Invasive mykoser hos børn
Epidemiologi & Diagnostik

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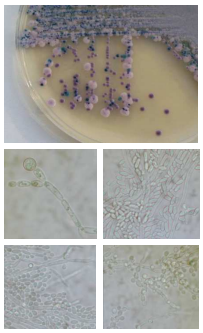
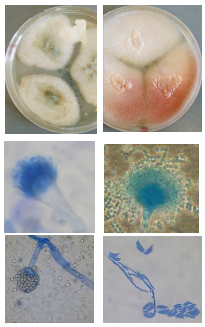
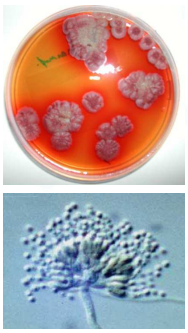
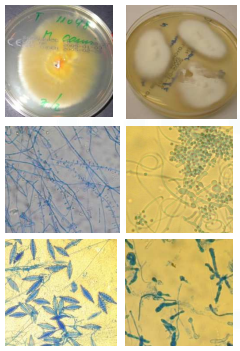
Speaker honoraria: Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida
Chair(wo)man for EUCAST-AFST

Agenda

- The FUNgal species and susceptibility
- Epidemiology
  - Candidaemia in children
    - DK nationwide study
  - Aspergillosis
  - Mucormycosis
- Diagnostics
  - conventional diagnostics
  - surrogate markers and molecular tests

Practical approach to Classification

Yeasts	Moulds	Dimorfic fungi	Dermatophytes
▪ *Candida*	▪ *Aspergillus*	▪ *Histoplasma*	▪ *Trichophyton*
▪ *Saccharomyces*	▪ *Fusarium*	▪ *Coccidioides*	▪ *Microsporum*
▪ *Malassezia*	▪ *Scedosporium*	▪ *P. marneffei*	▪ *Epidermophyton*
▪ *Trichosporon*	▪ *Mucor, Rhizopus..*	▪ *Sporotrix schenkii*	
▪ *Cryptococcus*			

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▪ *Candida*	▪ *Aspergillus*	▪ *Histoplasma*	▪ *Trichophyton*
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▪ *Trichosporon*	▪ *Mucor, Rhizopus..*	▪ *Sporotrix schenkii*	
▪ *Cryptococcus*			

Normal flora Mucositis, Pityriasis Invasive infection Haematogenous diss. Crypt.: pneumonia & meningitis	Ubiquitous Inhalation/inoculation Lung-infection Sinuses etc. *Fusarium*: fungaemia	Endemic Inhalation Lung-infection Dissemination Sporotrix: skin	Ringworm Nails, inguina, scalp

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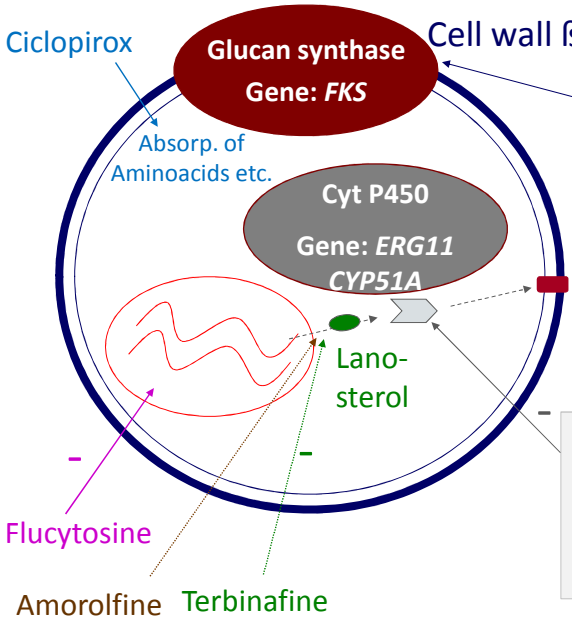
Licensed Antifungals: Mode of Action



Echinocandins
- Anidulafungin
- Caspofungin
- Micafungin

Polyenes
- Amphotericin B

Azoles
- Clotrimazole,Mi-, Ketoconazole
- Fluconazole
- Itra-, vori-, posa- & isavuconazole

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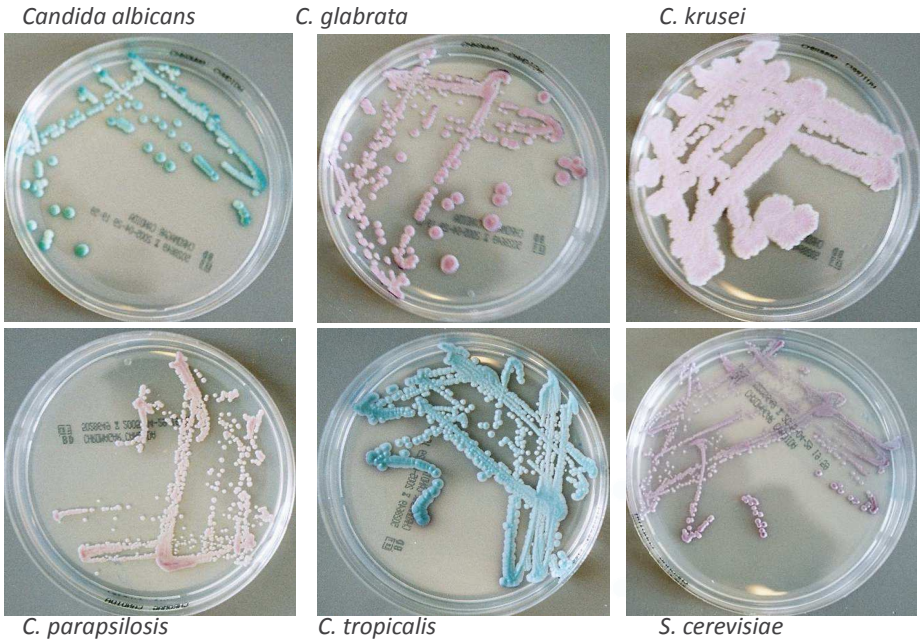
Candida arter på CHROMagar



