

# Svampeinfektioner i ører og øvre luftveje



Maiken Cavling Arendrup  
Unit of Mycology and Parasitology  
Staten Serum Institut  
Denmark  
maiken@arendrup.dk



## Disposition

- Generel mykologi
  - gær, skimmel etc
- Svampeinfektioner i
  - Øre
  - Næse & bihuler
  - Hals & oropharynx
- Svampefund øre-næse-hals 2008 til juni 2011 SSI
  - svampearter
  - resistens
- Diagnostik
  - prøvetagning og analyser
- Behandling

M. Cavling Arendrup



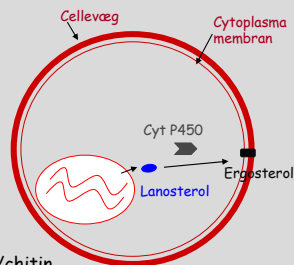
## Human-patogene svampe

### ■ 300/ ~100.000 svampearter

- I praksis:
  - ~10 af 500 gær
  - ~ 4 *Aspergillus*-species
  - ~ 3 hudsvampe slægter
  - ~ 5 dimorfe svampe

### ■ Eukaryoter

- Kerner med kernemembran
- Flere kromosomer
- Cellemembran med ergosterol
- Cellevæg med protein/glucan/mannan/chitin



M. Cavling Arendrup



## Sygdomme fremkaldt af svampe

- Toksiske
- Allergiske
- Egentlige infektioner
  - Superficielle
  - Subcutane
  - Invasive

M. Cavling Arendrup



## Practical approach to Classification

<b>Yeasts</b> <ul style="list-style-type: none"> <li><i>Candida</i></li> <li><i>Saccharomyces</i></li> <li><i>Malassezia</i></li> <li><i>Trichosporon</i></li> <li><i>Cryptococcus</i></li> </ul>	<b>Moulds</b> <ul style="list-style-type: none"> <li><i>Aspergillus</i></li> <li><i>Fusarium</i></li> <li><i>Mucor, Rhizopus..</i></li> </ul>	<b>Dimorphic fungi</b> <ul style="list-style-type: none"> <li><i>Histoplasma</i></li> <li><i>Coccidioides</i></li> <li><i>P. marneffeii</i></li> <li><i>Sporotrix schenckii</i></li> </ul>	<b>Dermatophytes</b> <ul style="list-style-type: none"> <li><i>Trichophyton</i></li> <li><i>Microsporum</i></li> <li><i>Epidermophyton</i></li> </ul>
<b>Normal flora</b> Mucositis Pityriasis  <b>Invasive infection</b> Haematogenous diss. Crypt.: meningitis	<b>Ubiquitous</b> Inhalation/inoculation  <b>Lung-infection</b> Sinuses, external ear etc. <i>Fusarium</i> : fungaemia	<b>Endemic</b> Inhalation  <b>Lung-infection</b> Dissemination <i>Sporotrix</i> : skin	<b>Tinea</b> Ringworm Nails, inguina, scalp..

M. Cavling Arendrup

## Yeasts at CHROMagar

<i>Candida albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>S. cerevisiae</i>

M. Cavling Arendrup

## Yeasts micro-morphology

<i>Candida albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>S. cerevisiae</i>

M. Cavling Arendrup

## Moulds

<i>Aspergillus fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>
<i>Zygomycetes (Mucor)</i>	<i>Fusarium</i>	<i>Scedosporium</i>

M. Cavling Arendrup

## Moulds

*Aspergillus fumigatus*      *A. flavus*      *A. niger*

*Zygomycetes (Mucor)*      *Fusarium*      *Scedosporium* Cavling Arendrup

## Species Identifikation !

- Koloni og mikro-morfologi
- Biokemisk profil el. Massespektrometri
- Temp. tolerance, vækst på special substrater

M. Cavling Arendrup

## Svampemidler - virkningsmekanisme

Cell wall  $\beta$ -1,3 glucan

**Echinocandiner**

- Anidulafungin
- Caspofungin
- Micafungin

**Lanosterol**

**Ergosterol**

**Polyener**

- Nystatin
- Amphotericin B

**Azoler**

- Clotrimazol, mi- & econazol
- Ketoconazol
- Flu- & itraconazol
- Voriconazol & posaconazol

