3° Simpozijuma Dijagnoza i Terapija Gljivičnih Oboljenja
3rd Symposium on Diagnosis and Therapy of Fungal Diseases

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Fusariosis

Anna Maria Tortorano
Dipartimento di Sanità Pubblica Microbiologia Virologia
Università degli Studi di Milano

PHOTO QUIZ

Philip A. Mackowiak, Section Editor

Foot Ulcer, Skin Nodules, and Blurred Vision

(See pages 736–7 for the Answer to the Photo Quiz.)

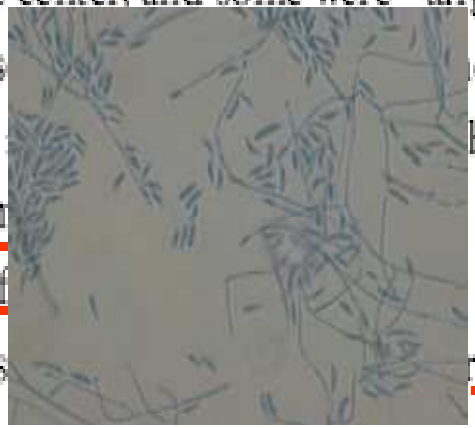
Highly immuno-compromised host

High fever

Rapid dissemination with metastatic skin lesions

Broken skin portal of entry

A 49-year-old female with acute lymphoblastic leukemia suffered from prolonged neutropenia after induction chemotherapy. On day 24 postchemotherapy, she developed a high fever of 40°C, and a small ulcer was noted on her right foot (Figure 1*A*). Soon, multiple painful skin nodules developed on her limbs (Figure 1*B*), trunk, and face. The lesions were characterized by a necrotic center, and some were "target-like." A few days later, she als d blurred vision; an examination halmitis. Despite empirical treatm antibacterial agents and anidulaf not controlled. The microorganis rown from her blood culture.



Positive bloodcultures

Resistance to echinocandins

Plant pathogens

Mycotoxins

Pathogens in immunocompetent hosts

Fusarium spp

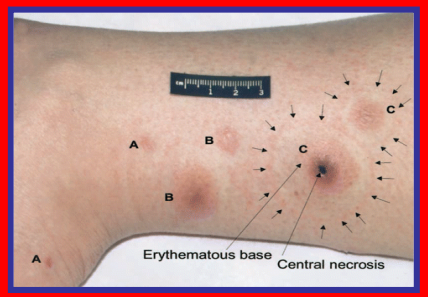
Pathogens in immunocompromised hosts

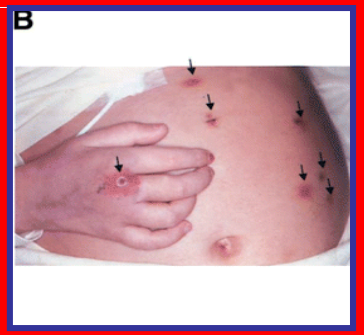
Portal of entry:

- ★ sino-pulmonary
- ★ broken skin
- ★ pre-existing onychomycosis

Rapid dissemination of infection due to sustained release of conidia

Metastatic skin lesions



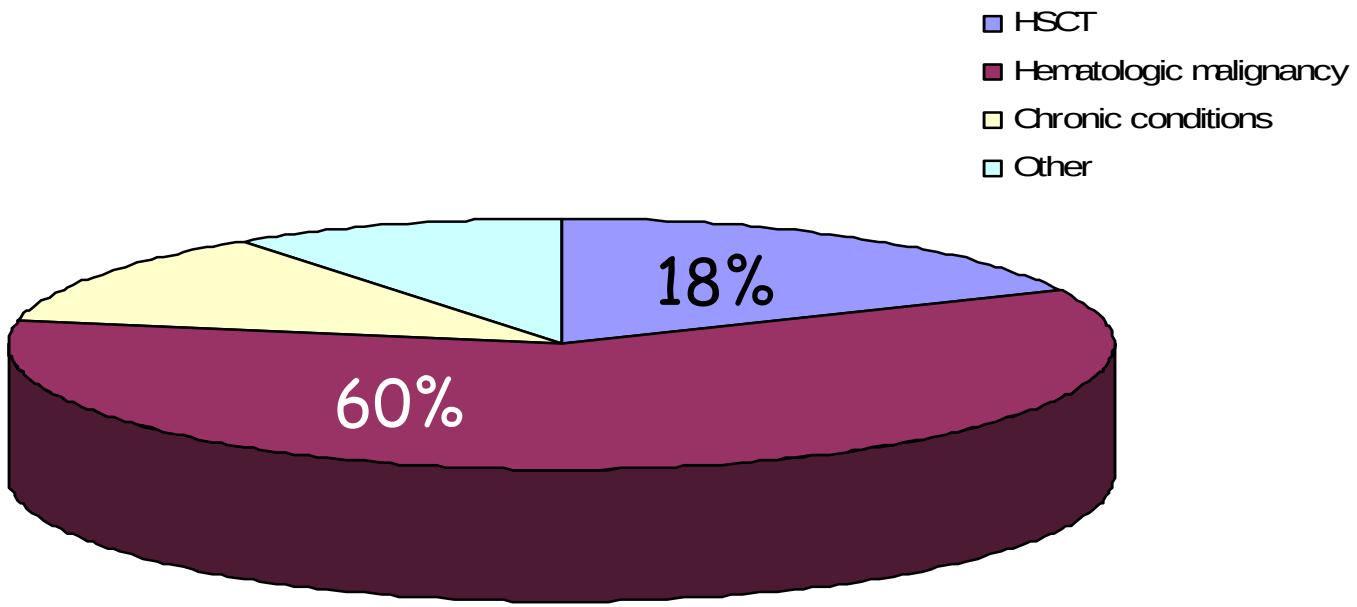


Nucci & Anaissie CID 2002

Positive bloodcultures

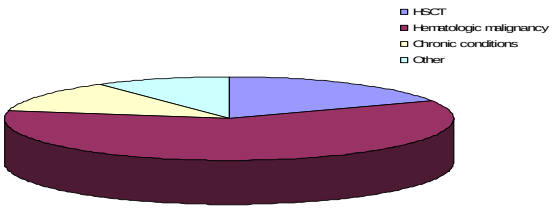
Poor prognosis of disseminated infection

Underlying diseases in patients with invasive fusariosis

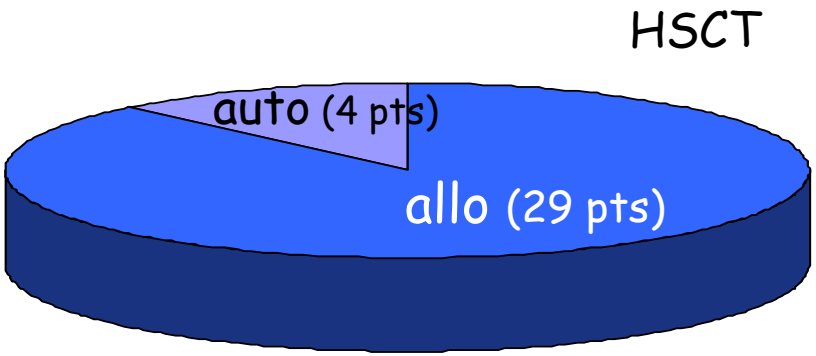


Lortholary et al AAC 2010 (73 patients)