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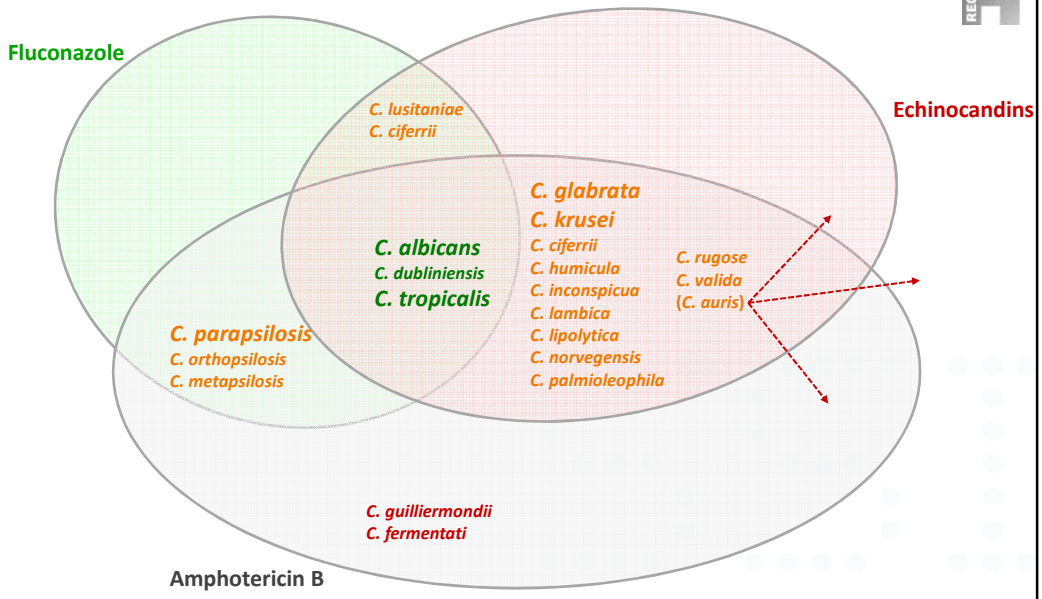
Candida spp. intrinsic susceptibility pattern



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Moulds: intrinsic susceptibility pattern

	Aspergillus				*Aspergillus* Cidal?	*Fusarium*	Zygo-mycetes
	fumigatus	*terreus*	*flavus*	*niger*			
Amph. B	+	-	-	+	+	(+)	(+)
Anidula	+	+	+	+	-	-	-
Caspo	+	+	+	+	-	-	-
Mica	+	+	+	+	-	-	-
Fluco	-	-	-	-	-	-	-
Itra	+	+	+	+/-	-	-	-
Posa	+	+	+	+	?	+/-	+/-
Isavu	+	+	+	+	?	+/-	+/-
Vori	+	+	+	+	+	+/-	-
5-FC	-	-	-	-	-	-	-

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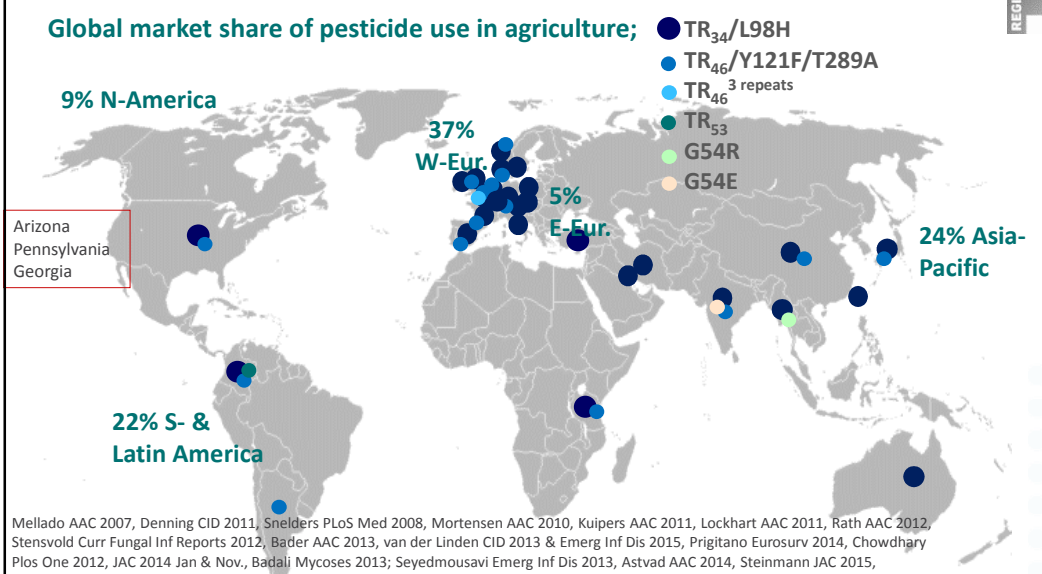
Fungicide use & Azole "R" *A. fumigatus* reports

Global market share of pesticide use in agriculture;



Mellado AAC 2007, Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Kuipers AAC 2011, Lockhart AAC 2011, Rath AAC 2012, Stensvold Curr Fungal Inf Reports 2012, Bader AAC 2013, van der Linden CID 2013 & Emerg Inf Dis 2015, Prigitano Eurosurv 2014, Chowdhary Plos One 2012, JAC 2014 Jan & Nov., Badali Mycoses 2013; Seyedmousavi Emerg Inf Dis 2013, Astvad AAC 2014, Steinmann JAC 2015, Brillowska-Dabrowska Acta Biochim Pol. 2015, Kidd Mycoses 2015, Lavergne AAC 2015, Wu Mycoses 2015, Chen AAC 2015, Pelaez New microbes New Inf 2015; Sharma J Global Antimicrob Res. 2015, Özmerdiven J Inf Chem 2015, Verweij CID 2016, Hagiwara J Infect Chemother 2016, Wiederhold JCM 2016, Le Pape Emerg ID 2016, Castanheira DMID 2016, Tangwattanachuleeporn Med Mycol In press 2016, Moore JIA 2017, Alvarez-Moreno Sci Rep. 2017, Toyotome J Inf Chemother 2017, Hurst JAC 2017, Zhang mBio 2017, Riat AAC 2018, Isla AAC 2018, AzoleResNet report 2019

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Candidaemia: Population Based Paediatric studies