**Flucytosin**

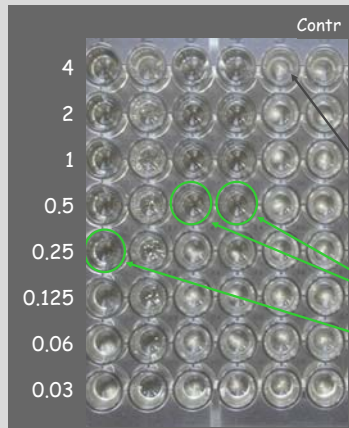
**Terbinafin**

## Resistensbestemmelse Skimmel (MIC)

1. Fortyndingsserie af svampemiddel
2. tilsætning af svamp
3. MIC = Minimum Inhibitory Concentration  
dvs laveste nødvendige konc for at hæmme svampen

M. Cavling Arendrup

## Resistensbestemmelse Skimmel (MIC)

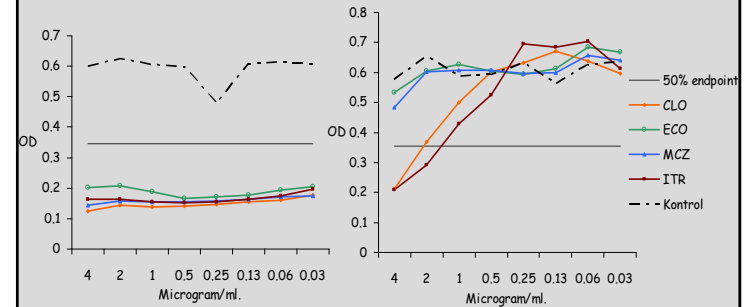


1. Fortyndingsserie af svampemiddel
2. Tilsætning af svamp
3. MIC = Minimum Inhibitory Concentration  
Dvs. laveste nødvendige konc. for at hæmme svampen

MIC >4 µg/ml  
MIC = 0.5 µg/ml  
MIC = 0.25 µg/ml

M. Cavling Arendrup

## *C. albicans* azole S and R



CLO	ECO	MCZ	ITR	FLU
≤0,03	≤0,03	≤0,03	≤0,03	≤0,125
S	S	S	S	S

CLO	ECO	MCZ	ITR	FLU
4	>4	>4	2	>16
R	R	R	R	R

M. Cavling Arendrup

## Svampemiddel spectrum *Candida*

	<i>C. albicans</i>	<i>C. parapsilosis</i> <i>C. tropicalis</i>	<i>C. glabrata</i> <i>S. cerevisiae</i>	<i>C. krusei</i> <i>C. norvegensis</i>
<b>Topikale</b>				
Amphotericin B/ Nystatin	+++	+++	+++	+++
Miconazol	+++	++	++	0
Econazol	+++	++	++	0
Clotrimazol	+++	+++	+0	++
<b>Systemiske</b>				
Fluconazol	+++	+++	+0	0
Itraconazol	+++	+++	+	+0
Voriconazol	+++	+++	++	++
Posaconazol	+++	+++	++	++

M. Cavling Arendrup

## Antifungals spectrum: Moulds

	<i>Aspergillus</i>				<i>Fusarium</i>	<i>Zygo-</i> <i>mycetes</i>
	<i>fumigatus</i>	<i>flavus</i>	<i>niger</i>	<i>terreus</i>		
Amph. B/ Nystatin	+	+/-	+	-	(+)	(+)
Fluconazol	-	-	-	-	-	-
Itraconazol	+	+	+/-	+	-	-
Posaconazol	+	+	+	+	+	+/-
Voriconazol	+	+	+	+	+	-

M. Cavling Arendrup

## Candida Disponerende faktorer

### ■ Del af den normale mikroflora

### ■ Lokale faktorer

- Lokal steroid /antibiotika
- Protese bærer
- Reflux
- Dårlig mundhygiejne
- Rygning
- Lav spytsekretion
- Lokal stråleterapi