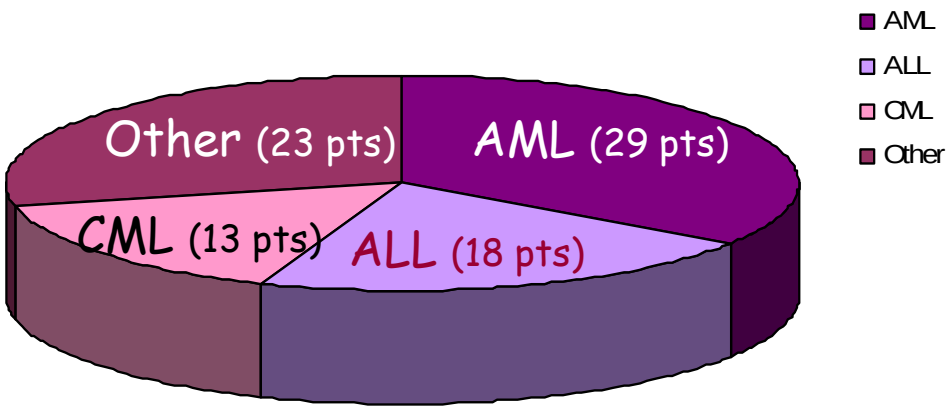
Underlying diseases in patients with invasive fusariosis



Lortholary et al AAC 2010 (73 patients)



Nucci et al Cancer 2003 (33 patients)



Nucci et al Cancer 2003 (84 patients)

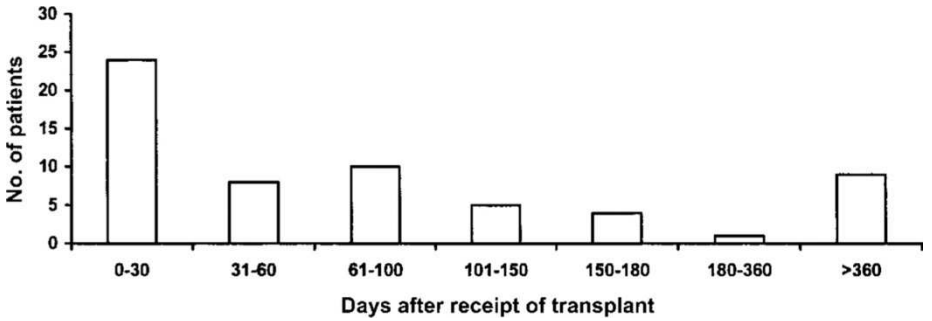
Underlying diseases in patients with invasive fusariosis

Fusarium infections in 61 hematopoietic stem cell transplant recipients from 9 hospitals

54 cases in allogeneic HSCT,
20.19 per 1000 recipients

7 cases in autologous HSCT,
4.21-5.0 per 1000 recipients

Trimodal distribution of cases diagnosed after allogeneic HSCT



Nucci M et al. CID 2004; 38: 1237-42

Treatment of disseminated fusariosis

★ Lack of data from clinical trials

Amphotericin B

Efficacy rates

- ★32% for amphotericin B
- ★46% for amphotericin B lipid formulations

Nucci et al Cancer 2003
Nucci et al CID 2004
Perfect CID 2005

Posaconazole as Salvage Treatment for Invasive Fusariosis in Patients with Underlying Hematologic Malignancy and Other Conditions

Issam I. Raad,[1] Ray Y. Hachem,[1] Raoul Herbrecht,[2] John R. Graybill,[3] Roberta Hare,[3] Gavin Corcoran,[4] and Dimitrios P. Kontoyiannis[1]

[1]The M. D. Anderson Cancer Center, Houston, and [3]The University of Texas Health Science Center at San Antonio, San Antonio, Texas; [2]Schering-Plough Research Institute, Kenilworth, New Jersey; [4]Steifel Laboratories, Coral Gables, Florida; and [5]Hôpitaux Universitaires de Strasbourg, Strasbourg, France

Background. Conventional amphotericin B–based antifungal therapy for invasive fusariosis in patients with a hematologic malignancy results in a ≥70% failure rate. Posaconazole is a broad-spectrum antifungal agent with in vitro and in vivo activity against *Fusarium* species.

Methods. In this retrospective analysis of patients from 3 open-label clinical trials, we evaluated posaconazole for the treatment of invasive fusariosis. Twenty-one patients with proven or probable invasive fusariosis who had disease refractory to or who were intolerant of standard antifungal therapy received oral posaconazole suspension (800 mg per day in divided doses) as salvage therapy.

Results. Successful outcome occurred in 10 (48%) of all 21 patients. Among patients with leukemia who received posaconazole therapy for >3 days, the overall success rate was 50%; for patients who recovered from myelosuppression, the success rate was 67%, compared with 20% for those with persistent neutropenia.

Conclusion. These results suggest that posaconazole is useful for the treatment of invasive fusariosis.

Clinical Infectious Diseases 2006; 42:1398–403

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2010, p. 4446–4450 Vol. 54, No. 10
0066-4804/10/$12.00 doi:10.1128/AAC.00286-10

International Retrospective Analysis of 73 Cases of Invasive Fusariosis Treated with Voriconazole▽

Olivier Lortholary,[1,2] Gaelle Obenga,[1] Pinaki Biswas,[3] Denis Caillot,[4] Elisabeth Chachaty,[5] Anne-Lise Bienvenu,[6] Muriel Cornet,[7] John Greene,[8] Raoul Herbrecht,[9] Claire Lacroix,[10] Frédéric Grenouillet,[11] Issam Raad,[12] Karine Sitbon,[1] Peter Troke,[13]* and the French Mycoses Study Group†

- ★ 47% success rate
- ★ Baseline neutropenia impacted success adversely
- ★ Success varied by underlying condition and infection site
- ★ Combination therapy (13 pts) was no better than treatment with voriconazole alone

Treatment of disseminated fusariosis

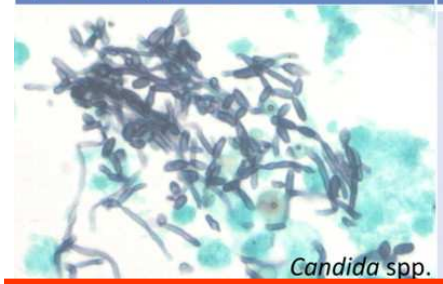
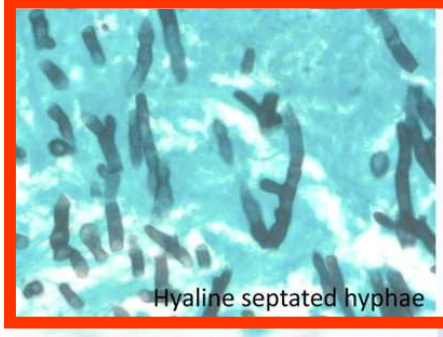
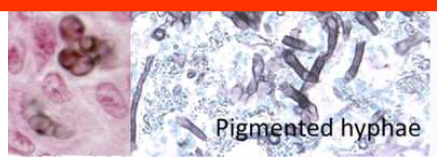
- ★ Lack of data from clinical trials
- ★ Potential efficacy of posaconazole and of voriconazole recently shown by retrospective analyses
- ★ Outcome improved by a rapid and efficient diagnosis in terms of identifying the aetiology of the infection