Neonates versus non-neonates

- ↑ ICU
- ↑ pre-maturity
- ↓ Surgery
- ↑ mortality

		Neonates				Non-neonates			
		ICU %	Pre-maturity %	Surgery %	Mortality %	ICU %	Malignancies %	Surgery %	Mortality %
	Benedict, 2018	99	78	9	16	48-83	2-15	20-38	11-13
	Blyth, 2009	94	94	19	22	13	31	21	10.1
Latin America	Santolaya, 2014	79	53	29	40	34	25	40	28
	Sutcu, 2016	*	*	*	*	63	*	59	22.2

*missing

Data from the four large studies of paediatric candidaemia patients

Benedict J Ped Inf Dis Soc 2018, Blyth Pediatrics 2009, Sutcu Am J Infect Control 2016

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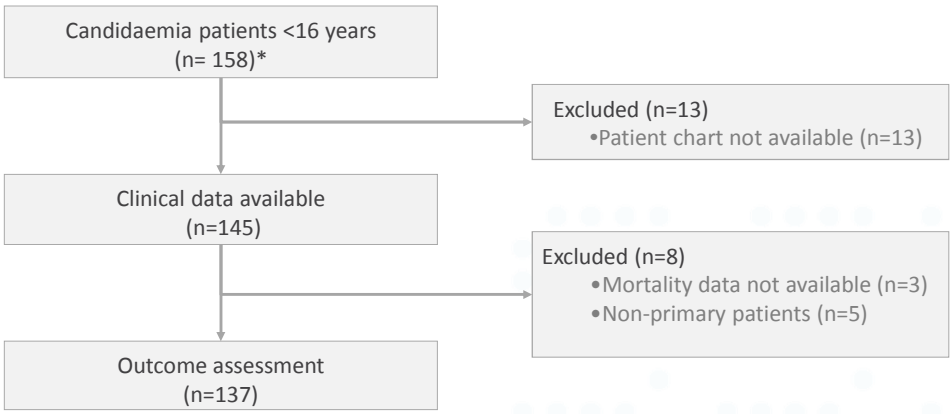
Paediatric candidaemia: Nationwide study DK 2004-14

- Flow chart, included patients
  - identified via nationwide surveillance programme



*153 patients with 158 episodes of candidaemia

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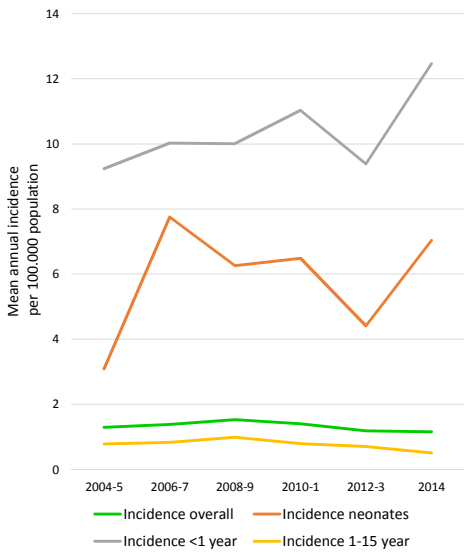
Candidaemia incidence: DK paediatric patients



- Low birth weight (<2500 g): 103.8/100.000 LBW births
- Very low birth weight (<1500 g): 442.0/100.000 VLBW births

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Paediatric candidaemia: Predisposing Host factors

	Total (145)	Neonates (35)	Toddlers * (60)	Older children (53)
Male sex	57%	60%	60%	53%
Prior hospital contact	68%	32%	73%	88%
BC drawn from internal catheter	66%	33%	71%	81%
LOS before BCC, median(IQR) (days)	13 (4-23.5)	11 (7-18)	17 (5-32)	12 (2-23)
CRP at BCC, median (IQR)	58 (19-123)	36 (18-73)	52 (13-135)	72 (30-160)
Temperature at BCC, median (IQR)	39 (38-39)	37 (37-39)	39 (38-40)	39 (38-39)
Intensive care unit	38%	51%	43%	22%
Colonised with *Candida*	67%	82%	57%	68%
Bacteriaemia	26%	37%	20%	24%
Sepsis	39%	60%	38%	25%

*1m – 2y of age

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Paediatric candidaemia: Predisposing Risk factors

	Total (145)	Neonates (35)	Toddlers (60)	Older children (53)
Antibiotic, %(n)	80%	77%	82%	80 %
Chemotherapy, %(n)	29%	0	33%	45%
Corticosteroid, %(n)	24%	0	30%	35%
CVC, %(n)	83%	68%	88%	89%
Surgery, %(n)	30%	26%	44%	18%
TPN, %(n)	37%	60%	26%	32%
Mechanical ventilation, %(n)	30%	60%	30%	10%

*1m – 2y of age

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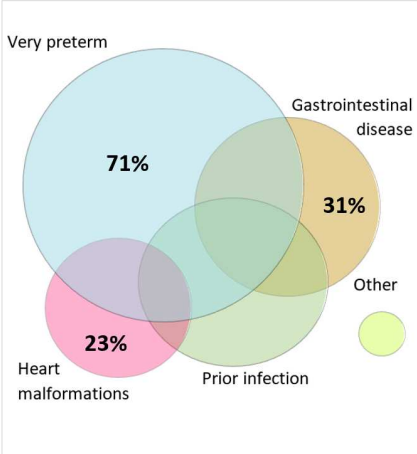
Underlying diseases among neonates



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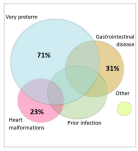
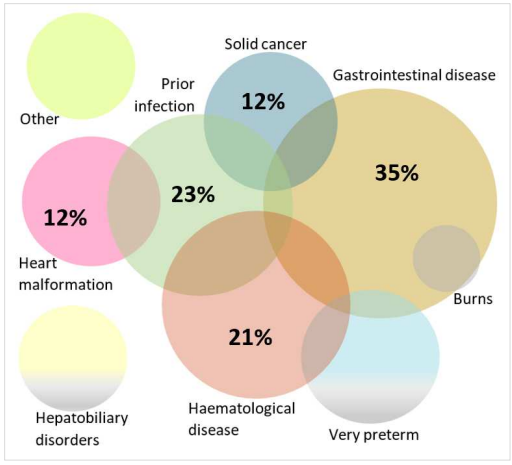
Underlying diseases among infants (1m-2y)