Kilde: James Heilman, MD

### ■ Systemiske faktorer

- Alder
- Diabetes
- Immundefekt
- Systemisk antibiotika /steroid

M. Cavling Arendrup

## Skimmelsvampe patogenese

### ■ Ubiquitært forekommende

- > Jord & Plantedele
- > Byggematerialer
- > Ventilationssystemer

### ■ Lokale faktorer

- > Fugtigt miljø
- > Lokal steroid /antibiotika
- > Beskadiget lungearkitektur

### ■ Systemiske faktorer

- > Immuninkompetent
- > Steroidbehandling
- > Dysreguleret Diabetes



M. Cavling Arendrup

## Disposition

### ■ Generel mykologi

- gær, skimmel etc

### ■ Svampeinfektioner i

- Øre
- Næse & bihuler
- Hals & oropharynx

### ■ Svampesvamp fund øre-næse-hals 2008 til juni 2011 SSI

- svampesvamp
- resistens

### ■ Diagnostik

- prøvetagning og analyser

### ■ Behandling

M. Cavling Arendrup

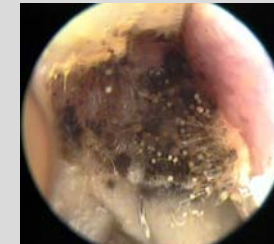
## Otomycosis

### ■ Dispositioner:

- Fugtigt miljø
- Tidl. bakteriel infektion
- Antibiotika & steroid

### ■ Patogener:

- Skimmel:
  - *A. niger*
  - *A. flavus*
  - *A. fumigatus*
- Gær:
  - *C. parapsilosis*
  - *C. albicans*



Kilde: Kevin Kavanagh, MD, www.entusa.com

Vennewald Clinics in Dermatology 2010, Richardson & Warnock. 2003 Fungal infection. Diagnosis and Management. M. Cavling Arendrup  
Magbor & Gugrani. 2001. Mycosis 44: 395-397

## Otomycosis

Table 1 Etiology of 390 otomycosis cases

Species	Number	Percentage	Procent
<b>Yeasts</b>			
<i>Candida parapsilosis</i>	145	63.6	56%
<i>Candida albicans</i>	78	34.2	34%
<i>Candida glabrata</i>	3	1.3	3%
<i>Candida tropicalis</i>	2	0.9	5%
<b>Filamentous fungi</b>			
<i>Aspergillus flavus</i>	78	42.4	32%
<i>Aspergillus niger</i>	66	35.9	39%
<i>Aspergillus fumigatus</i>	23	12.5	19%
<i>Aspergillus candidus</i>	13	7.1	-
<i>Aspergillus terreus</i>	3	1.6	-
<i>Paecilomyces variotii</i>	1	0.5	-
Total	412*	100	

\* Mixed culture in 22 cases

Garcia-Agudo Mycopathologica 2011 & SSI data

M. Cavling Arendrup

## Fungal Rhinosinusitis: Classification

Currently, there are more questions than answers concerning the categorization of FRS...

Table 1 Classification of fungal rhinosinusitis.

Category	Host immune status	Role of fungus
<b>A. Invasive (invasion of the mucosal layer)</b>		
1. Granulomatous invasive	Immunocompetent	Pathogen
2. Chronic invasive	Often diabetes mellitus, steroid therapy	Pathogen
3. Acute invasive	Immunocompromised	Pathogen
<b>B. Non Invasive (no invasion of mucosal layer)</b>		
1. Saprobic infestation (localized colonization)	Immunocompetent	Saprobe
2. Fungal ball	Immunocompetent	Saprobe
3. Allergic	Atopic	Allergen
4. Eosinophilic	majority non-atopic	May take role in deposition of toxic eosinophilic major basic protein [23]

Chakrabarti Med Mycol 2009

M. Cavling Arendrup

## Fungal Rhinosinusitis

Patogener: Skimmelsvampe

### ■ Invasive rhinosinusitis

- *Aspergillus fumigatus* og *flavus*
- Zygomyceter
- Diabetes, immun-inkompetent...

### ■ Non-invasiv

- *Aspergillus flavus, fumigatus*
- Dematiaceous moulds
  - *Alternaria*
  - *Exophiala dermatitidis*
  - *Bipolaris*
  - *Curvularia*

Chakrabarti Med Mycol 2009 (Suppl. 1)

M. Cavling Arendrup

## Oral candidose

### ■ Pseudomembranøs candidiasis

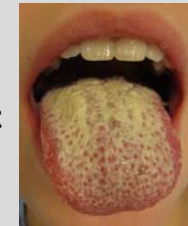
- hvide flødeskinds belægninger

### ■ Erythematøs candidosis / Atrofisk

- rødme, atrofi

### ■ Stomatitis prothetica

- Delvis mekanisk betinget



Kilde: James Heilman, MD

### ■ Angulær Cheilitis

- Jernmangel, vitaminmangel eller immundefekt
- Associeret med Stomatitis prothetica

