



BELGISCHE VERENIGING VOOR MENSELIJKE EN DIERLIJKE MYCOLOGIE  
SOCIÉTÉ BELGE DE MYCOLOGIE HUMAINE ET ANIMALE

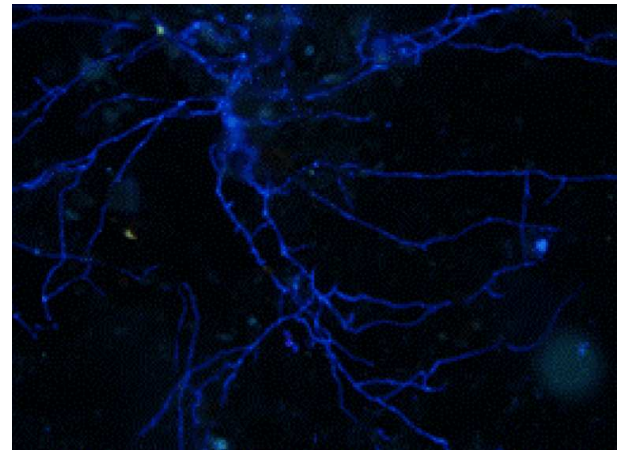
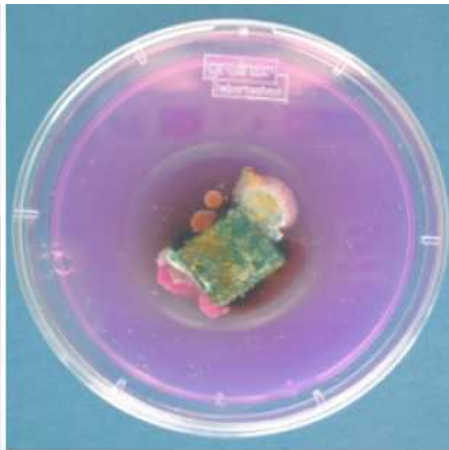
# FILAMENTOUS FUNGI IN HOSPITAL WATER DISTRIBUTION SYSTEMS: WHAT IS THE RISK?