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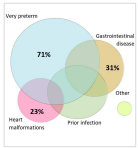
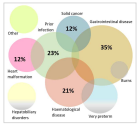
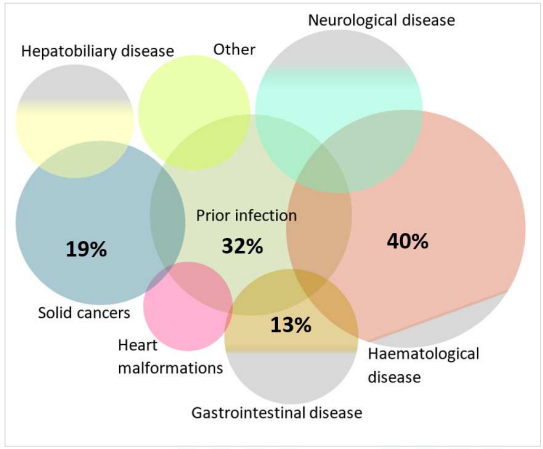
Underlying diseases among older children (3-15y)







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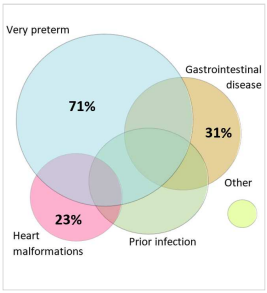
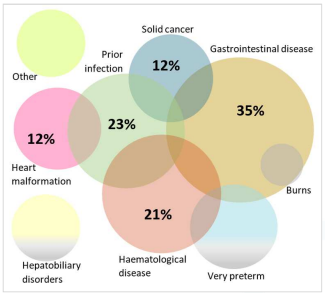
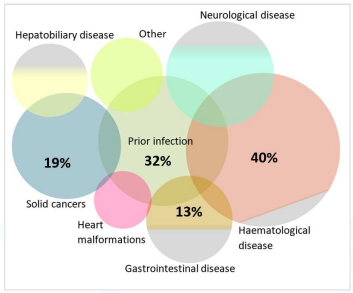
Underlying diseases by age group







Neonates | Infants | Older children

→ **Multi-morbidity (≥2) in 43.5% of patients**

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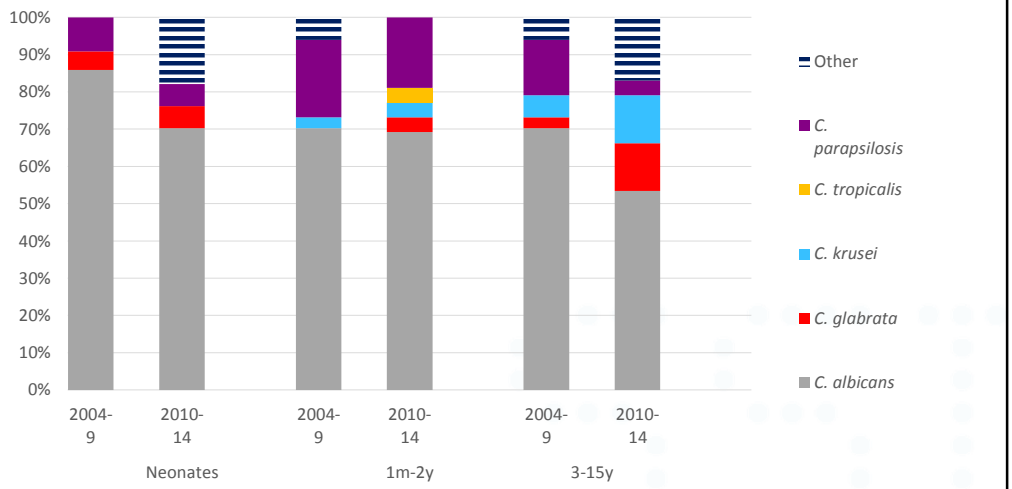
Species distribution by age group and time period



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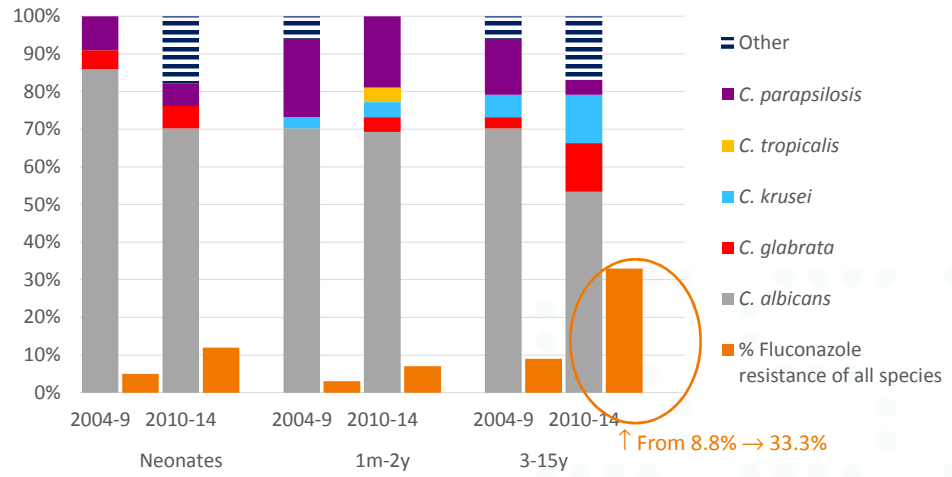
Species distribution - fluconazole resistant species



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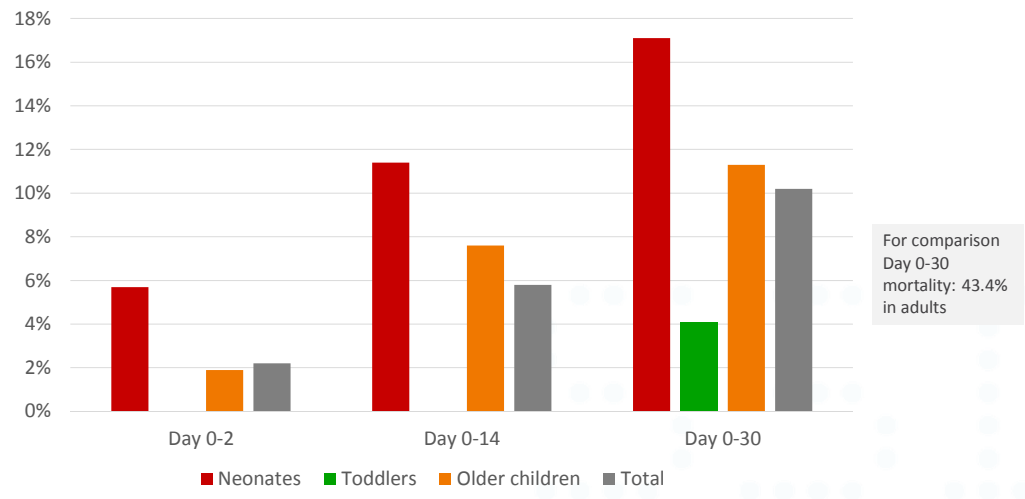
Paediatric candidaemia: Overall Mortality



