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The importance of *Candida* and other yeast infections in neonates: Epidemiology data and diagnostic options

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Declarations of interest

- Lena Klingspor has been adviser to Astellas Pharma, Gilead Sciences, Schering-Plough and has received research grants from Gilead Sciences, Schering-Plough, Merck Sharpe & Dohme, and has received honoraria for educational lectures from , Gilead Sciences, Pfizer, Schering-Plough, Merck Sharpe & Dohme, Abbott and Janssen.

Invasive fungal infections (IFIs) in neonatal patients

- **Invasive candidiasis (IC) is the most frequent neonatal IFI and *C.albicans* and *C .parapsilosis* are the most common species isolated**
- In some neonatal intensive care units, IC it is the third most common cause of nosocomial bloodstream infections (Jinjian Fu et al, 2016)
- **IFIs are probably underestimated because IFIs are often difficult to discriminate from other conditions in combination with laboratory diagnostic difficulties**

The importance of neonatal IC

- Candidemia presents a leading cause of late-onset sepsis in very-low-birth-weight (VLBW) infants (birth weight < 1,500 g) and is associated with significant morbidity and mortality
- It is reported that IC develops in 2%-5% of VLBW infant*
- Especially in preterm infants, the hematogenous spread of *Candida* spp. in the CNS may cause a syndrome, known as hematogenous *Candida* meningoencephalitis (HCME)**
- It is a serious condition associated with an increase mortality rate and neurodevelopmental impairment.

*Jinjian Fu et al, 2016, **Steinbach WJ, et al. 2012, Schmidt J, et al 2011

Epidemiology: IC in neonates

- *Candida albicans* is still the most frequent fungal species most commonly transmitted vertically (from mother to infant)
- However, the emergence of non-*albicans* species such *parapsilosis*, *tropicalis*, *krusei* and *glabrata* with resistance to azoles is of great concern
- Among *Candida* species associated with neonatal sepsis, ***Candida parapsilosis*** is an increasingly reported pathogen especially in preterm neonates with a history of indwelling catheters
- **Mortality is higher for *C. albicans* than *C. parapsilosis***
- Jinjian Fu et al, 2016, Steinbach Wj et al 2012, Schmid J,et al 2011

*Results from a Prospective, International, Epidemiologic Study of Invasive Candidiasis in Children and Neonates**

- The report from the International Pediatric Fungal Network (PFN) is the largest prospective, multi-center observational study dedicated to pediatric and neonatal invasive candidiasis.
- From 2007 to 2011, 196 pediatric (200 isolates) and **25** neonatal (≤ 28 days of age) patients (**26 isolates**) with invasive candidiasis were enrolled.

* Steinbach et al., with the International Pediatric Fungal Network (PFN) 2012

Candida Species Isolated

Species	Pediatric (n=200 isolates)	Neonatal (n=26 isolates)
<i>Candida albicans</i>	87 (44%)	12 (48%)
<i>Candida parapsilosis</i>	45 (22%)	7 (28%)
<i>Candida glabrata</i>	21 (11%)	1 (4%)
<i>Candida lusitaniae</i>	7 (4%)	0
<i>Candida krusei</i>	6 (3%)	0
<i>Candida guilliermondii</i>	3 (2%)	0
<i>Candida dubliniensis</i>	3 (2%)	0
Other	27 (14%)	6 (24%)
Unkown	1 (1%)	0

Steinbach et al. 2013 (PFN=The International Pediatric Fungal Network : a Working in ISHAM)

Outcomes of Pediatric and Neonatal Candidiasis

Outcome	Pediatric (n=196 patients)	Neonatal (n=25 patients)
Success, Complete response	132 (67%)	20 (80%)
Success, Partial response	17 (9%)	3 (12%)
Failure, Stable response	4 (2%)	0
Failure, Progression of disease	4 (2%)	0
Failure, Death	38 (19%)	2 (8%)
Unknown	1 (1%)	0

Antifungal prophylaxis

A recent multicenter study reported a significant decrease in the incidence of neonatal IC over a 14-year period

It was attributed to:

- fluconazole prophylaxis and empirical antifungal therapy
 - and decreased use of broad-spectrum antibacterial antibiotics
-
- Pana ZD, et al 2015

Emerging *Candida* species

Candida auris

- can cause bloodstream infections
- *C. auris* infections have been reported from over a dozen countries **mostly in adults but also in neonates (Venezuela) and infants**
- It has caused outbreaks in healthcare settings
- **Most *C. auris* infections are treatable with echinocandins**
- BUT some *C. auris* infections are resistant to all three main classes of antifungals
- Multiple classes of antifungals at high doses may be required to treat the infection