Diagnosis of disseminated fusariosis

- ★ Positive bloodcultures due to adventitious sporulation
- ★ Interpretation of growth from other biological materials depends on the clinical context
- ★ Radiological findings are not specific
- ★ Skin lesions often the initial clue to diagnosis
- ★ Morphological discrimination at histology is difficult

Morphology, description, diagnosis, and comment for fungal infections that present with hyphae or pseudohyphae in tissues

Guarner, J. et al. 2011. Clin. Microbiol. Rev. 24(2):247-280

Morphology of hyphae and pseudohyphae	Description, diagnosis and comment
 *Candida* spp.	**Description:** Small yeasts (3- 5 microns in size) intermingled with pseudohyphae and hyphae. **Diagnosis:** Yeasts with pseudohyphae. **Comment:** The morphology is consistent with *Candida* spp.; however, *Aspergillus* spp. and other hyaline fungi can be confused histologically.
 Hyaline septated hyphae	**Description:** Non-pigmented (hyaline), septated hyphae with acute angle branching. **Diagnosis:** Non-pigmented (hyaline), septated hyphae. **Comment:** The morphology is consistent with *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Trichoderma* spp., *Paecilomyces* spp. and others. Mucorales genera can sometimes have this morphology.
	Description: Non-pigmented (hyaline), pauciseptate branching. e), pauciseptate hyphae. sistent with *Mucorales* and other septated e this morphology.
 Pigmented hyphae	yphae and yeast-like yphae with septations. sistent with durella spp, *Fonsecaea* spp, *Cladophialophora* spp, *Exophiala* spp, *Curvularia* spp, *Bipolaris* spp, and others.

- Immunohistochemestry
- In situ hybridization

useful to identify the fungus

Diagnosis of disseminated fusariosis

- Positive bloodcultures due to adventitious sporulation
- Interpretation of growth from other biological materials depends on the clinical context
- Radiological findings are not specific
- Skin lesions often the initial clue to diagnosis
- Morphological discrimination at histology is difficult
- 1,3-β-D-glucan test cannot distinguish *Fusarium* from other fungal infections
- Galactomannan assay is sometimes positive in case of disseminated fusariosis

Cross-reactivity of *Fusarium* in *Aspergillus* GM assay

9 out of 11 patients with disseminated infection had repeated positive GM

All 12 *Fusarium* tested isolates produced positive GM

Tortorano et al J Clin Microbiol 2012; 50: 1051-3

Plant pathogens

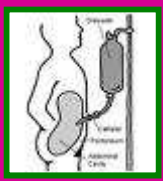
Pathogens in immunocompromised hosts

Mycotoxins

Fusarium spp

Pathogens in immunocompetent hosts

Peritonitis in association with peritoneal dialysis



Arthritis/osteomyelitis following trauma

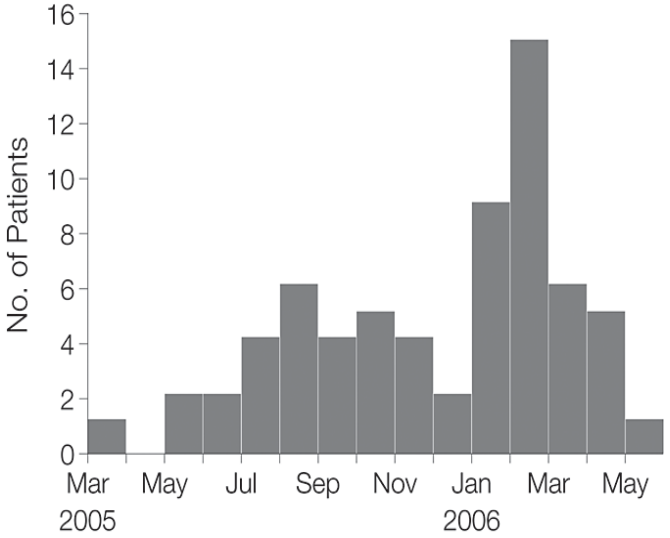
Keratitis
consequence of corneal trauma associated with contact lens wear

Onychomycosis

Hay & Baran AJAD 2011

2005-2006 outbreaks of *Fusarium* keratitis associated with contact lens wear

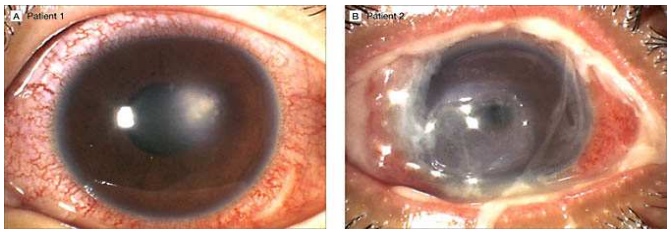
Epidemic curve for the outbreak of *Fusarium* keratitis associated with contact lens wear (Singapore).



Khor, W. et al. JAMA 2006;295:2867-2873

JAMA

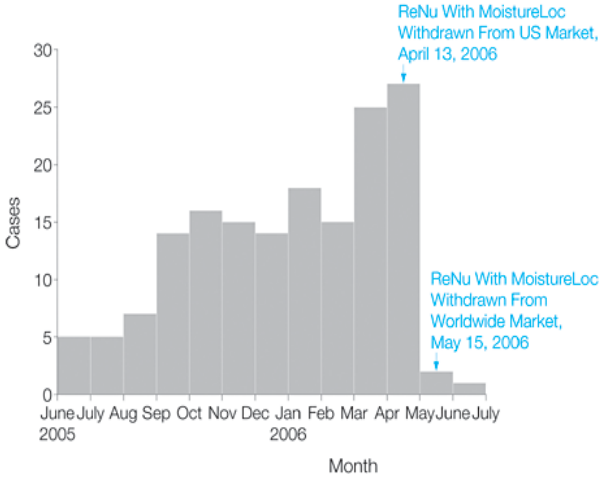
Patients with fungal keratitis.
Patient 1 has classic characteristics of fungal keratitis, which include stromal infiltrate with indistinct, feathery edges, and satellite lesions.