M. Cavling Arendrup



## Dyrkningsfund SSI 3½ år

SSI 2008-juni 2011

	Mund	Mund- vig	Læbe	Oeso- phagus	Øre	Sinus	Larynx (incl asp)	Næse	Total
In total	998	21	24	28	256	13	29	61	1430
No growth	458	10	18	9	149	5	2	58	709
<i>Candida</i>	534	11	6	18	49	1		1	620
<i>Aspergillus</i>	1				43	6	24	1	75
Both					8			1	9
Others*					5		1		6
Others -probably contaminants**	5			1	2	1	2		11

\* *Fusarium*, *Scedosporium* & zygomycetes.

\*\* *Penicillium*, *Alternaria*, *Bipolaris*, *Scopulariopsis*, *Geotrichum*, *Rhodotorula*.

M. Cavling Arendrup

## Candida Species distribution

SSI 2008-juni 2011

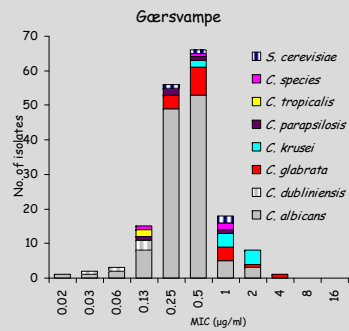
	Mund (537*)	Mund- vig (8)	Læbe (4)	Oeso- phagus (23)	Øre (61)	Sinus (1)	Næse (1)	Total (635)
<i>C. albicans</i>	65%	8/8	4/4	57%	34%			62%
<i>C. dubliniensis</i>	5%							4%
<i>C. glabrata</i>	17%			43%	3%			16%
<i>C. krusei</i>	5%				2%	1/1		5%
<i>C. parapsilosis</i>	2%				56%			7%
<i>C. tropicalis</i>	6%				5%		1/1	6%

\* Antal *Candida* fund med species ID

M. Cavling Arendrup

## Amphotericin B

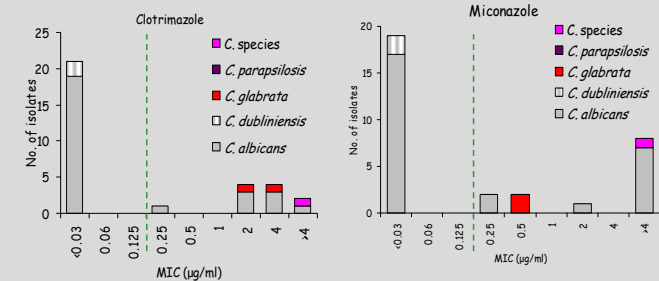
170 øre-næse-hals isolater DK 2008-juni 2011



M. Cavling Arendrup

## Candida topikale azoler

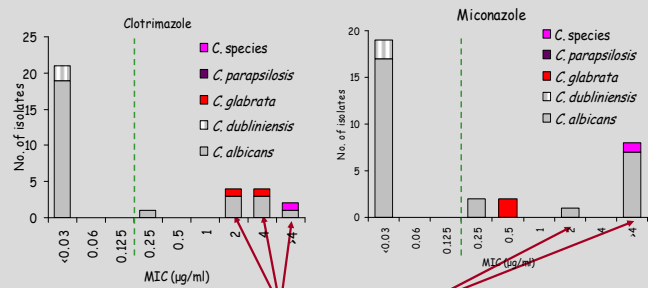
32 (øre-næse)-hals isolater DK 2008-juni 2011



M. Cavling Arendrup

## Candida topikale azoler

32 (øre-næse)-hals isolater DK 2008-juni 2011

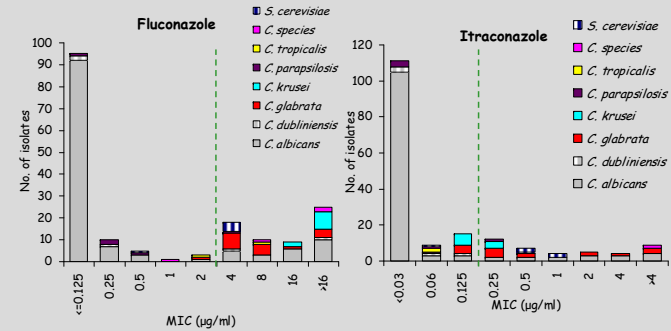


Erhvervet azol resistens!!!