MARIE-PIERRE HAYETTE

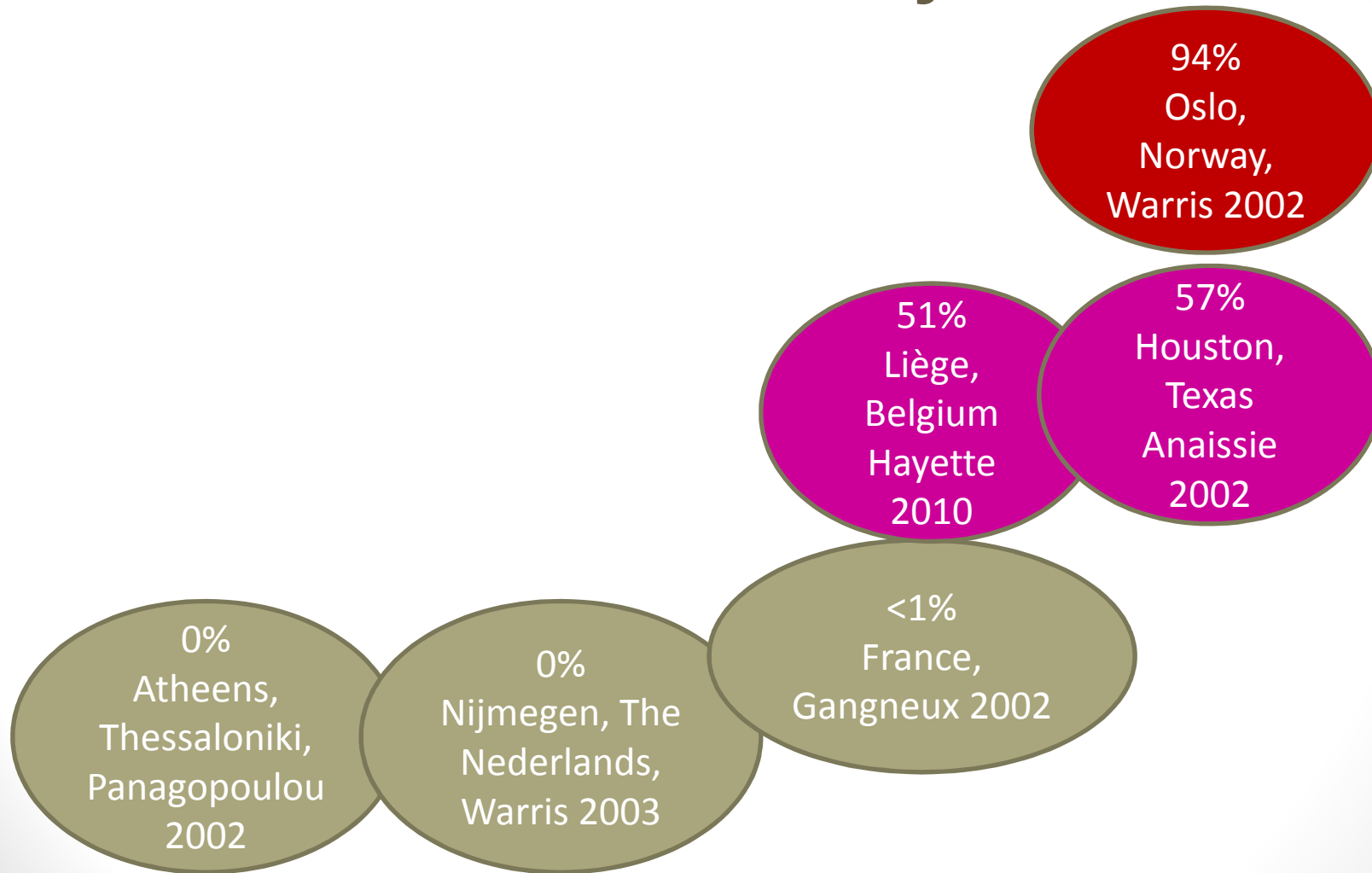
University Hospital of Liège



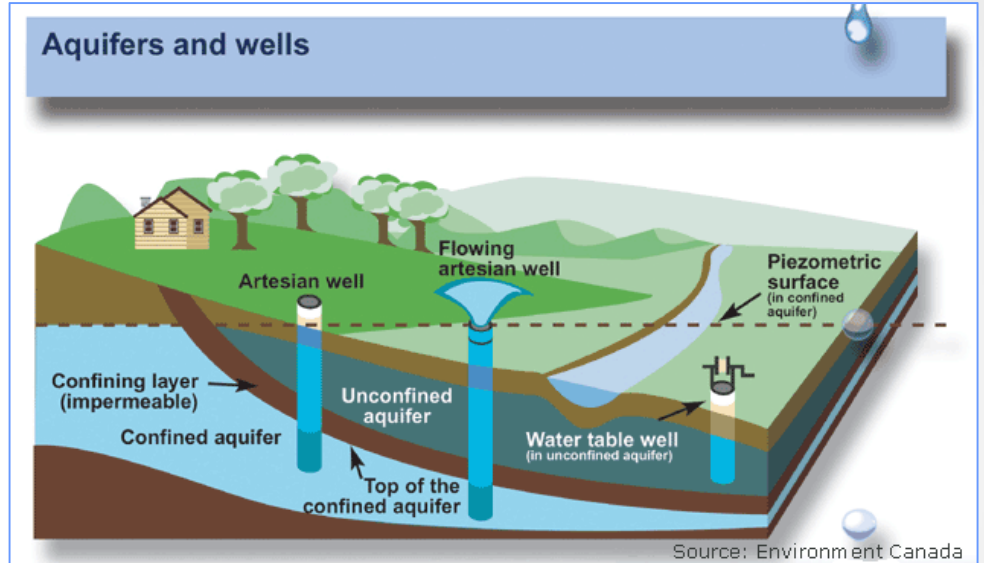
# Biofilms



# Variable situations in hospital water distribution systems



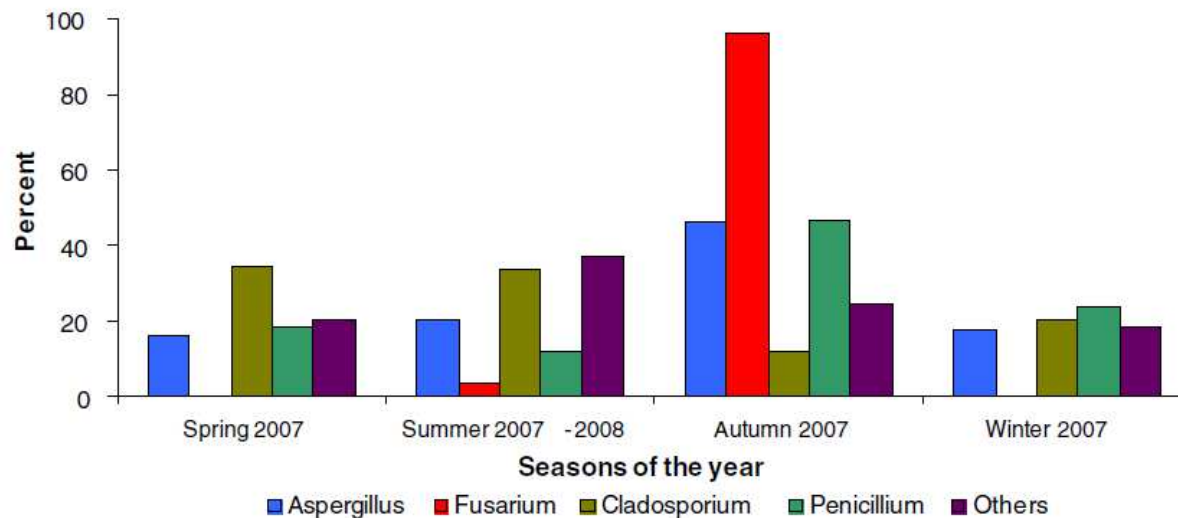
# Origin of the water



Water  
storage tanks

Warris A CID 2002

# Highest concentration in autumn



**Figure 2** Distribution of fungal propagules in water samples collected from 4 different seasons of the year.



# Surveys : 1996-2003

Country, Place, Year	Period of time	Type of water	Main isolation method	Most frequent fungal isolates
United Kingdom, 1996	Autumn and Spring	Surface water and network	Membrane filtration, Direct plating and Bating	<u>Aspergillus</u> , <u>Cladosporium</u> , <u>Epicoccum</u> , <u>Penicillium</u> and <u>Trichoderma</u>
Greece, Thessaloniki, 1998	One collection (126 samples)	Tap water (hospital and community)	Membrane filtration	<u>Penicillium</u> , <u>Aspergillus</u> and <u>Acremonium</u>
Greece, 85 haemodialysis units, 1998	One collection (255 samples)	Municipal water supplies of haemodialysis centres	Membrane filtration	<u>Penicillium</u> and <u>Aspergillus</u>
Germany, North Rhine-Westphalia, 1998/9	12 months	Drinking water	Pour-plating	<u>Acremonium</u> , <u>Exophiala</u> , <u>Penicillium</u> and <u>Phialophora</u>