Khor, W. et al. JAMA 2006;295:2867-2873

JAMA

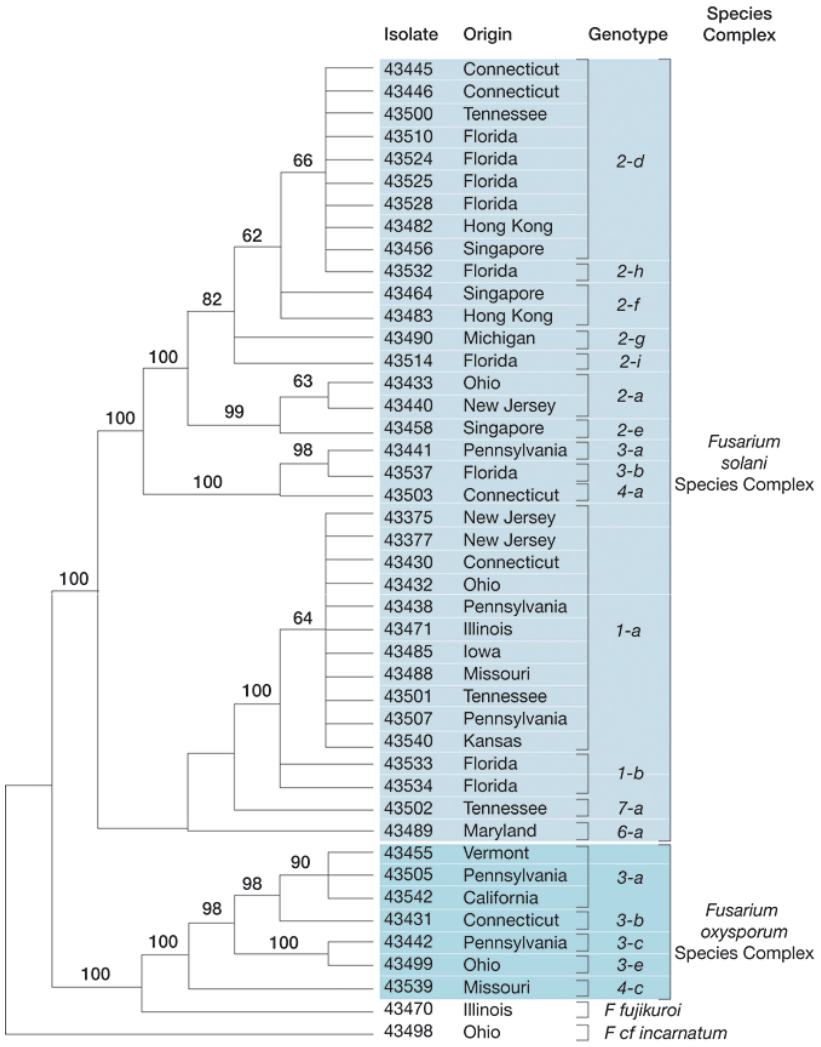
Confirmed cases of *Fusarium* keratitis in the United States, June 2005-July 2006 Month of illness onset of confirmed cases of *Fusarium* keratitis reported to the Centers for Disease Control and Prevention.



Chang, D. C. et al. JAMA 2006;296:953-963

JAMA

2005-2006 outbreaks of *Fusarium* keratitis associated with contact lens wear



Phylogenetic diversity of 59 isolates (39 corneal isolates from 38 US confirmed case patients, and 20 isolates from the keratitis outbreaks in Singapore and Hong Kong) inferred from multilocus DNA sequence data

US isolates represent 19 unique genotypes:

- 12 genotypes (30 isolates) within the *Fusarium solani* species complex (FSSC),
- 5 genotypes (7 isolates) within the *Fusarium oxysporum* species complex (FOSC),
- 1 genotype (1 isolate) *Fusarium fujikuroi*
- 1 genotype (1 isolate) *Fusarium incarnatum*

Chang, D. C. et al. JAMA 2006;296:953-963

Fusarium superficial infections: possible acquisitions ?

Fusarium species isolated from

- ★ surfaces of public swimming pool facilities

  Bobichon H et al 1989
  Brandi G et al 2007

- ★ area of a hospital physical therapy swimming pool (associated with *Fusarium* onychomycosis in two patients attending the pool)

  Buot G et al 2010

Fusarium infections: community acquired or hospital acquired?

Fusarium species

- ★present in outdoor air
- ★recovered from hospital water system (water, water storage tanks, shower and sink drains, shower heads, sink faucet aerators)
- ★present in hospital air