M. Cavling Arendrup

## Candida systemiske azoler

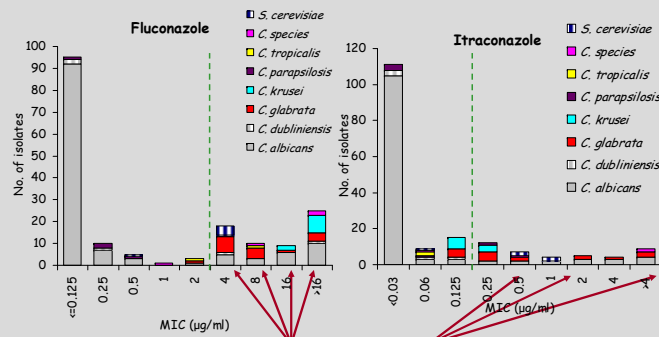
176 øre-næse-hals isolater DK 2008-juni 2011



M. Cavling Arendrup

## Candida systemiske azoler

176 øre-næse-hals isolater DK 2008-juni 2011



Erhvervet azol resistens!!!

M. Cavling Arendrup

## Aspergillus Species distribution

SSI 2008-juni 2011

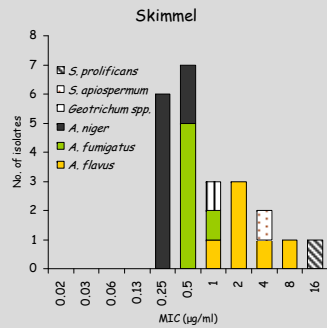
	Mund (1)	Mund vig (0)	Læbe (0)	Oeso-phagus (0)	Øre (58)	Sinus (6)	Larynx (incl sug) (28)	Næse (2)	Total (95)
<i>A. flavus</i>					32%	1/6	11%		24%
<i>A. fumigatus</i>	1/1				19%	3/6	61%	2/2	35%
<i>A. nidulans</i>					2%				1%
<i>A. niger</i>					39%	2/6	18%		31%
<i>A. terreus</i>							4%		1%
<i>A. species</i>							4%		1%
<i>Fusarium</i> spp							4%		1%
<i>S. apiospermum</i>					5%				3%
<i>S. prolificans</i>					2%				1%
Zygomycet					2%				1%

M. Cavling Arendrup



## Amphotericin B

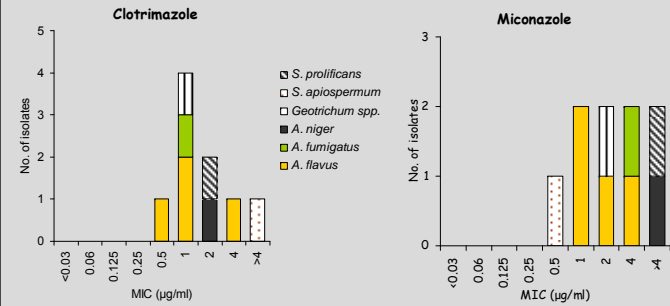
170 øre-næse-hals isolater DK 2008-juni 2011



M. Cavling Arendrup

## Skimmel Topikale azoler

9 øre-næse-hals isolater DK 2008-juni 2011

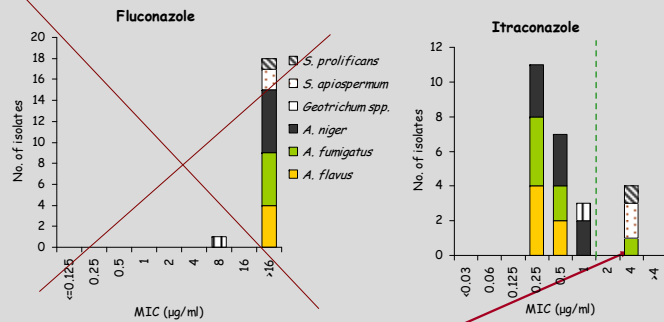


Clotrimazole > Miconazole til *Aspergillus* ??