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Aspergillus: Host interaction & spectrum of disease

Normal Host	Overreacting immune system	Impaired (lung) tissue architecture	Compromised immune system
Transient colonisation	ABPA Sinusitis Asthma w fungal sensitisation	TB Sarcoidosis COPD CF Aspergilloma Chronic forms of aspergillosis	Haematological disease HSCT, GVHD Wegener's granulomatosis etc Steroids Influenza… Semi → acute invasive aspergillosis

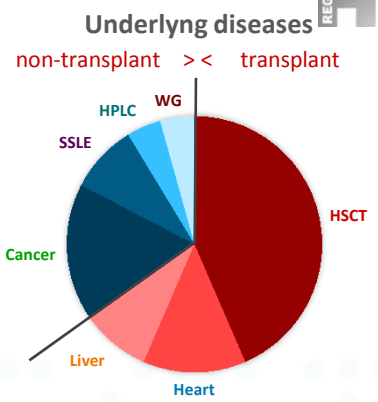
Paediatric Invasive pulmonary aspergillosis

- Retrospective study (2004- 2013)
  - Tertiary care children's hospital in New York City
- Methods: Searches
  - Microbiology
    - *Aspergillus* cultures and S- Aspergillus galactomannan results
    - Excl.: CF sputum Aspergillus, Skin without evidence, external ear canal; and ABPA.
  - Dept Inf Prev Control surveillance records
  - Pathology & Radiology, string searches
    - %spergil%
    - Histopath specifically
      - septated hyphae, hyaline hyphae, septated hyaline hyphae, acute angle branching, forty-five degree branching, vascular invasion, angioinvasion, local invasion, Aspergillus, aspergillosis, consistent with Aspergillus, and consistent with aspergillosis.
    - Radiology specifically
      - nodule, halo, cavity, consistent with Aspergillus, consistent with aspergillosis, and air-crescent sign

Nadimpalli Am J Inf Control 2016 M Cavling ARENDRUP

Paediatric Invasive pulmonary aspergillosis

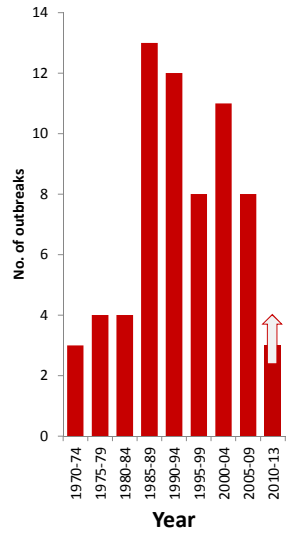
- 22 children met IA criteria
  - 5 (23%) only identified by string searches.
- Clinical characteristics
  - 13 (59%) were
  - Mean age 11.2 years (0.6-18 years)
  - Underlying diseases
    - 14 (63.6%) transplants
      - 9 (41%) hematopoietic stem cell transplant
      - 2 (9%) cardiac transplant
      - 2 (9%) liver transplant
      - 1 (4.5%) hematopoietic stem cell transplant & cardiac transplant
    - 8 (36%) immunosuppressive treatment for
      - cancer (n = 4)
      - severe systemic lupus erythematosus (n = 2)
      - hemophagocytic lymphohistiocytosis (n = 1)
      - Wegener granulomatosis (n = 1).

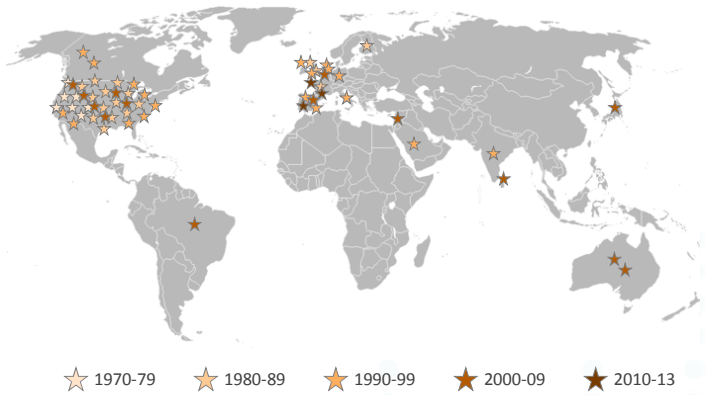


NadNadimpalli Am J Inf Control 2016 M Cavling ARENDRUP

Aspergillus outbreaks reported in the literature

- 65 outbreaks involving 536 patients (1970-2013)





Vonberg 2006; Weber 2009; Sessa 1996; Kronman 2007, Saracli 2007, Raviv 2007, Panackal 2007, Gunarathne 2007, Chang 2008, Freitas 2008, Kidd 2009, Menotti 2010, Etienne 2001, Pelaez 2012