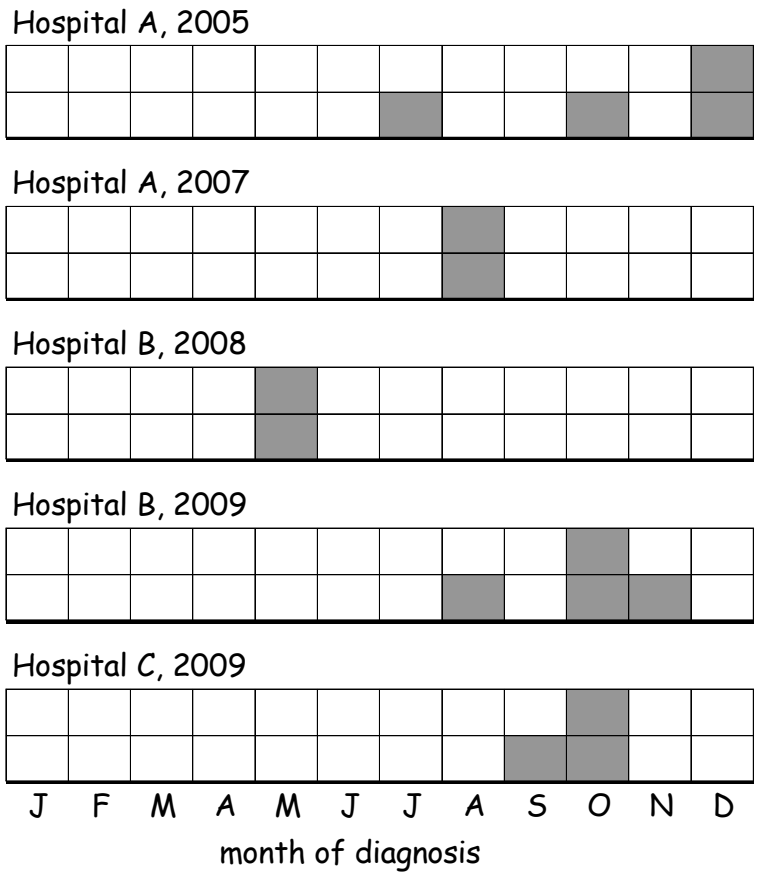
Anaissie et al CID 2001

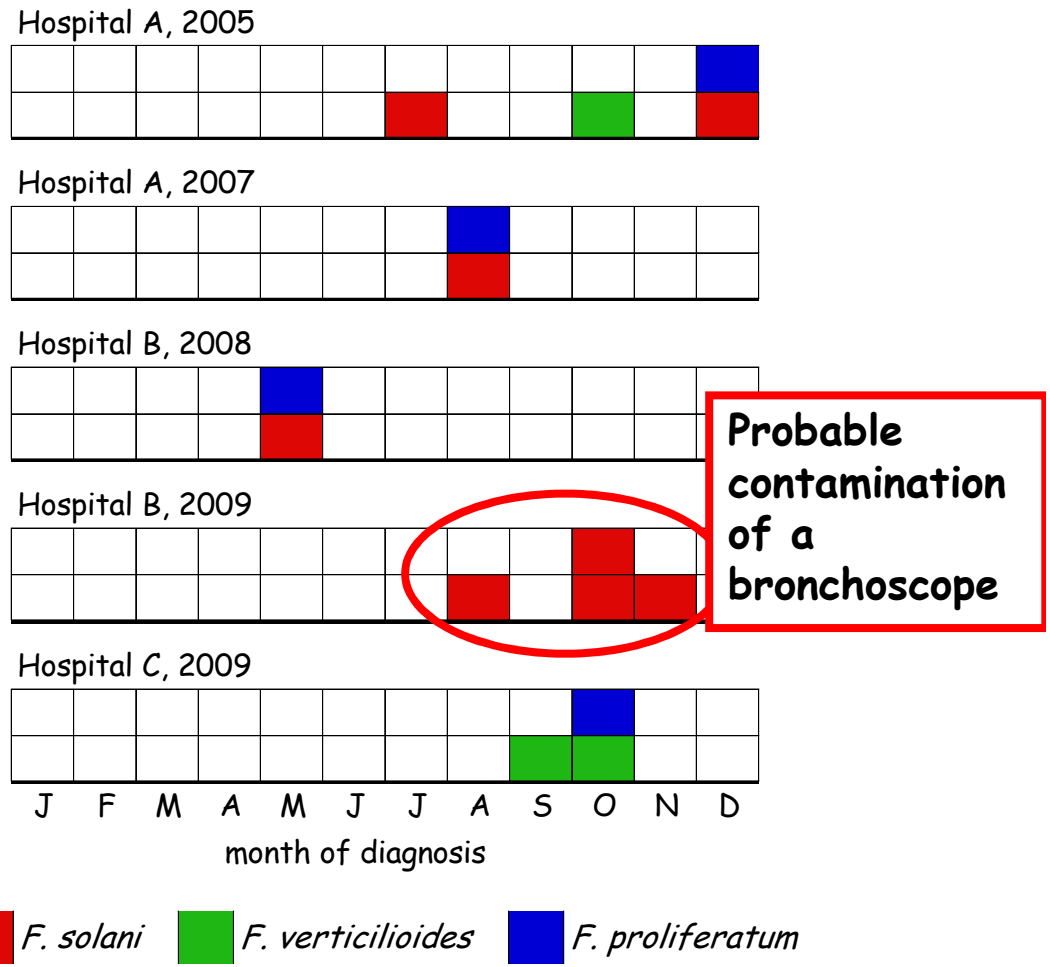
Fusarium infections: protection of high risk individual from exposure

- ★ HEPA-filtered air
- ★ Drinking of sterile water
- ★ Bed bath instead of showering
- ★ Cleaning and drying showerheads, bathroom floor and walls
- ★ Evaluation by a dermatologist

Fusarium infections: outbreaks or clusters?

Distribution of cases according to month of diagnosis in different Italian hospitals





Fusarium spp

Pathogens in immunocompetent hosts

Pathogens in immunocompromised hosts

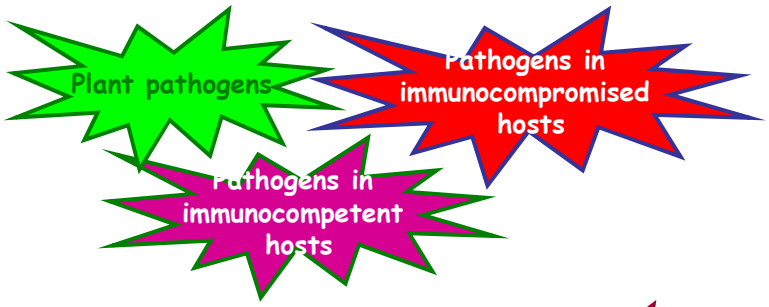
Mycotoxins

Plant pathogens

Fig. 1. Typical disease symptoms caused by various species of *Fusarium*. A, Fusarium wilt of banana caused by *F. oxysporum* f. sp. *cubense*. B, Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. C, Stem rot of vanilla caused by *F. oxysporum*. D, Mango malformation caused by *F. manginifera*. E, Fusarium wilt of Canary Island date palm caused by *F. oxysporum* f. sp. *canariensis*. F, Bakanae disease of rice caused by *Fusarium fujikuroi*. G, Stalk rot of sorghum caused by *F. thapsinum*. H, Root rot of *Aglaonema commutatum* caused by *Fusarium solani*. I, Cob rot of maize caused by *F. verticillioides*. All photos by authors except D, by Randy Ploetz, E, by Suzanne Bullock, and G, by Larry Claflin.

Plant Disease / Vol. 87 No. 2

Fusarium spp



Mycotoxins

Fumonisins ➡

Leukoencephalomalacia in equines and rabbits

Neural tube defects

Link with esophageal cancer ??

Zearalenone
Nonsteroidal estrogen

Trichothecenes
(>60 sesquiterpenoid metabolites)

Potent inhibitors of eukaryotic protein synthesis

➡ Alimentary toxic aleukia

Trichothecenes

➡ Bioterrorism ??

Identification of *Fusarium* species

★ Classical morphological identification

underestimates
species diversity

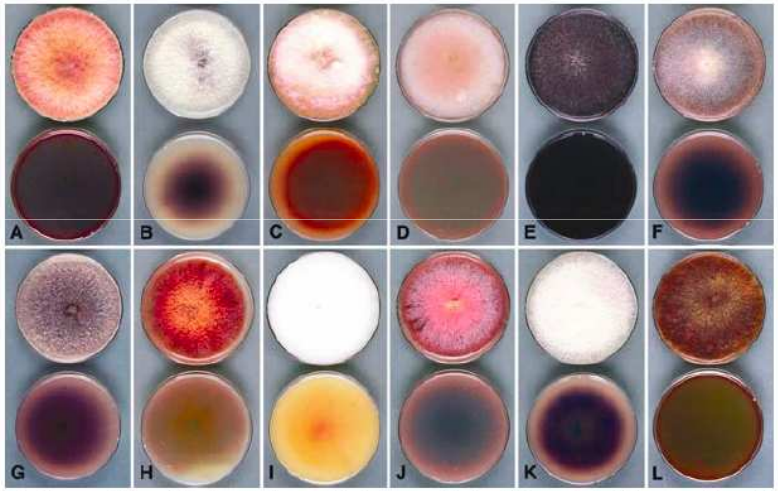


Fig. 2. Colony morphology of *Fusarium* species on potato dextrose agar. The top plate in each pair is the upper surface and the lower plate is the under surface. A, *F. poae*. B, *F. oxysporum*. C, *F. acuminatum*. D, *F. nelsonii*. E, *F. subglutinans*. F, *F. nygamai*. G, *F. pseudonygamai*. H, *F. lateritium*. I, *F. thapsinum*. J, *F. decemcellulare*. K, *F. verticillioides*. L, *F. culmorum*.