M. Cavling Arendrup

## Skimmel systemiske azoler

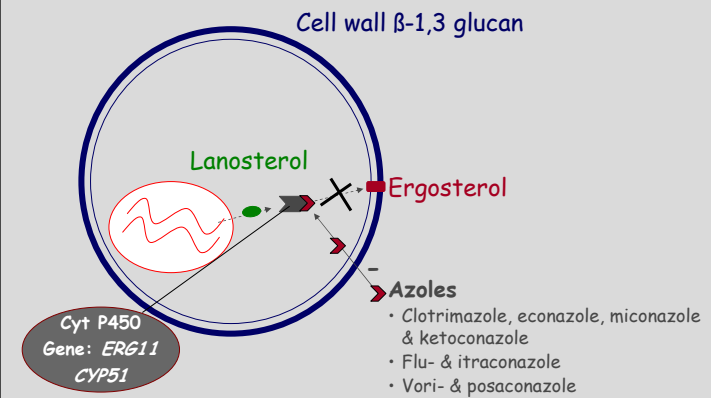
25 øre-næse-hals isolater DK 2008-juni 2011



Erhvervet azol resistens!!!

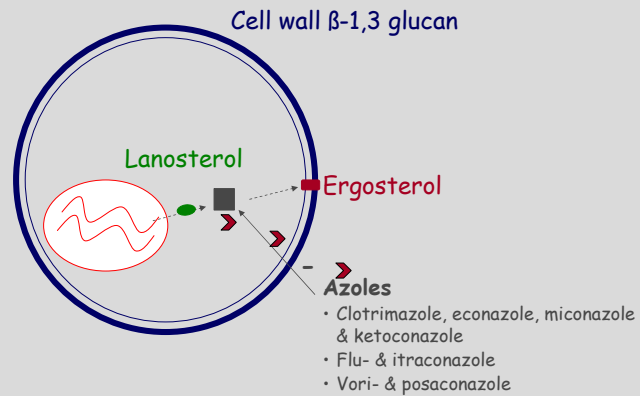
M. Cavling Arendrup

## Azoles: mechanism of action

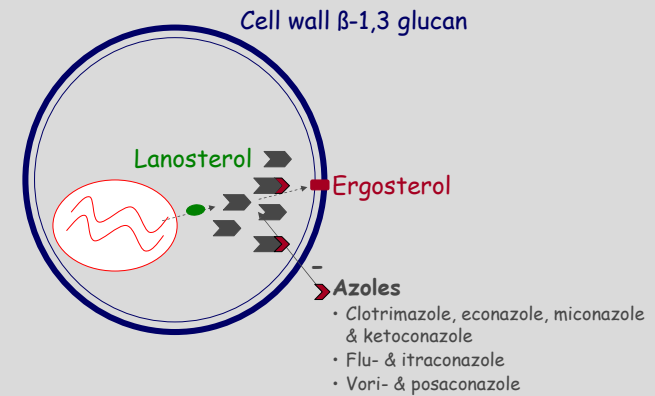


M. Cavling Arendrup

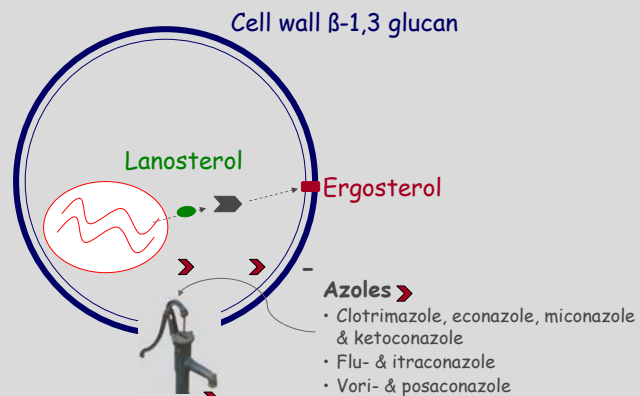
## Resistance 1: Target mutation



## Resistance 2: Up-regulation of target



## Resistance 3: Efflux pumps



## Candida isolates with azole resistance

7 sequential isolates from AIDS patient with oropharyngeal candidiasis

→ Nov 1998: Amb 2 months → Itraconazole 5 months

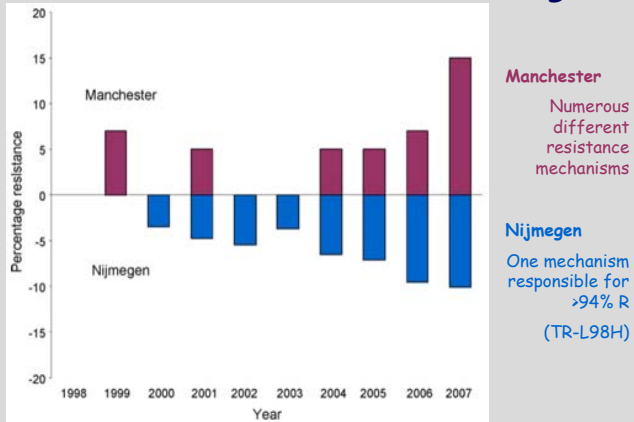
Nov 1998 - June 1999: Posaconazole 6 months