Rare yeasts in neonates

- ***Malassezia*** (Underdiagnosed?)
- *Trichosporon* (rare)
- *Magniomyces* (former *Geotrichum Blastoschizomyces capitatus*) (rare)
- *Saccharomyces cerevisiae* (rare)
- *Komodea Ohmeri* (rare)
- *Cryptococcus neoformans/gattii* (extremely rare)

- May cause infection with similar clinical picture as *Candida* and may be cultured from blood (rarely *Malassezia*) or septical lesions in different organs

Malassezia

- *Malassezia furfur* is a lipophilic yeast belonging to the normal skin flora and is a common colonizer of the skin in the neonatal period. (Devlin, RK 2006)
- It may cause a chronic superficial skin infection (Pityriasis (tinea) versicolor), and a systemic infection
- *Malassezia furfur* and *Malassezia pachydermatidis* are the two most common *Malassezia* species that may cause infections in neonates

Malassezia pachydermatis

- Nosocomial outbreaks from human to human has been reported
- *Malassezia pachydermatis* is not strictly lipid dependent
- Outbreaks has been associated with health care worker's hands after being colonized from pet dogs at home (Chang HJ, et al 1998)
- Risk factors for invasive *Malassezia* infection in newborns and infants includes:
 - prematurity, underlying complications
 - the presence of CVC,
 - the use of broad-spectrum antibiotics,
 - and long time treatment with parenteral lipids

Implications for current practices and recommendations

It is of outmost importance that we gain more knowledge regarding contemporary national epidemiology:

- in each country
- in different hospitals and wards

and that we follow the epidemiology over time!

More studies are needed in neonates!

Laboratory methods to diagnosing yeasts

- **Direct microscopy** (Calco-flour white)
 - Cultures: on Sabourad's media, chrom agar, BHI, specialised media (Malassezia) etc.
 - Histopathology- Fungal stains
 - Serologi : Antigen and antibodies

 - **MOLECULAR METHODS**
-

Fungal diagnostics in neonates

- **Culture**
- of blood or other normally sterile body fluid –is still the golden standard for diagnosing IC
- In neonates it is difficult to obtain an appropriate volume for analysis
- ESCMID guidelines recommend daily blood culture from three separate sites with a total culture volume of 2–4 ml for infants <2 kg.
- Even with optimal volumes the sensitivity is poor around, 50%-75%

Pediatric blood cultures vials are not optimized for fungi!!

Diagnosis of candidosis

- When systemic candidiasis is suspected, urine and CSF culture are always indicated
- **Microscopy (and PCR)** of CSF and skin abscesses can give an earlier diagnoses

Fungal diagnostics in neonates

- **Urine**
- Urine cultures positive for *Candida* spp. are highly significant, and should be treated as invasive disease
- ELBW infants with candiduria have almost the same rates of neurodevelopmental impairment and death as those with positive blood cultures suggesting that candiduria is a sign of IC.
(Wynn JL, et al, *Clin Infect Dis* 2012)

