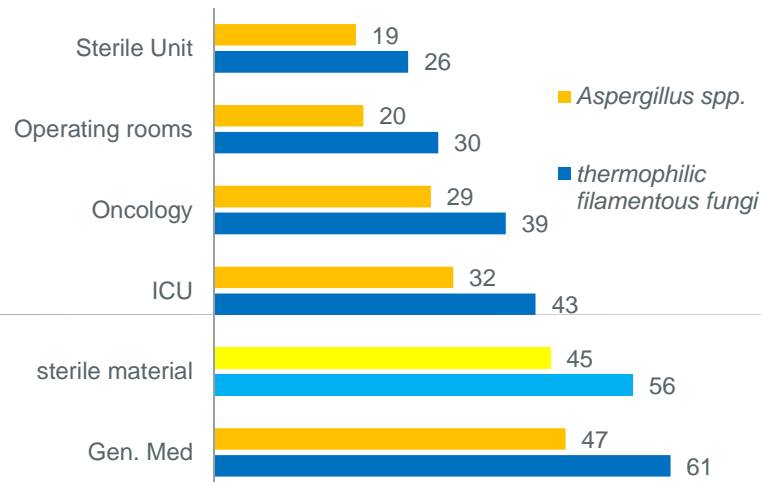


- 8 Surveys in sterile stock room material and OR (April -> November 2011- 306 samples)
- % samples contaminated with
  - *A. fumigatus* = 34-84%  
(mean values Af OR = 10%)
  - *Monilia sitophila* = 0%-59%  
« bread mould »

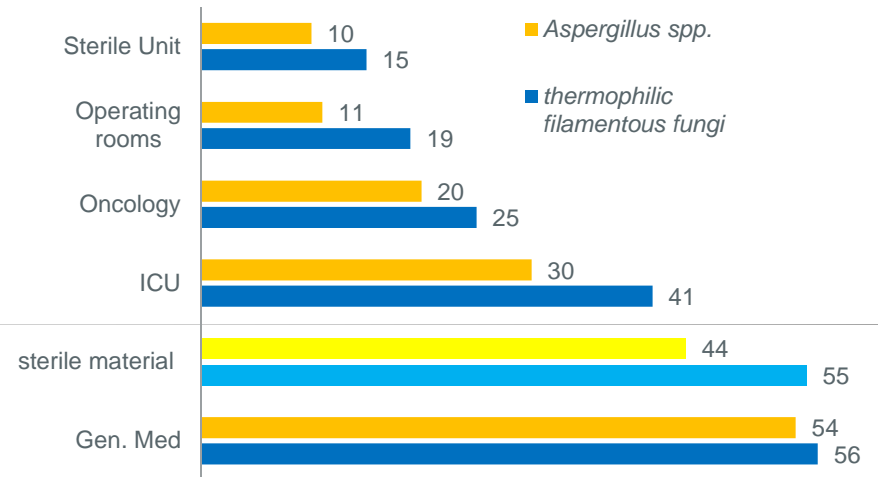


## % of positive samples and mean value of CFU of filamentous fungi and *Aspergillus* spp. (air and surfaces) different hospital settings / sterile stock room & adjacent OR

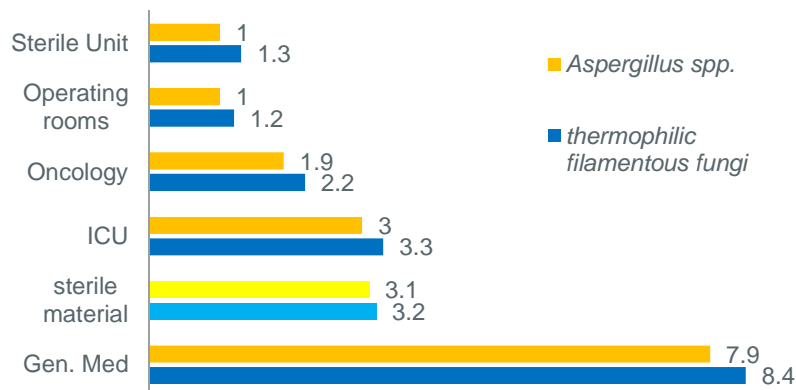
**% surface samples containing filamentous fungi and *Aspergillus* in different hospital settings and storage room for sterile material**



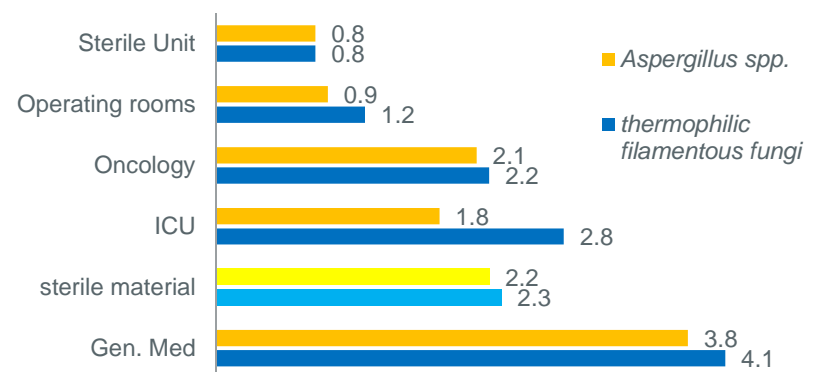
**% air samples containing filamentous fungi and *Aspergillus* in different hospital settings and storage room for sterile material**



**Mean values CFU/m<sup>3</sup> thermotolerant filamentous fungi and *Aspergillus* spp. in the air from different hospital settings and storage room for sterile material**



**Mean values CFU/plate thermotolerant filamentous fungi and *Aspergillus* spp from different hospital settings and storage room for sterile material**



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## 2. Contamination of sterile stock room and adjacent operating rooms

- *Monilia sitophila* rarely reported as agent invasive mycose
- Danger as associated with material used for injection
- Measures were taken, no case reported
- Rare pathogenic fungus *Exserohilum rostratum* -> agent outbreak fungal meningitis in 2012 in US injection of contaminated steroids

## Conclusion

- The different units of the hospital and particularly sterile unit and operating rooms must be regularly controlled to have base line values of levels of fungal contamination
- Control must be more frequent in cases of renovation work or other particular situations
- All thermotolerant filamentous fungi must be taken into account

The image features a complex, branching network of thin, light-colored lines that radiate from a central point, resembling a tree or a neural network. At the center of this network is a dense cluster of small, dark, circular dots. The background is a light, pale blue color. The text is overlaid on the upper portion of the image.

Thanks to Suzanne Heinemann  
Surveys from 1995-2006

**Thank you for your attention**