Norway, 14 networks, 2002/3	December, June and September	Drinking water (surface and groundwater)	Membrane filtration	<u>Penicillium</u> , <u>Trichoderma</u> and <u>Aspergillus</u>

# SURVEYS 2004-2010

Country, Place, Year	Period of time	Type of water	Main isolation method	Most frequent fungal isolates
Portugal, Braga, 2003/4	12 months	Tap water	Membrane filtration	<i>Penicillium</i> and <i>Acremonium</i>
Pakistan, Karachi, 2007	One collection (30 samples)	Water (and fruit juice)	Direct plating	<u><i>Aspergillus niger</i></u> and <i>A. clavatus</i>
Australia, Queensland, 2007/8	18 months	Municipal water	Membrane filtration	<i>Cladosporium</i> , <i>Penicillium</i> , <u><i>Aspergillus</i></u> and <i>Fusarium</i>
Brazil, Recife, 2009/10	5 months	Water treatment plant; tap water	Membrane filtration	<i>Penicillium</i> , <u><i>Aspergillus</i></u> and <i>Phoma</i>
Portugal, Lisbon, 2010	4 months	surface water; spring water; groundwater	Membrane filtration	<u><i>Aspergillus</i></u> , <i>Cladosporium</i> , <i>Penicillium</i>
Belgium, Liège	4 months	Tap water+MDS	Membrane filtration	<i>Fusarium</i> , <u><i>Aspergillus</i></u> , <i>Penicillium</i> , <i>Paecilomyces</i>