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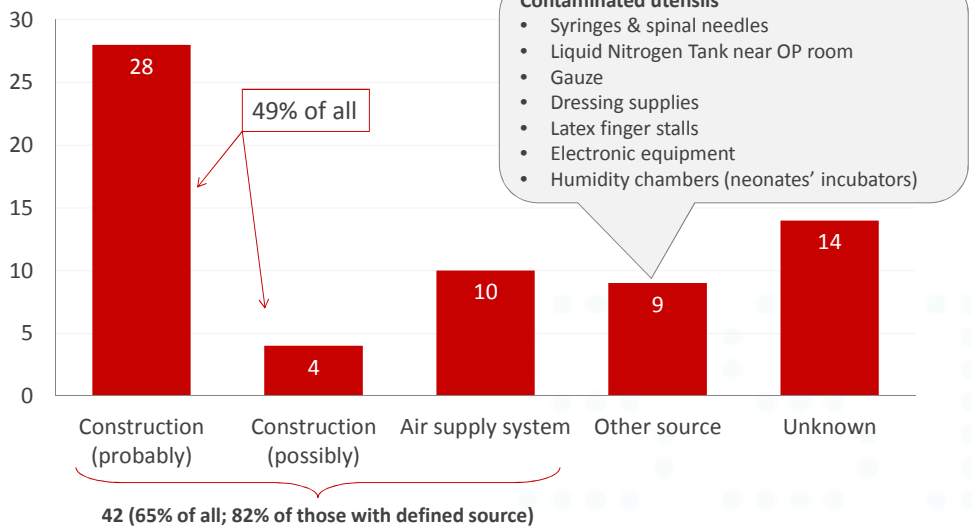
Aspergillus outbreaks reported in the literature

- Source of infection, defined in 78% (51/65) of the outbreaks



Contaminated utensils

- Syringes & spinal needles
- Liquid Nitrogen Tank near OP room
- Gauze
- Dressing supplies
- Latex finger stalls
- Electronic equipment
- Humidity chambers (neonates' incubators)

Vonberg 2006; Weber 2009; Sessa 1996; Kronman 2007, Saracli 2007, Raviv 2007, Panackal 2007, Gunarathne 2007, Chang 2008, Freitas 2008, Kidd 2009, Menotti 2010, Etienne 2001, Pelaez 2012

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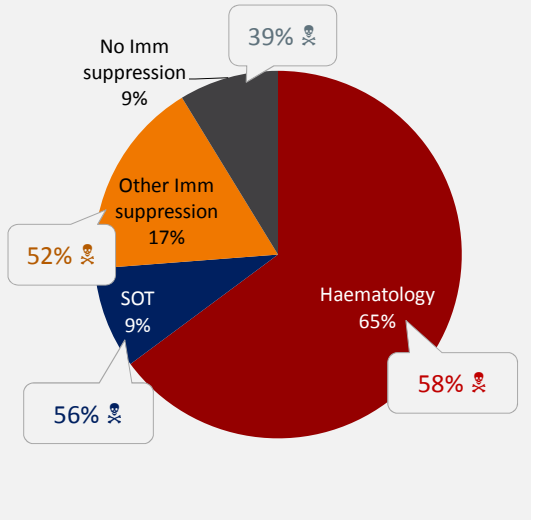
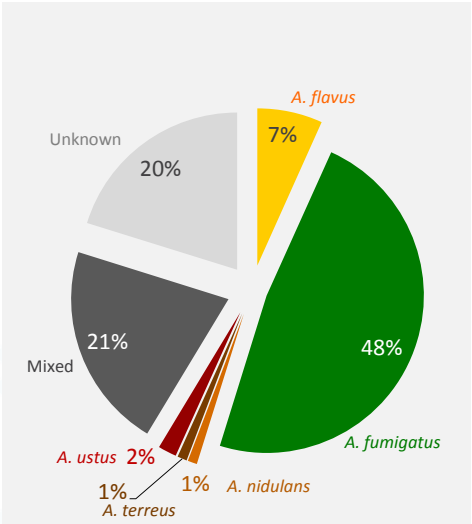
Aspergillus outbreaks reported in the literature

Patient categories & mortality



Species distribution



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Paediatric Mucormycosis Multicentre study Italy 2009-16

15 proven mucormycosis

- male/female: 8/7;
- median age 14.1 years
- range 7.7-18.6 years

Underlying malignancy

- 12 acute leukaemia/ lymphoma
- 3 allo stem cell transplantation
- 13/15 steroid
- 10/15 prolonged neutropenia

Site of infection

- 14/15 Paranasal sinus combined with
  - 9 orbital involvement
  - 8 CNS
  - 4 lung
  - 1 thyroid gland & kidney

Fungal species

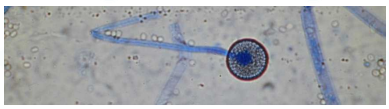
- *Rhizopus oryzae* 4,
- *Lichtheimia corymbifera* 3
- *Mucor spp.* 8.

Therapy

- 15/15 AmBisome (3-10 mg/kg),
- 14/15 surgical debridement
- 11/15 maintenance treatment
  - 9 posaconazole
  - 2 isavuconazole

Outcome

- 8/15 (53.3%) died, after 3-6 months



Muggeo Mycoses 2019

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European Diagnostics Guidelines

ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: diagnostic procedures

M. Cuenca-Estrella[1†], P. E. Verweij[2†], M. C. Arendrup[3†], S. Arikan-Akdagli[4†], J. Bille[5†], J. P. Donnelly[2†], H. E. Jensen[6†], C. Lass-Flörl[7†], M. D. Richardson[8†], M. Akova[9], M. Bassetti[10], T. Calandra[11], E. Castagnola[12], O. A. Cornely[13], J. Garbino[14], A. H. Groll[15], R. Herbrecht[16], W. W. Hope[17], B. J. Kullberg[2], O. Lortholary[18,19], W. Meersseman[20], G. Petrikkos[21], E. Roilides[22], C. Viscoli[23] and A. J. Ullmann[24] for the ESCMID Fungal Infection Study Group (EFISG)

ECIL-3 classical diagnostic procedures for the diagnosis of invasive fungal diseases in patients with leukaemia

MC Arendrup[1], J Bille[2], E Dannaoui[3], M Ruhnke[4], C-P Heussel[5] and C Kibbler[6]

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RADS Rådet for Anvendelse af Dyr Sygehusmedicin

http://www.regioner.dk/sundhed/medicin

Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline

A Ullmann et al

Cuenca-Estrella et al CMI 2012, www.escmid.org, Arendrup et al BMT 2012, Ullmann CMI 2018

ECIL-3: Min. Lab-criteria for haem pts

Technique	Minimum criteria
Direct Microscopy	Optical brighteners and fluorescent microscopy
Culture	Fungal agar should be used for primary culture; Sufficient volume Prolonged incubation where appropriate Species identification ≤ 2 days for *Candida* ≤ 3 days for *A. fumigatus*
Susceptibility testing:	Reference methodology (CLSI or EUCAST) for all licensed antifungals ≤ 1 week
Indirect tests	*Aspergillus* galactomannan Ag *Cryptococcus* Antigen *Candida* mannan Ag and antimannan antibody B-D-Glucan PCR Depending on local epidemiology and clinical presentation