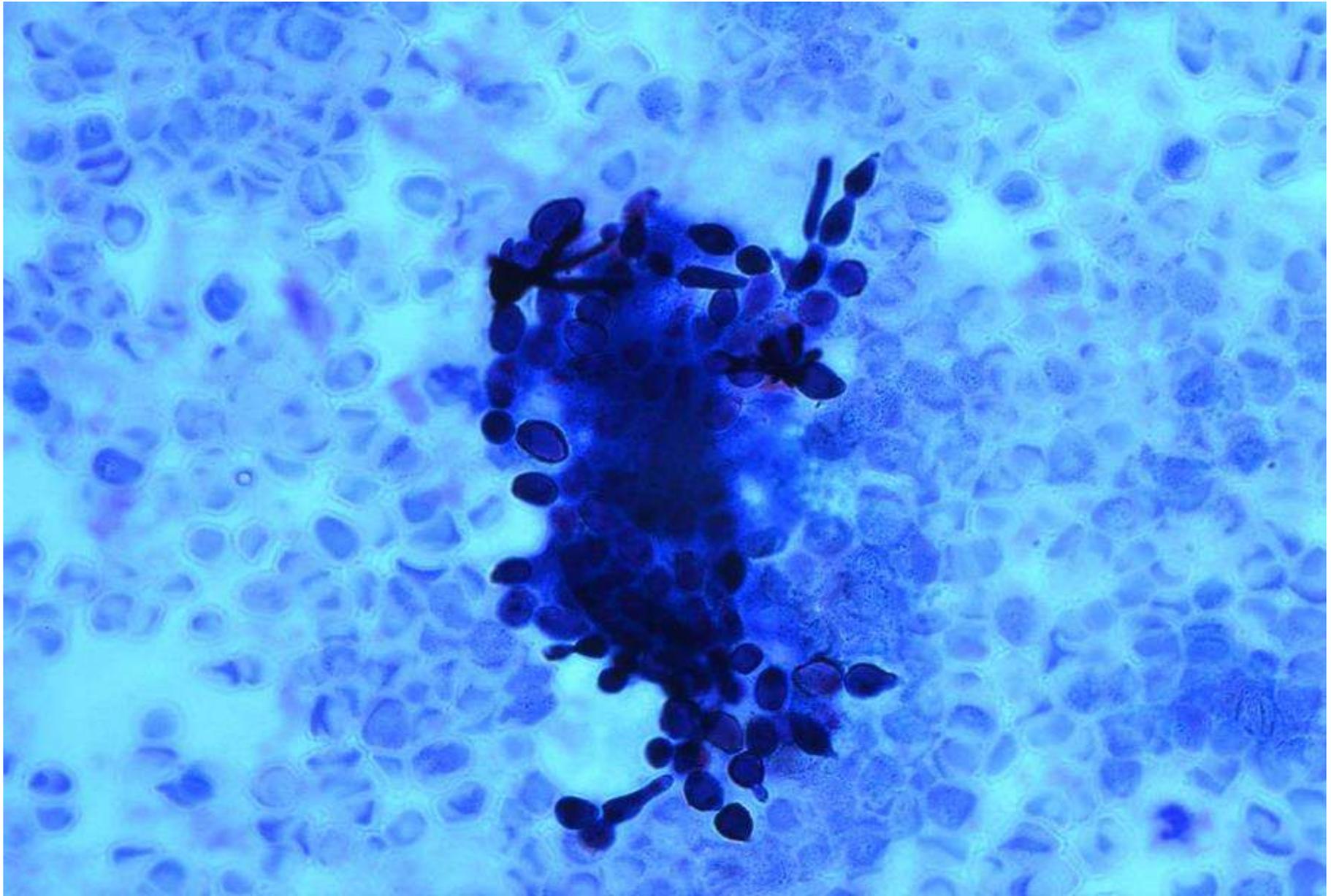
Congenital candidiasis

- Is acquired prenatally from ascending maternal infection (associated with cerclage and intra-conceptive devices) and may lead to intra-uterine death or present at or around the time of birth
- Clinically the infection usually manifests as a widespread vesiculopustular or erythematous skin eruption, which in a term infant largely follows a benign course
- Congenital candidiasis in preterm infants can evolve into severe pneumonia, sepsis, disseminated disease and death
- **Diagnoses is made by: Direct microscopy and culture from the umbilical cord and placenta and from skin lesions and blood cultures from the baby**

Direct microscopy from blood culture



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Identification of *Candida* and other yeast to species level is important!

Molecular tests direct from **blood cultures** such as :

- PNAFish (YTL) (1.5 hours) (*C. albicans* ,*C. glabrata* ,*C. krusei*, *C. parapsilosis* *C. tropicalis*)
- FilmArray Blood Culture Identification Panel by BioFire (1 hour) (*C. albicans* ,*C. glabrata* ,*C. krusei* ,*C. parapsilosis* *C. tropicalis*)

Molecular tests from **blood directly** such as:

- Various in house In- house PCR methods (WB, serum, plasma?ml?)
- T2Candida Panel by T2 Biosystems (4 ml blood) (3-4 hours)

Conventional tests **from cultures** such as:

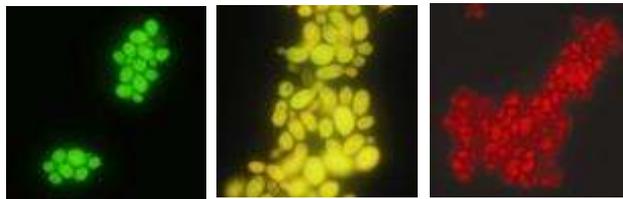
API®/ID32, Vitek®, latexagglutination

- Spectrophotometric: **MaldiTOF**
-

Traffic Light™ PNA FISH®-test

- When direct microscopy show yeast cells in blood (directly from blood culture vials), in situ hybridisation hybridising (Traffic Light™ PNA FISH®-testet) may give a rapid preliminar diagnos to species level for some of the most common **Candida** species. (1,5 thour) (32).