**Fusariosis Associated with Pathogenic *Fusarium* Species Colonization of a Hospital Water System: A New Paradigm for the Epidemiology of Opportunistic Mold Infections**

Elias J. Anaissie,<sup>1</sup> Robert T. Kuchar,<sup>2</sup> John H. Rex,<sup>3</sup> Andrea Francesconi,<sup>4</sup> Miki Kasai,<sup>4</sup> Frank-Michael C. Müller,<sup>4</sup> Mario Lozano-Chiu,<sup>2</sup> Richard C. Summerbell,<sup>2</sup> M. Cecilia Dignani,<sup>1</sup> Stephen J. Chanock,<sup>4</sup> and Thomas J. Walsh<sup>1</sup>

Houston university Hospital, Texas, 2001  
Numerous cases of *Fusariosis*  
over a 10- year period

- 162/283 (57%) *Fusarium* sp. in water samples
- 18 strains of *F. solani* from patients/17 *F. solani* from environment

**Table 2. Molecular biotyping profiles of related strains of *Fusarium solani* isolated from patient and environmental samples from a hospital in Houston, Texas.**

Type of matched isolate, by source; isolate no. <sup>a</sup>	Pattern score, by laboratory and testing method <sup>b</sup>				Relatedness <sup>c</sup>
	Laboratory A			Laboratory B: RAPD	
	RAPD	RFLP	IR-PCR		
Patient-environment					
1381, 1370	Highly probable	Probable	Probable	Highly probable	Probably related
1379, 1369	Probable	Highly probable	Probable	Probable	Possibly related
Patient-patient					
1328, 1379	Probable	Highly probable	Highly probable	Probable	Probably related
1242, 1319	Highly probable	Probable	Probable	Highly probable	Probably related
1317, 1377	Highly probable	Probable	Probable	Highly probable	Probably related
Environment-environment					
1368, 1370	Probable	Highly probable	Probable	Probable	Possibly related



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Water System: A New Paradigm  
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Houston university Hospital, Texas, 2001  
Numerous cases of *Fusariosis*  
over a 10- year period

1. This study demonstrates that hospital water is a **reservoir** for opportunistic fungi (*Fusarium*)
2. Genetically diverse strains of *F. solani* can contaminate the water system and persist for years
3. WDS can disseminate the fungi by way of aerosols from shower and sink
4. Isolates of *Fusarium* can cause nosocomial infections

# Pathogenic *Aspergillus* Species Recovered from a Hospital Water System: A 3-Year Prospective Study

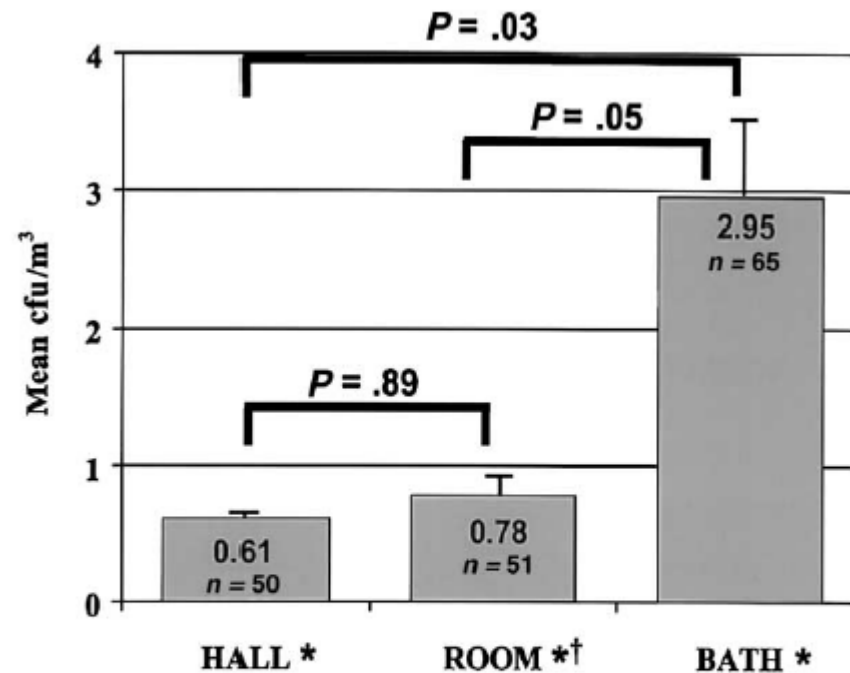
Elias J. Anaissie,<sup>1</sup> Shawna L. Stratton,<sup>1</sup> M. Cecilia Dignani,<sup>1</sup> Richard C. Summerbell,<sup>3</sup> John H. Rex,<sup>4</sup> Thomas P. Monson,<sup>2</sup> Trey Spencer,<sup>1</sup> Miki Kasai,<sup>5</sup> Andrea Francesconi,<sup>5</sup> and Thomas J. Walsh<sup>5</sup>