+ recommendations regarding availability for bronchoscopy, imaging and histopath

Arendrup, ECIL-3 guidelines, BMT 2012

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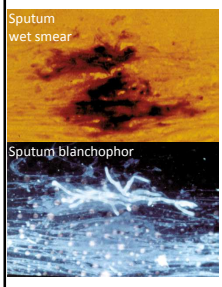
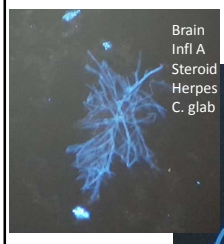
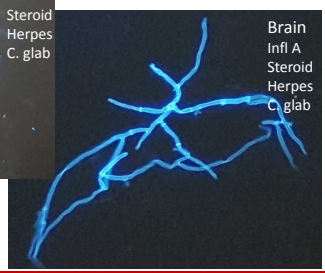
Blanchophor: Recent real life Examples

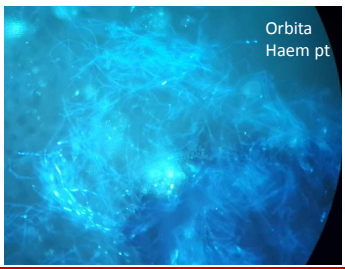




Specimen	Laboratory	Microscopy	Culture
Brain biopsy	Routine lab	Negative	+ mould
	Ref lab	Septate dichotonic branching hyphae	*A. fumigatus**
Liver abscess	Routine lab	Negative	No fungi
	Ref lab	Pauci-septate hyphae	*Lichtheimia ramosa**
Orbita-tissue	Routine lab	Negative	No fungi
	Ref lab	Septate and pauciseptate hyphae	*Circinella Muscae, Acremonium pinkertoniae* *Fusarium **
Sinus biopsy	Routine lab	Negative	No fungi
	Ref lab	Septate dichotonic branching hyphae	*A. fumigatus**

* Confirmed by direct PCR





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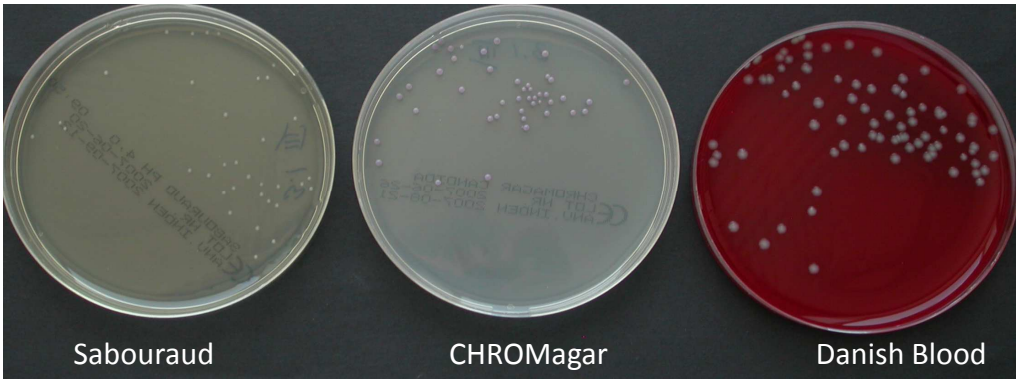
Selective vs blood agar

C. glabrata + *S. maltophilia*, 2 days of incubation



Mortensen, Fernandez, Chryssanthou, Gaustad, Sandven, Arendrup, NRMM report 2007

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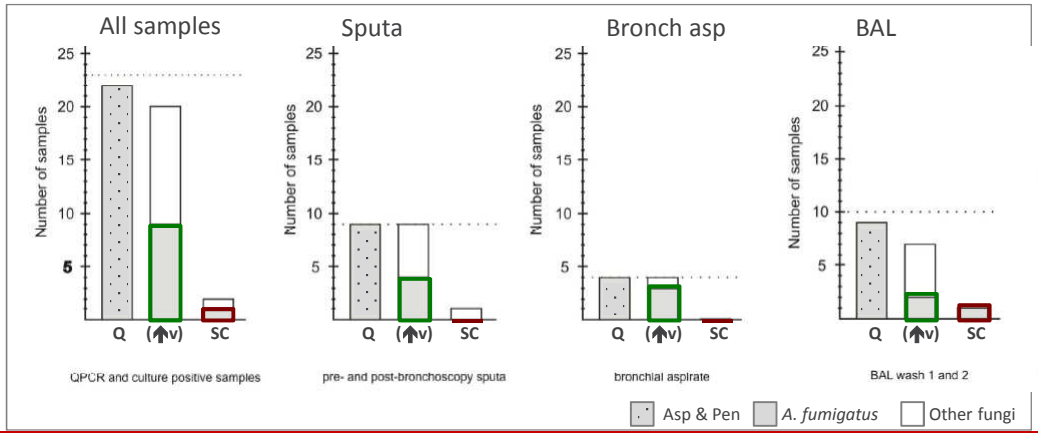
Aspergillus culture: Volume matters

5 aspergillosis patients (ABPA 2; Bronchitis 2, prev IPA 1)

- 4 pre-bronchoscopy sputa
- 4 broncial aspirates
- 10 BAL
- 5 post-bronchoscopy sputa

23 samples;
Q-PCR >< ↑ volume culture >< standard culture
(Q) (↑v) (SC)



Fraczek Mycoses 2014

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Arendrup, ECIL-3 guidelines, BMT 2012

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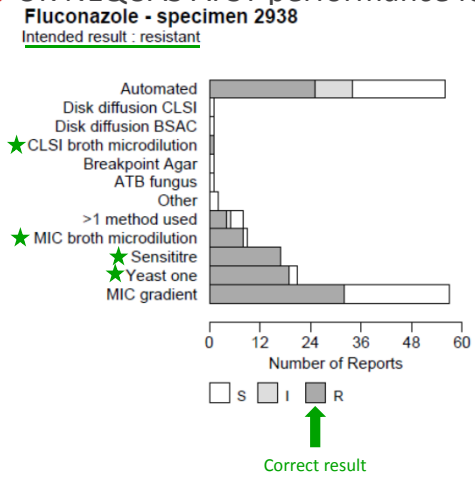
Routine testing not always sensitive in detecting R

- UK NEQUAS AFST performance for a resistant *C. albicans* isolate



	S	I	R	% concordance
score				
All	62	10	124	63.3
DK	1	0	2	66.7

37% errors
32% very major errors!!