Yeast Traffic Light® PNA FISH®



C. albicans

C. parapsilosis

C. tropicalis

C. glabrata

C. krusei



087

CHROMagar Candida

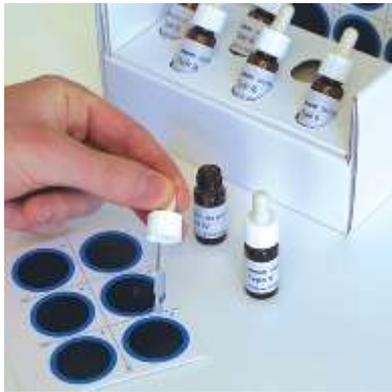
YEAST

C. tropicalis *C. albicans*

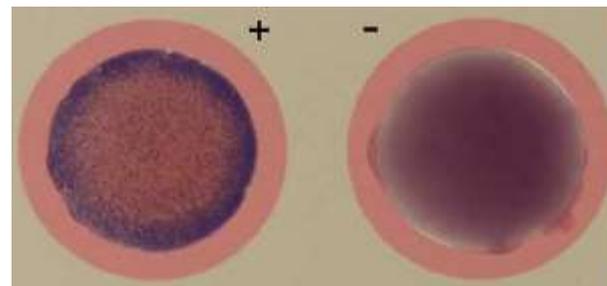
C. parapsilosis

C. glabrata
(*C. krusei*)

- BICHRO-DUBLI FUMOUCZE® - Coagglutination test



C.
dublinskiensis



C. albicans

Tests to identify *Candida* species-but how to Identify *Candida auris*?

Results

- AuxaColor tm 2 : misidentified as *Saccharomyces cerevisiae*
- API ID20C: misidentified as *C. sake*
- Vitek MS IVS: misidentified as *C.lusitaniae*, *C. haemulonii*
- Vitek MS RUO: ***C. auris***
- ITS molecular sequencing: ***C. auris***

- Diagnostic devices based on MALDI-TOF * can differentiate ***C. auris*** from other *Candida* species, (but not all the reference databases included in MALDI-TOF devices allow for detection)

Biomarkers

Serum (1-3)- β -d-glucan (BDG)



BDG is a component of the cell wall

The method does not identify the infection to genus or species level

- The glucan component in the cell wall differ in different fungi
 - *Cryptococcus* , 6%
 - *Mucor*, *Rhizopus* species <10%
 - *Aspergillus* and *Candida*: most of the cell walls component
- Glucan may occur in blood from patients with infections caused by : *Candida* * *Aspergillus*, *Fusarium*, *Saccharomyces*, *Trichosporon*, *Acremonium* species.and *Pneumocystis jiroveci*

(NOT *Cryptococcus neoformans* or *Zygomycetes* species)

Biomarkers

Serum (1-3)- β -d-glucan

- Detection of BDG in serum has a FDA approved commercially available assay (Fungitell®, Associates of Cape Cod, Falmouth, MA) and has been validated in adults.
- Cut of level \geq **80 pg/mL** (adults)
- Only two studies using the Fungitell assay have so far been carried out to assess the utility of serum BDG in diagnosing neonatal IC
- The existing data is currently too limited for clinical decision making based on neonatal BDG levels

Serum (1-3)- β -d-glucan

- There are evidence for BDG values being significantly elevated during neonatal fungal infections
- In one study a relatively high threshold value for BDG positivity in neonates of 125 pg/ml gave a sensitivity of 84% and specificity of 75%. (Goudjil S, et al. 2013)
- Age-specific threshold values are probably needed but are yet to be determined
- **Lower sensitivity of serum BDG for the diagnosis of candidaemia due to *Candida parapsilosis*** (Miluska et al Clin Microbiol Infect. 2016)

Serum (1-3)- β -d-glucan

BDG levels may be "false positive"*

- Concomitant treatment with Betalactams
- Bacteremia
- Hemodialyses (cellulosa filter)

Patients receiving:

- coagulations-factors
- Albumin
- immunoglobulins

*Ellis M, et al 2007.