120 Plant Disease / Vol. 87 No. 2

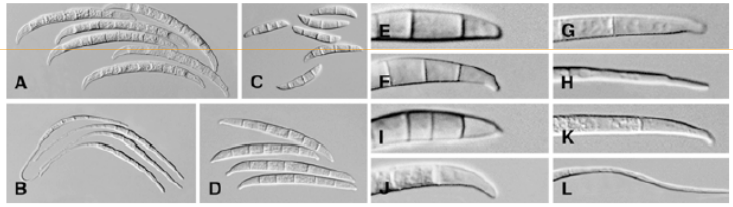


Fig. 6. Macroconidia of *Fusarium* species. A to D, Variation in macroconidial shape and length. A, *F. decemcellulare*. B, *F. longipes*. C, *F. culmorum*. D, *F. chlamydosporum*. E to H, Variation in basal cells of macroconidia. E, *F. culmorum*. F, *F. crookwellense*. G, *F. avenaceum*. H, *F. longipes*. I to L, Variation in apical cells of macroconidia. I, *F. culmorum*. J, *F. decemcellulare*. K, *F. verticillioides*. L, *F. longipes*.

124 Plant Disease / Vol. 87 No. 2

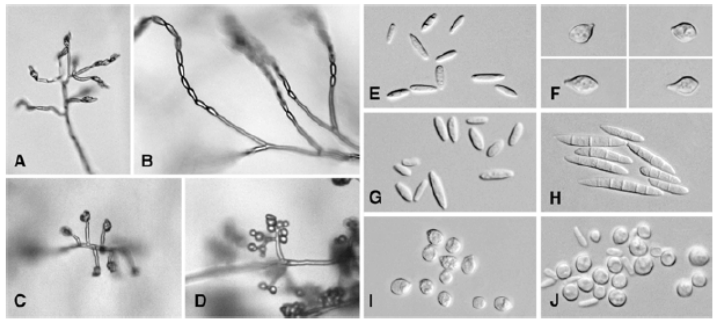


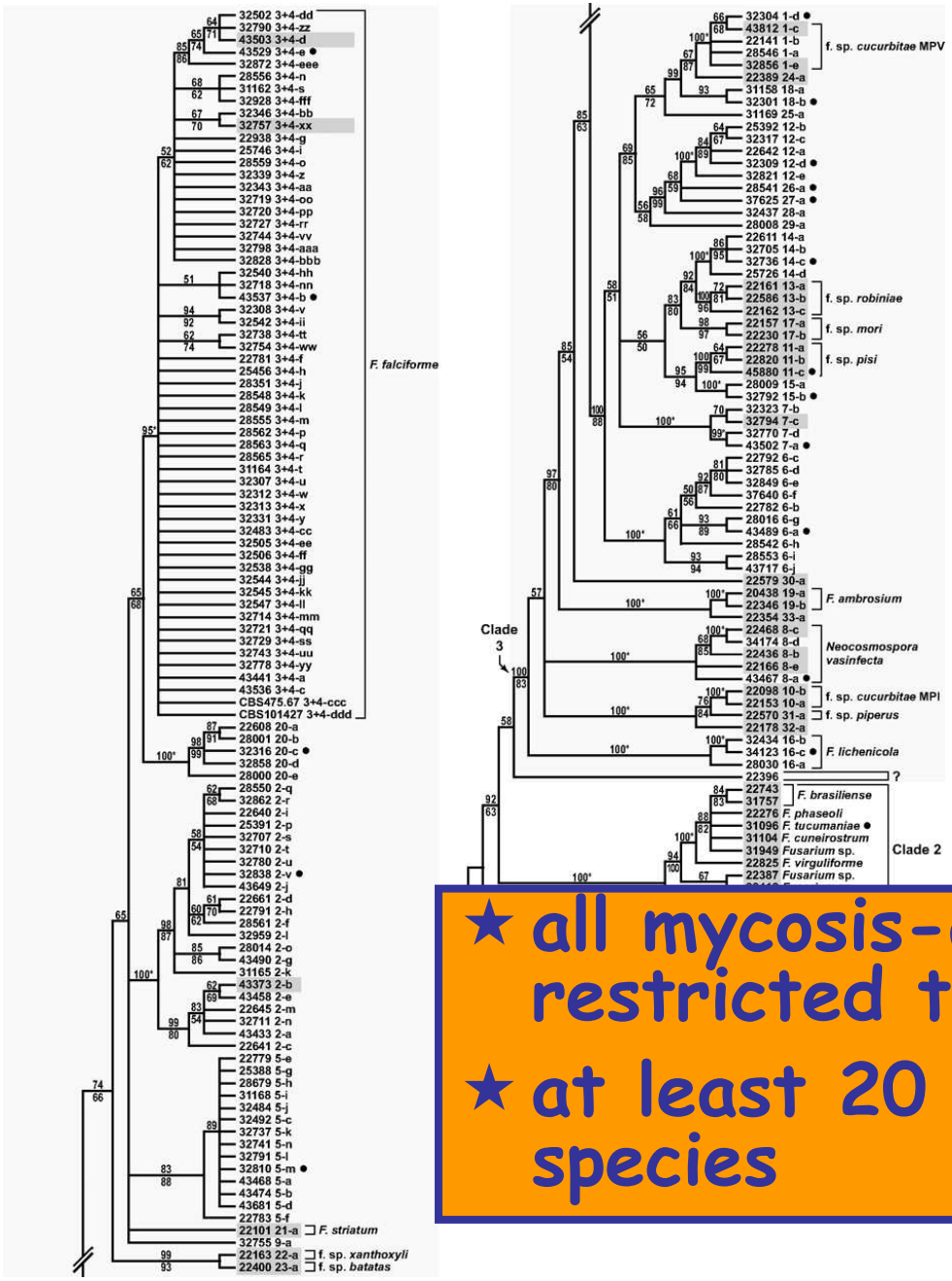
Fig. 4. Formation and types of microconidia produced by *Fusarium* species. A, Microconidia produced in short chains (*F. brevicatenulatum*). B, Microconidia produced in long chains (*F. decemcellulare*). C, Microconidia produced in false heads (*F. circinatum*). D, Napiform microconidia in false heads (*F. konzum*). E, Oval microconidia (*F. babinda*). F, Pyriform microconidia (*F. anthophilum*). G, Clavate microconidia (*F. anthophilum*). H, Fusiform microconidia (*F. semitectum*). I, Napiform microconidia (*F. poae*). J, Globose microconidia (*F. anthophilum*).