July 1999 - August 1999: Voriconazole but no response

August 1999 - May 2000: Posaconazole - clinical failure in May 2000

Isolate	Date of isolation	MICs (mg/L)					Amino acid substitutions in Erg11p
		POS	FLZ	ITZ	VOR	AMB	
C43 <sup>a</sup>	-	0.03	0.125	0.06	0.03	0.5	
C369	25 November 1998	0.25	32	1	2	0.25	D116E, K128T, Y132H, D278N, G464S
C378	29 December 1998	0.25	32	1	2	0.25	D116E, K128T, Y132H, D278N, G464S
C371	28 January 1999	0.25	>64	1	4	0.25	D116E, K128T, Y132H, D278N, G464S
C372	8 April 1999	0.25	>64	1	2	0.25	D116E, K128T, Y132H, D278N, G464S
C373	24 January 2000	2	>64	16	4	0.25	D116E, K128T, Y132H, D278N, G464S, P230L <sup>a</sup>
C376	24 April 2000	2	32	16	2	0.25	D116E, K128T, Y132H, D278N, G464S, P230L
C375	10 May 2000	2	48	16	2	0.25	D116E, K128T, Y132H, D278N, G464S, P230L

## Azole-resistance over time: *A. fumigatus*



Verweij Drug Resist Updates 2009; Howard Emerg Infect Dis 2009

M. Cavling Arendrup

## Multi-azole resistant *A. fumigatus*

Site (no.) Sample ID.	EUCAST MIC (µg/ml)			<i>Cyp51A</i>
	ITC	VRC	POS	
Rigshospitalet (27)				
RH-13	0.06	0.12	0.5	TR-L98H
Tivoli Gardens (23)				
T-11				TR-L98H
T-18				TR-L98H
T-22	>8	2-4	0.25-0.5	TR-L98H
Control isolate				
CM-237	0.25	0.5	0.06	Wild type

**4/50 soil samples in Cph ~ 8%**

Mortensen, AAC, 2010

M. Cavling Arendrup

## Imported via compost bags???

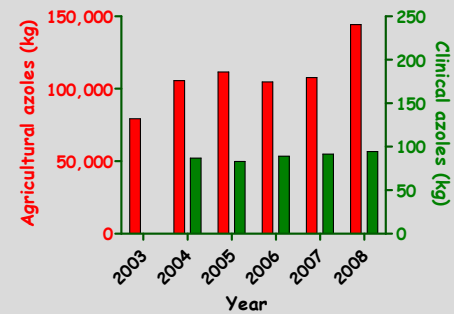
### Commercial compost bags

Country of purchase	No. bags tested	<i>A. fumigatus</i>	
		No. (%)	Azole resistant
Denmark	26	17 (65.4%)	0
Austria	25	24 (96.0%)	0
Spain	28	23 (82.1%)	0
In total	79	64 (81%)	0

Mortensen, AAC, 2010

M. Cavling Arendrup

## Agricultural vs. clinical azoles in DK



Mortensen, AAC, 2010

M. Cavling Arendrup

### Origin of Azole resistance: In >> Ex vivo

Date	ITC	AMB	cyp51A alteration	2A	2B	2C	3A	3B	3C	4A	4B	4C
<b>CF Patient 4</b>												
17.07.2007	1	0.5	Not found	18	23	16	35	13	18	15	9	10
05.12.2007	0.5	0.5	Not found	18	23	16	35	13	18	15	9	10
09.07.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	9	10
16.07.2009	>4	0.25	M220I + V101F	18	23	16	35	13	18	15	12	10
21.07.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	12 & 9	10
10.08.2009	>4	0.25	M220I + V101F	18	23	16	35	13	18	15	9	10
07.09.2009	>4	1	M220I + V101F	18	23	16	35	13	18	15	12	10
23.10.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	12	10
24.11.2009	>4	1	M220I + V101F	18	23	16	35	13	18	15	9	10
26.11.2009	0.5	1	Not found	18	23	16	35	13	18	15	9	10
14.12.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	9	10
<b>CF Patient 5</b>												
01.08.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
16.11.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
20.11.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
06.12.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
12.05.2009	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
28.07.2009	0.25	0.5	Not found	23	24	15	34	12	19	13	9	5
31.08.2009	>4	1	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
13.11.2009	0.25	0.5	Not found	13	20	9	34	9	10	10	10	19
24.11.2009	>4	0.5	TR+L98H	23	10	9	10	10	6	8	10	20