Study conducted in  
the Hospital of Little  
Rock, Arkansas

- Comparison of genotypic profile of environmental strains and patients isolates

1. 21% *Aspergillus* positive water samples from patients care areas
2. Significantly higher concentration of air-borne propagules were found in bathrooms

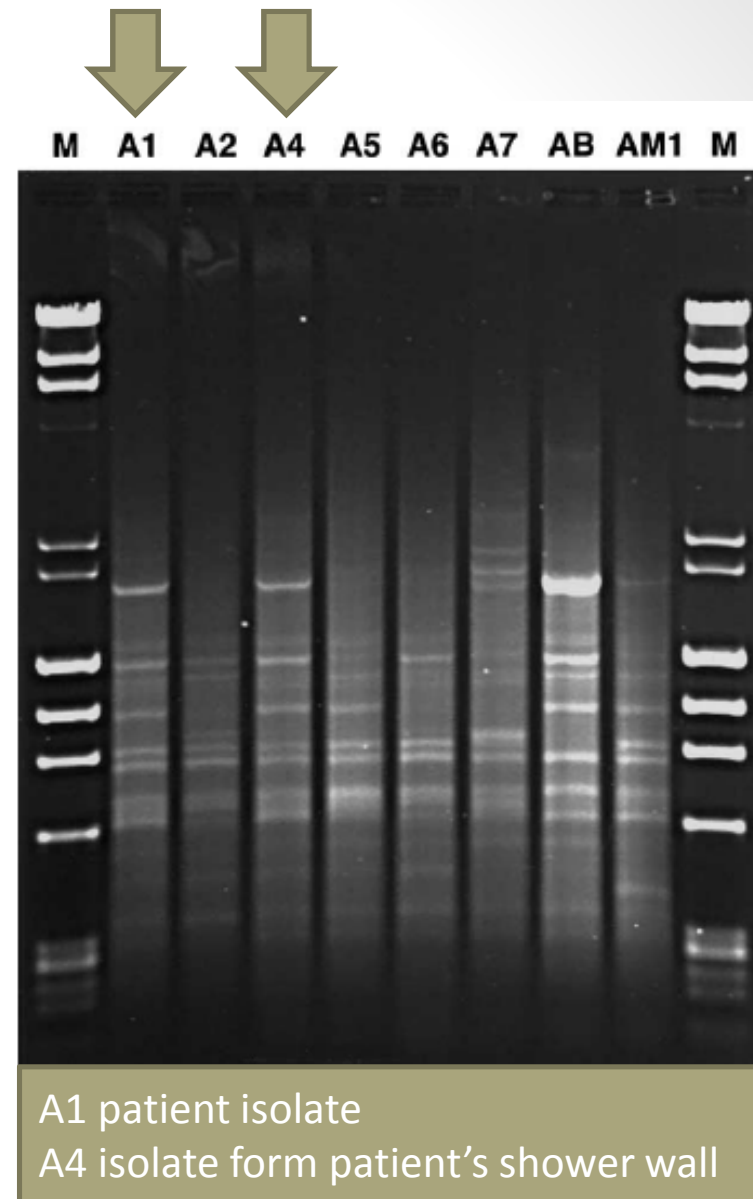
# Higher rates in the shower



\*vs. OUTDOOR  $P = .004$   $P = .005$   $P = .006$   
( $n = 9$ )  
11.33 cfu/m<sup>3</sup> (SEM 2.67)

† HEPA-filtered rooms. 16 samples from 3 LAF rooms were not included in this analysis.