122 Plant Disease / Vol. 87 No. 2

Identification of *Fusarium* species

- ★ Classical morphological identification underestimates species diversity
- ★ ITS region sequencing not very useful in differentiating cryptic species (insufficient hypervariability)
- ★ Sequencing of elongation factor 1α gene (EF-1α), β-tubulin (β-TUB), calmodulin (*CAM*), and RNA polymerase II second largest subunit (RPB2), revealed the presence of multiple cryptic species

≈ 70 medically relevant species



Fusarium solani species complex

Bootstrapped ML out-group-rooted cladogram inferred from the combined DNA sequence data from three loci for 180 unique STs

O'Donnell, K. et al. 2008. J. Clin. Microbiol. 46(8):2477-2490

★ all mycosis-associated isolates restricted to clade 3

★ at least 20 phylogenetically distinct species

Journal of Clinical Microbiology

Identification of *Fusarium* species

- Classical morphological identification underestimates species diversity
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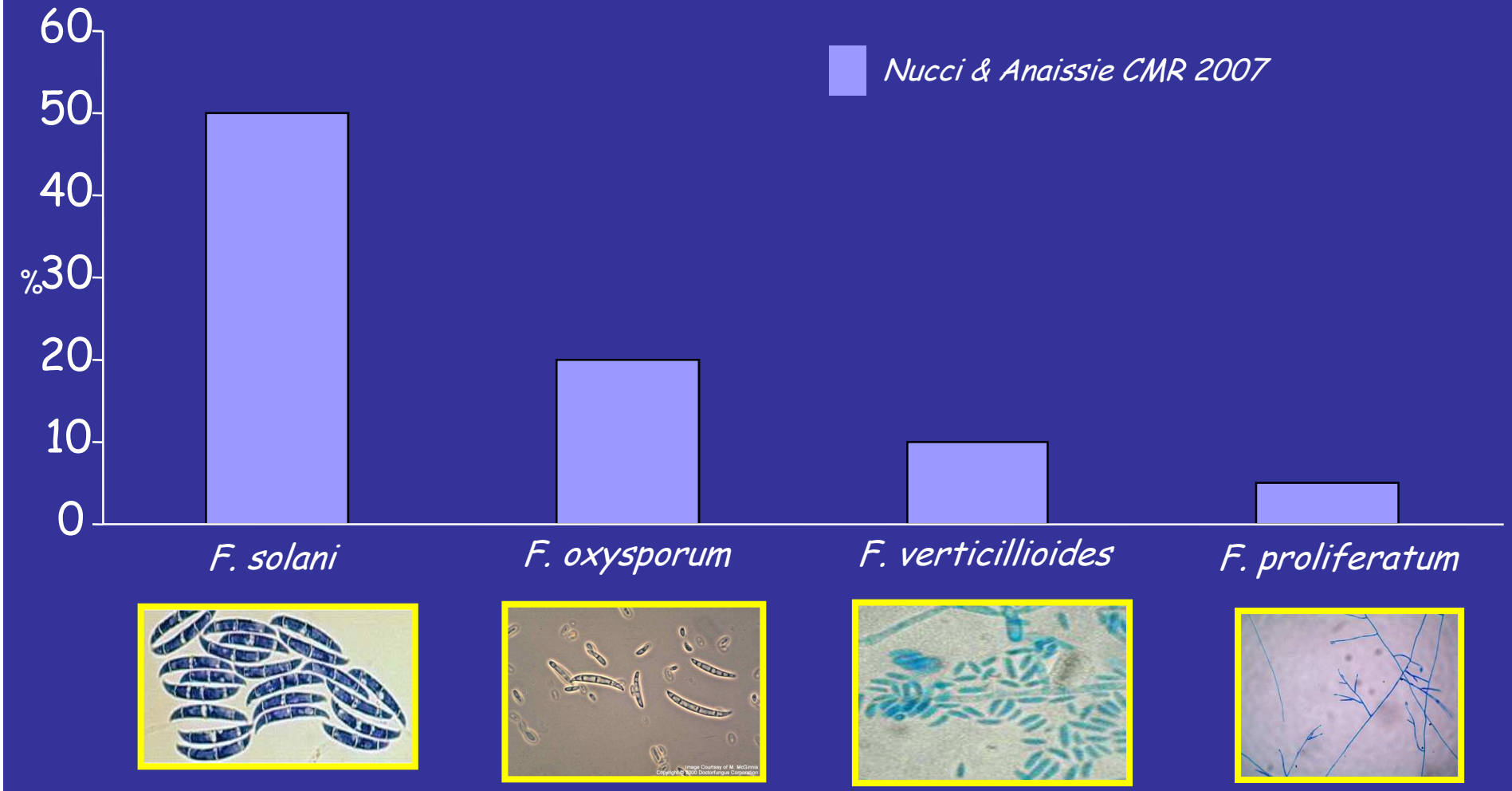
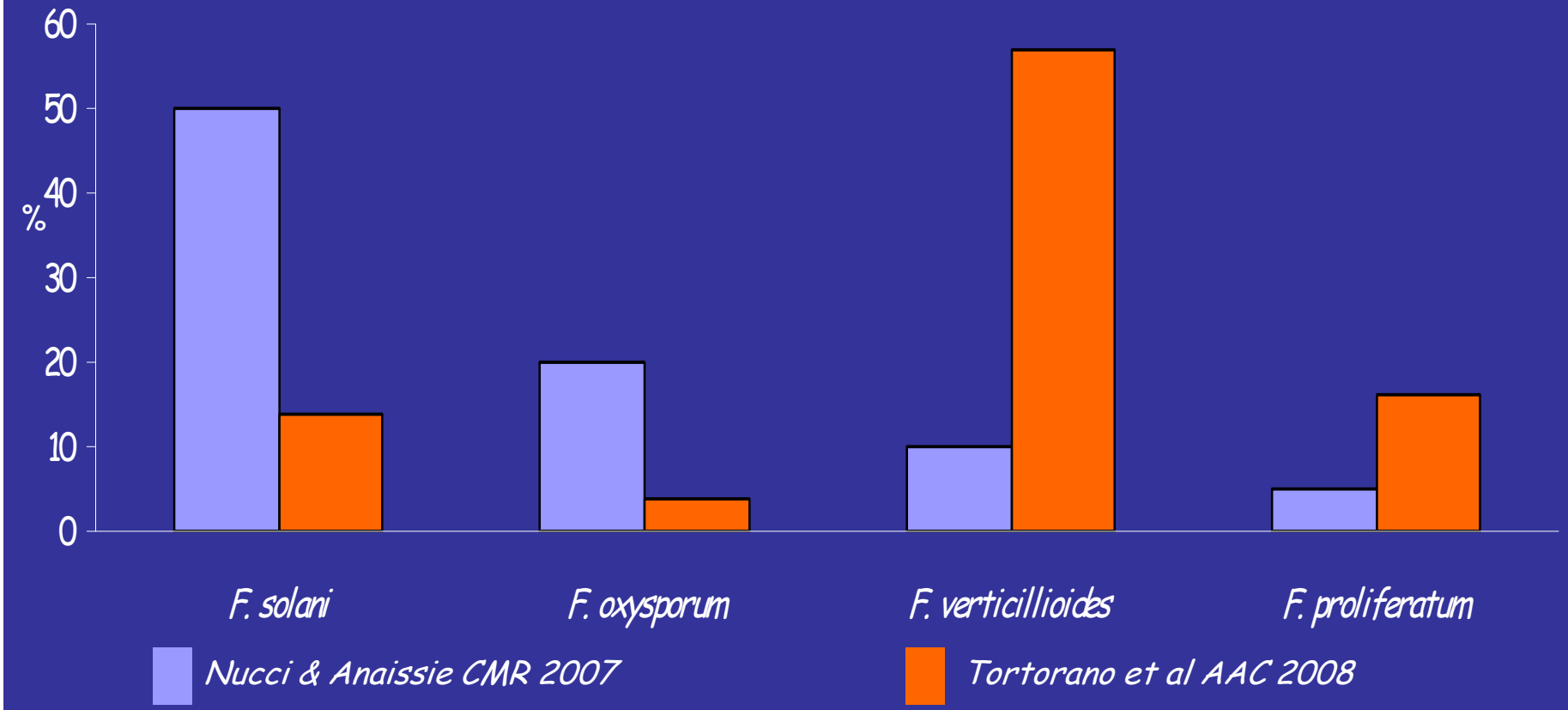
- Sequencing of elongation factor 1α gene (EF-1α), β-tubulin (β-TUB), calmodulin (*CAM*), and RNA polymerase II second largest subunit (RPB2), revealed the presence of multiple cryptic species
- MALDI-TOF: promising results