Mortensen JCM E-pub 2011

M. Cavling Arendrup

## Diagnostik af svampeinfektioner

### ■ Mikrobiologi

- Prøvetagning
- Analyser
- Resistensbestemmelse

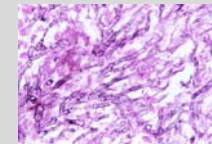
### ■ Histopatologi

### ■ Billeddiagnostik:

- CT
- MR

### ■ Indikation:

- Sinusitis
- Invasiv infektion
- Underliggende malignitet



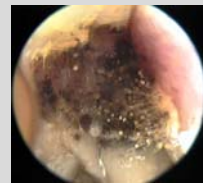
Kaminski

M. Cavling Arendrup

## Diagnostik af svampeinfektioner

### ■ Prøvetagning

- Spritserviet
- Skarpske
- Skalpel
- Pincet
- Kulpedepind
- Objektglas
- Biopsi



Kilde: Kevin Kavanagh, MD, www.entusa.com

### ■ Forsendelse

- Stuarts transport medium
- ellers: Kølet, obs. udtørring

M. Cavling Arendrup

## Analysér: Blanket 11

### ØVRIGE SVAMPEINFEKTIONER

#### Øvrige svampeinfektioner: Dyrkning

- 063  *Candida* og *Aspergillus*: Dyrkning og resistensbestemmelse (svampemidler til vaginitis, otitis og hudinfektioner)
- 052  Gær- og skimmelsvampe (*Candida*, *Cryptococcus* og *Aspergillus*): Påvisning, identifikation og resistensbestemmelse (svampemidler til systemiske infektioner)
- 051  Gær- og skimmelsvampe (*Candida* og *Aspergillus*): Påvisning og identifikation

M. Cavling Arendrup

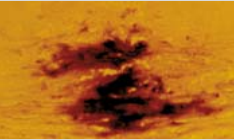
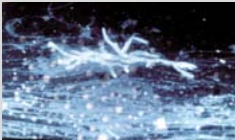
## Analyser WEB req


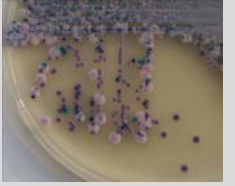
Statens Serum Institut & Klinisk kemi & Normalvisning

M. Cavling Arendrup

## Diagnostik af svampeinfektion

- Mikroskopi
  - Pus
  - Biopsi
  - Skrab
  - Ej podedind
- Dyrkning
  - incl ID

M. Cavling Arendrup

## Betydning af svampeidentifikation

*Gær vs. Skimmel* ej tilstrækkeligt

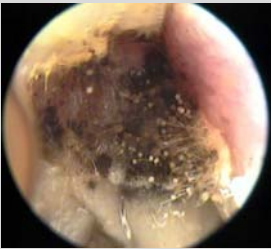
Species Identifikation → information om

- forurening ≠ klinisk betydning
- ± naturlig resistens
- relaps eller ny infektion

M. Cavling Arendrup

## Behandling af svampeinfektioner

- Antimykotikum
  - species ID
  - resistensforhold
  - topikal / systemisk
  - (clotrimazol, miconazol, tolnaftat og nystatin uden ototox i dyr)
- Kirurgi etc.?
  - Oprensning af bihuler
  - Fjern debris & nekrose



Kilde: Kevin Kavanagh, MD, www.entusa.com

M. Cavling Arendrup

## Konklusion

- Mange svampearter
- Mange sygdomsbilleder
- **Primær svampebehandling afhængig af art**
  - *C. glabrata* og *C. krusei* har medfødt nedsat følsomhed overfor azolerne.
  - *A. terreus* og *A. flavus* har medfødt nedsat følsomhed for amphotericin B.
- **Erhvervet Azol Resistens**
  - Hos tidligere behandlede patienter
  - *Aspergillus* også hos azol naive patienter (azol pesticid)

M. Cavling Arendrup

Thank you for your attention