Despite the fact that:
EQA typically overestimate performance
Many laboratories don't participate

UK NEQUAS, Distribution : 3789, Jan 2016.

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Antigen tests: Spectrum and prevalence of target diseases

	Estimate: invasive inf. (n)		Antigen tests				
	DK*	Cancer/Tx*	β-D-glucan	Galactom.	*Asp*LFD	Mannan	*Cryptococcus*
Mould							
Aspergillus	294	38	✓	✓	✓		
Fusarium	≤ 2	≤ 2	✓	(✓**)			
Scedosporium	≤ 1	≤ 1	✓				
Mucorales	1 (≤5)	1 (≤5)					
Yeast							
Candida	527 + 176	174 (120)	✓			✓	
Cryptococcus	2 (≤2)	0 (≤1)					✓
Trichosporon	≤1	≤1	✓	(✓**)			(✓)
Neither or							
Pneumocystis	82	38	✓				

*Mortensen Mycoses 2015, adapted/elaborated by MC Arendrup

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Surrogate markers- Pro et Cons

	Pro	Con
Asp GM	Detects *Aspergillus* *Fusarium, Trichosporon* *P. marneffei* Voriconazole 1st line for all Index correlates w outcome BAL, Biopsies, CSF, sterile fluids	↓ Sensitivity mould active treatment non-neutropenic population few samples Other IFI not detected Susceptibility not provided
AspLFD	Detects: "growing" *Aspergillus* Point of care format	As above for Asp GM Weak bands difficult to read, not quantitative
β-D-glucan	Almost pan-fungal	Asp → voriconazole PCP → Bactrim *Candida* → echinocandin Crypto/zygomycetes not detected ↑ Many contamination sources ↑ Cost
Mannan Ag+Ab	Specific for *Candida* Echinocandin 1st line for all Hepatosplenic candidiasis CSF	Less well studied/documented ↓ Sensitivity *C. parapsilosis* & *C. krusei* ↑ False positivity if heavily colonised? Other IFI not detected

Which AF?

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PCRs available in DK

- T2Candida (at RH)
  - 5 (9) most common *Candida* spp.
  - grouped by susceptibility pattern
- AsperGenius (Kit, available at Skejby))

Species multiplex PCR	Resistance multiplex	
A. fumigatus	L98H	Pan azole "R"
A. terreus	Tandem repeat 34	TR_{34}L98H
Aspergillus sp.	T289A	Voriconazole "R"
Internal Amplification Control (IAC)	Y121F	TR_{46}/Y121F/T289A

- SSI
  - Asp PCR incl. species ID and environmental resistance markers
    - validated against AsperGenius & extended w more species
  - *Mucorales* specific PCR
  - *Fusarium* PCR
  - *Candida* PCR
  - ITS – pan fungal PCR
  - Microbiome (pan bacterial and eucaryotic PCR)

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Candida PCR: Specification of the T2Candida test

- Specific detection of
  - *C. albicans* & *C. tropicalis*
  - *C. glabrata* & *C. krusei*
  - *C. parapsilosis*

Amphotericin B	Echinocandins	Fluconazole
S	S	S
S	S	I/R
S	I/R	S

- … and
  - *S. cerevisiae* & *C. bracarensis,*
    - genetically related to *C. glabrata*
    - susceptibility ~ *C. glabrata*
    - *C. glabrata*/*C. krusei* channel
  - *C. orthopsilosis* & *C. metapsilosis*
    - previously *C. parapsilosis* Group II and Group III
    - susceptibility ~ *C. parapsilosis*
    - *C. parapsilosis* channel

These species accounted for **91.7%** Candidaemia isolates in DK 2014-15 (838/914 in total) Or 90.8% of fungaemia isolates

- No other among 80 species tested, were positive when tested at concentrations ≤ 100 CFU/mL

Sens 91.1% **Spec 99.4%**

Mylonakis CID 2015

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Diagnostic A; B; C… !!

A. Ensure the relevant samples before starting AF TX

- **Invasive yeast**:
  - BC incl. Fungal medium improves sensitivity for BACTEC & BacT/ALERT
  - T2Candida
  - *Candida* mannan and anti-mannan
  - Focal symptoms → specific samples (CSF, fluids, urine, aspirates… swabs)
  - Colonisation samples
  - Susceptibility testing of significant findings (reliable method) - Particularly in AF exposed patients!!

Combined testing ↑ performance

- **Invasive mould**:
  - Resp samples (BAL >> Tracheal secretions >> sputum)
  - Susceptibility testing for all *A. fumigatus* cultures (even in azole naïve patients)
  - Asp GM: serum, Note lower sensitivity in non-neutropenic patients and patients on mould active TX
  - Asp GM BAL - <0.5 almost rules disease out; ≥ 3 almost rules disease in
  - Asp PCR BAL – allows speciation and detection of resistance
  - Mucorales PCR when clinical indication
  - Asp AB - relevant in chronic cases. Obs high level / rising titres and imaging
  - Imaging - CT >> X-ray

Combined testing ↑ performance

- **CNS**: Include *Cryptococcus* Ag testing

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Diagnostic A; B; C… !!

B. Notify your lab that you are looking for fungal infection!

- Optical brighteners for direct microscopy
- Fungal stains for tissue sections
- Selective agar for primary plating (Chromogenic agar (or Sab))
- Appropriate inoculum size
- Prolonged incubation
- Specific mould PCRs

↑ sensitivity

C. Follow up:

- Metastatic foci (endophtalmitis; spondylitis, hepatosplenic candidiasis, endocarditis…)
- Repeat airway samples culture/PCR incl. susceptibility (co-infection S & R moulds)
- TDM for mould active azoles and 5FC
  - Until stable therapeutic level, and if changes in co-medication, compliance issues, toxicity or failure
- Antigen titres when pos initially
  - Asp GM may turn neg. before inf is cured
  - *Cryptococcus* and *Candida* mannan Ag titres often pos for a long time
- Other (imaging etc)

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(in alphabetic order):

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