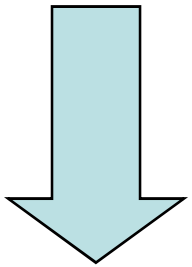
Fusarium species most frequent cause of invasive infection



Fusarium species most frequent cause of invasive infection



High resistance of *Fusarium* strains
to the available drugs



Treatment of fusariosis in
immunosuppressed and immunocompetent patients
is a frustrating task

High resistance of *Fusarium* strains to the available drugs

Fusarium species

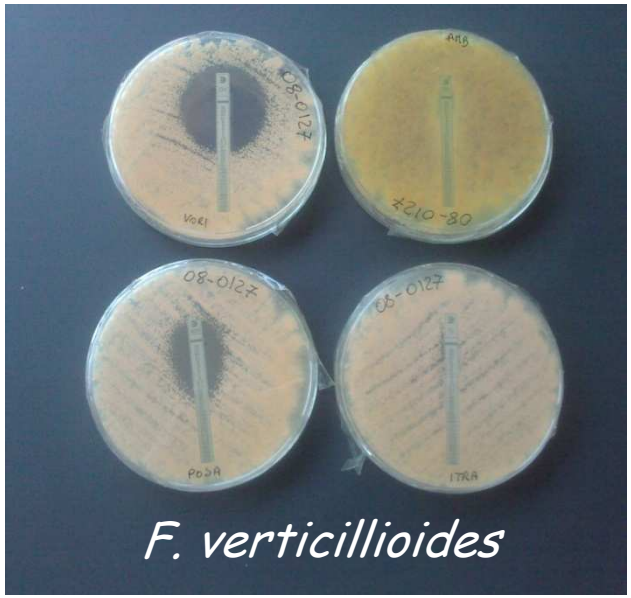
- resistant to fluconazole
- resistant to echinocandins
- high MICs of amphotericin B

High resistance of *Fusarium* strains to the available drugs

F. solani shows the highest MICs

no significant differences among the cryptic species

Azor M et al AAC 2007



F. verticillioides

F. verticillioides shows a higher susceptibility than the other important species of the genus

ECMM survey: *FUSARIUM* INFECTIONS in EUROPE

Country		**National Coordinator**	
Centre		City	

PATIENT

Patient code ..... Sex ☐ M ☐ F Birthdate (mm/yyyy) ☐☐ / ☐☐☐☐

Country of birth ..... Country of residence ..... Occupation .....

Ward of hospitalization ☐ Hematology ☐ ICU ☐ other, *specify*

***FUSARIUM* PATHOLOGY**	**Date of diagnosis** (dd/mm/yy) ☐☐ / ☐☐ / ☐☐

☐ Disseminated infection ☐ Localized infection ☐ Colonization

Involved site/s

☐ blood ☐ lung ☐ pleura ☐ peritoneum ☐ cornea ☐ skin ☐ nails ☐ other, *specify*

Detailed data (clinical manifestations, localization, imaging .....)

UNDERLYING DISEASE/FACTORS

- Autoimmune disease, *specify* date of diagnosis
- Leukemia / Lymphoma, *specify* date of diagnosis
- Solid cancer *specify* date of diagnosis
- Hemopoietic stem cell transplant date autologous
  - autologous allogeneic (matched related; matched unrelated; mismatched; aplotype; umbelical cord)
  - myeloablative (related; unrelated) non myeloablative (related; unrelated)
- Bone marrow transplant date
- Graft versus host disease (acute; chronic) date of diagnosis
- Severe neutropenia (<500/mm^3), *specify time and duration of neutropenia*
- Immunosuppressive drugs, *specify drugs, dosage, period of treatment*
- Corticosteroids, *specify drugs, dosage, period of treatment*
- Chronic obstructive pulmonary disease, *specify grade*
- Surgery, *specify* date
- Solid organ tranplant, *specify* date
- Diabetes (type I; type II) date of diagnosis
- AIDS CD4 number/mm^3 date of diagnosis
- Accidental trauma, *specify* date
- Dialysis (hemo; peritoneal)
- Stay in ICU APACHE II score SAPS III score period of stay
- Use of contact lens, *specify type* Contact lens solution used
- Other, *specify*

TREATMENT OF *FUSARIUM* INFECTION

Patient weight

Antifungal therapy

Drug 1	daily dose	from *(dd/mm/yy)*	to (dd/mm/yy)
Drug 2	daily dose	from *(dd/mm/yy)*	to (dd/mm/yy)
Drug 3	daily dose	from *(dd/mm/yy)*	to (dd/mm/yy)

Surgery, *specify* *date*

OUTCOME OF *FUSARIUM* INFECTION

Cure, date Death, date Lost, date Relapse, date

Last culture/s positive for *Fusarium* *(specify sample and date)*

MYCOLOGY

Direct microscopy and culture

	Direct microscopy	Culture
Blood	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done, *specify system* ☐ neg. ☐ pos.**, date
Bronchial secretions	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Oral secretions	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Nasal secretions	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Pleural fluid	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Peritoneal fluid, *specify if dialytic*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Biopsy, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Skin, *specify site*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Corneal scraping, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Nails, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
Other, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*	☐ not done ☐ done ☐ neg. ☐ pos.**, date
	* presence of hyphae	**specify identification

Histopathology *(specify examined sample/s and date)*

Biopsy, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*
Autopsy, *specify*	☐ not done ☐ done ☐ neg. ☐ pos.*
	* presence of hyphae

Note

ECMM survey: *FUSARIUM* INFECTIONS in EUROPE

AUSTRIA	GERMANY	PORTUGAL
BELGIUM	GREECE	SERBIA
CZECHOSLOVAKIA	IRELAND	SPAIN
DENMARK	ITALY	SWEDEN
FRANCE	NORWAY	TURKEY

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