3. An isolate of *A. fumigatus* of a patient with IPA **was genotypically identical to an isolate recovered from the shower wall** of patient's room





# Molecular analyses of *Fusarium* isolates recovered from a cluster of invasive mold infections in a Brazilian hospital

Christina M Scheel<sup>1\*†</sup>, Steven F Hurst<sup>1†</sup>, Gloria Barreiros<sup>2†</sup>, Tiayomi Akiti<sup>2†</sup>, Marcio Nucci<sup>2†</sup> and S Arunmozhi Balajee<sup>3†</sup>



2005, University Hospital  
of Rio de Janeiro  
Increase of cases of  
fusariosis

SEPT 2005 → OCT 2009  
Patients isolates  
Sampling of air-water-  
water related surfaces

BONE MARROW  
TRANSPLANT UNIT

DERMATOLOGIC  
CLINIC

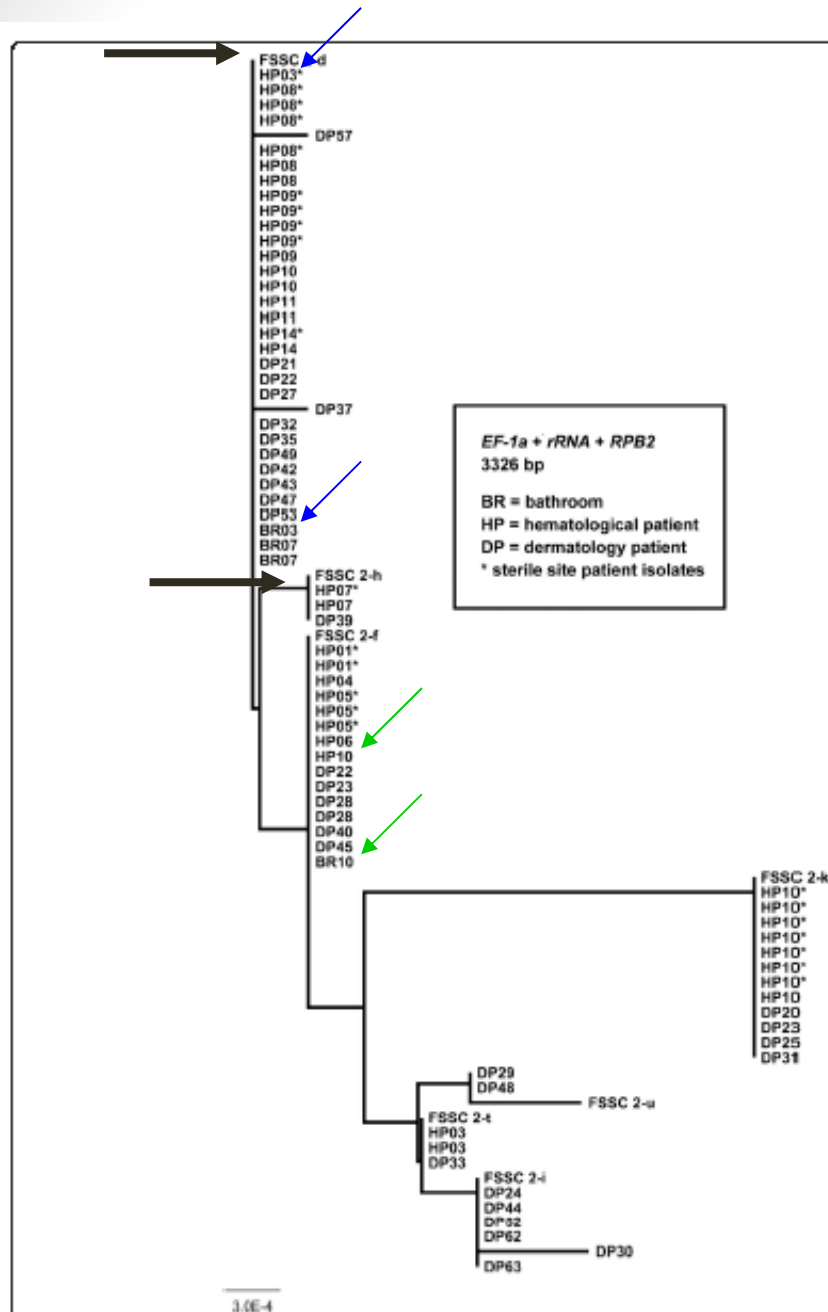


Figure 2 Neighbor-joining phylogeny of *Fusarium solani* species complex (FSSC) species 2 isolates ( $n = 74$ ). Bootstrap values were 100% between individual taxa (1000 iterations, data not shown). Included are previously described FSSC species 2 voucher sequences (labeled FSSC 2-d, FSSC 2-f, FSSC 2-h, FSSC 2-i, FSSC 2-k, and FSSC 2-u) that were retrieved from the RUSARUM ID database [8].

Total: 166 *Fusarium* isolates

→ PATIENTS: 68% *F. solani* species complex (FSSC) in clinical samples (BMTU, dermatology clinic outpatients)

→ ENVIRONMENT: 50% *F. oxysporum* species complex FOSC

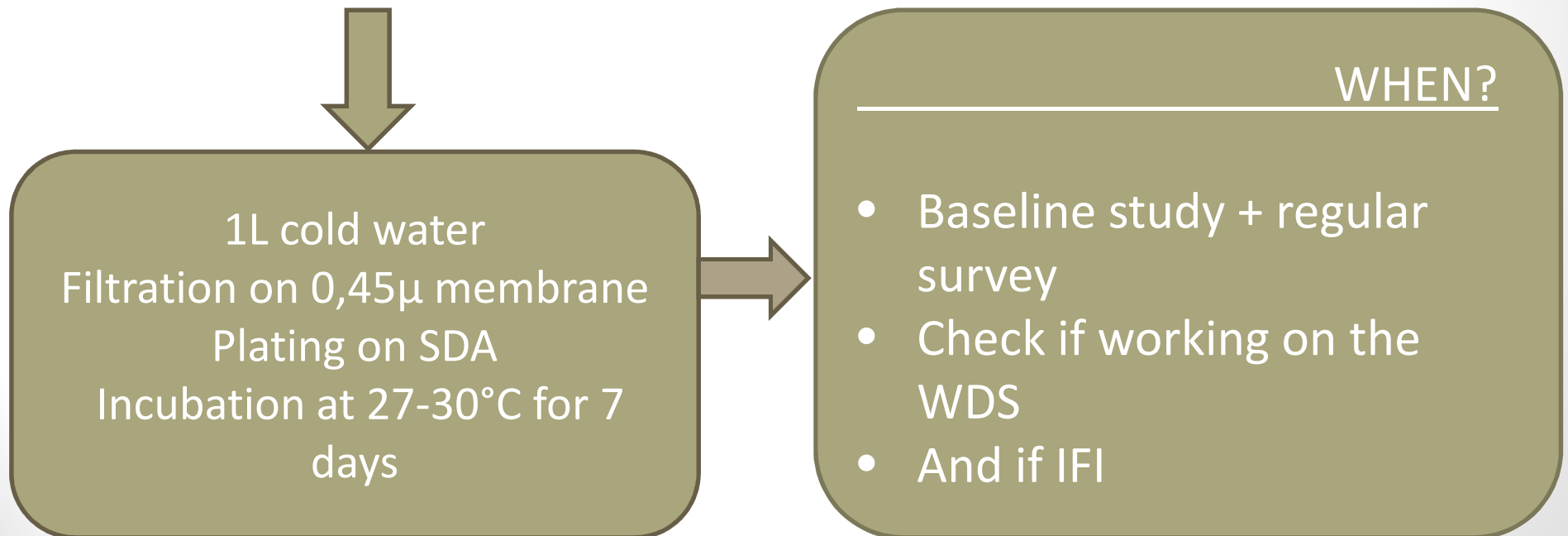
Two identical profiles between one strain from a BMTU patient and the bathroom but no temporal association

# Sampling methodology: « guidelines? »



Kauffmann-Lacroix C, P Med 2008

- French multicentric study from Feb 2004 → March 2005
- → no difference in colonisation between cold and hot water
- → no *Aspergillus fumigatus* but dematiaceous fungi +++



# Sampling methodologies: media

	Glucose (g)	pH	Antibiotiques
<b>CMA/2</b> Corn meal agar half streng	0	5.8-6.2	No
<b>CZ</b> Czapec agar	30	6.0-6.4	No
<b>DG18</b> Dichloran 18% glycerol agar			
<b>DRBC</b> Dichloran RoseBengale Chloramphenicol agar			
<b>NGRBA</b> Neopeptone glucose rose Bengale aureomycine	10	6.3-6.7	Aureomycine
<b>PDA</b> Potato dextrose agar	20	5.4-5.8	No
<b>MEA</b> Malt extract agar	20	5.0-5.5	No
<b>SDA</b> Sabouraud dextrose agar	40	5.4-5.8	No

DG18: recommended medium: 2 advantages

→ characteristic colony appearance

→ inhibits overgrowth of fast growing fungi (*Trichoderma*, *mucorales*)



# Study at the University Hospital of Liège



- CHU Liège: 955 Beds, 3 sites, Surface and underground water
- Filtration + chlorination before to enter the hospital and  $>65^{\circ}\text{C}$  for hot water.
- Study during 4 months from Feb 2005- March 2006
- Methodology: 197 sampling points
  - 500 ml cold and 500 ml hot water
  - filtration on  $0,45\mu$  Millipore membranes
  - Sabouraud agar medium (+ATB)
  - Incubation at  $30^{\circ}\text{C}$  for 1 month

# Results

- contamination rate: 51%

Site 1

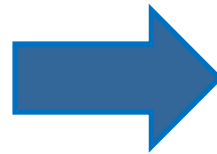
Site 2

Site 3

Filamentous fungi	Sampling sites				<i>P</i> (ST, OA, NDB)
	Three sites ( <i>N</i> = 197) <i>n</i> (%)	ST ( <i>N</i> = 107) <i>n</i> (%)	OA ( <i>N</i> = 40) <i>n</i> (%)	NDB ( <i>N</i> = 50) <i>n</i> (%)	
<i>Acremonium</i> spp.	3 (1.5)	1 (0.9)	1 (2.5)	1 (2)	NS
<i>Alternaria</i> spp.	1 (0.5)	1 (0.9)	–	–	NS
<i>Aspergillus</i> spp.	12 (6)	8 (7.4)	4 (10)	–	NS
<i>A. flavus</i>	2 (1)	2 (1)	–	–	NS
<i>A. fumigatus</i>	4 (2)	2 (1)	2 (5)	–	NS
<i>A. nidulans</i>	2 (1)	2 (1)	–	–	NS
<i>A. niger</i>	2 (1)	–	2 (1)	–	NS
<i>A. sydowii</i>	2 (1)	2 (1)	–	–	NS
<i>Cladosporium</i> spp.	6 (3)	4 (3.7)	1 (2.5)	1 (2)	NS
<i>Fusarium</i> spp.	23 (11.6)	3 (2.8)	6 (15)	14 (28)	≤0.001
<i>Monilia</i> spp.	7 (3.5)	2 (1.8)	3 (7.5)	2 (4)	NS
<i>Paecilomyces</i> spp.	14 (7)	6 (5.6)	–	8 (16)	≤0.05
<i>Penicillium</i> spp.	22 (11.2)	9 (8.4)	6 (15)	7 (14)	NS
<i>Sterile mycelia</i>	10 (5)	5 (4.6)	1 (2.5)	4 (8)	NS
<i>Trichoderma</i> spp.	4 (2)	1 (0.9)	2 (5)	1 (2)	NS
Contamination rates	102 (51)	40 (37.3)	24 (60)	38 (76)	≤0.01

ST, Sart Tilman; OA, Ourthe-Amblève; NDB, Notre-Dame des Bruyères; *N*, total number of tested samples; *n*, number of samples; NS, not significant; (–), no observation.

# Conclusion of the study and preventive measures



- Implementation: quite easy
- Regular replacement more difficult to implement !!!

# Conclusion



- There is a need for guidelines
- Every hospital with severe immunosuppressed patients should be aware of the potential danger
  - sampling tap water and shower walls
  - implement point-of-use filtration systems and organize replacement
  - avoid showering during severe immunosuppression
  - replace showers by baths