Mannan and anti-mannan antibodies

- Two studies have included neonatal patients showing that mannan and antimannan antibodies become positive in neonates with IC. (Oliveri S, et al. CMI, 2008 . Verduyn Lunel FM, et al. 2004)
- Its use in neonatal patients may be of restricted value as the performance of the test is species dependent **and lower for C. parapsilosis**. (Sendid B, et al. 2002. Montagna MT, et al. 2011)
- Data is still lacking to support the use of mannan and anti-mannan antibodies in the diagnosis of neonatal IC

Candida PCR in neonates

- Could potentially enhance an earlier detection of IC

Candida PCR assays need standardization

- Which blood fraction is best to use? WB, serum or plasma?
- The WB/plasma/serum volume needed in neonates is not know
- PCR testing is probably about 2-3 ml and cant be a drawback of its use in ELBW infants
- Promising data exists supporting the diagnosis of IC using PCR amplification of *Candida* DNA from serum or plasma (Avni T, et al 2011)

Metabolites (D- and L-arabinitol in urine*)

Serum D-arabinitol/L-arabinitol (DA/LA) ratios is determined by gas chromatography mass spectrometry

Detects: ***C. albicans*** and many other ***Candida*** species

(**NOT** *C. krusei* and not *C. glabrata* (in vitro))

*G. Sigmundsdottir, et al. Scand J Infect Dis. 2007

*Arendrup et al. Clin Microbiol Infect.2010

Antifungal prophylaxis and treatment

Decreases the sensitivity of the diagnostic
tests!

Diagnosis of Malassezia

Microscopy

- Microscopy of blood and biopsies from cutaneous lesions

Culture

- Conventional blood culture media has low sensitivity,
- but blood cultures drawn from a CVC used for parenteral nutrition (intralipid) or
- culturing on agar plates containing lipids increase the likelihood to isolate *Malassezia furfur*

- *Malassezia pachydermatidis* are not strictly lipid dependent and can grow in blood culture media

- Nelson SC, et al . J Clin Microbiol 1995

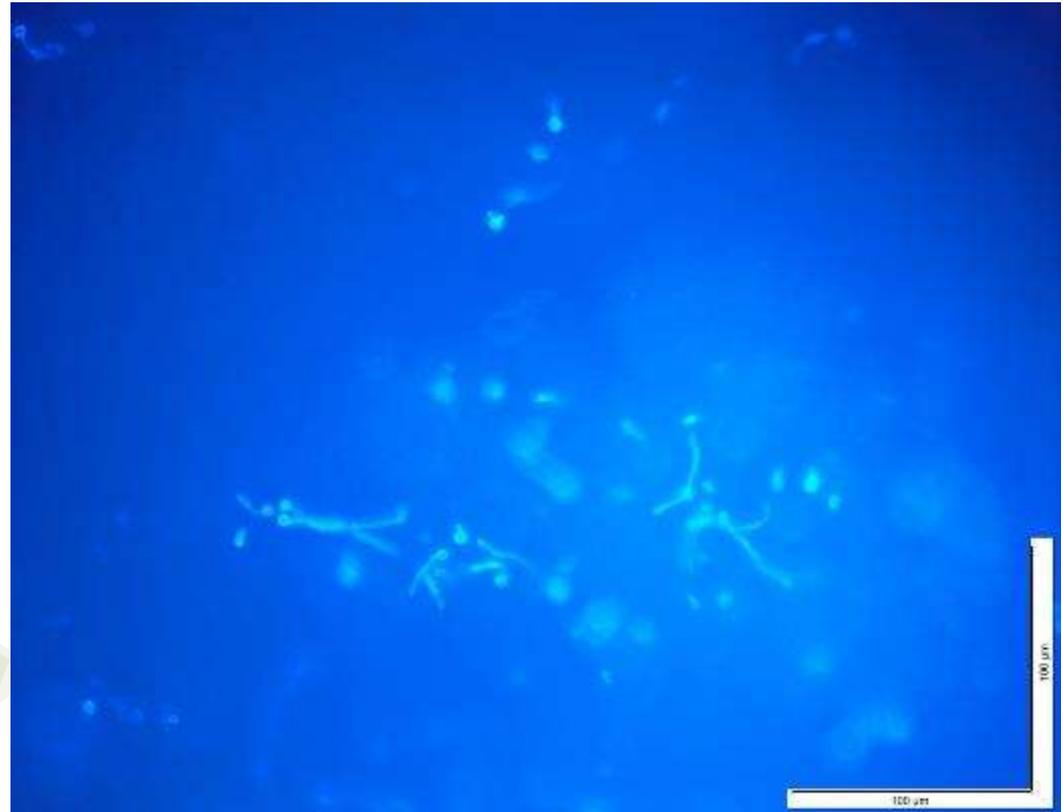
Antifungal susceptibility testing for *Candida* and other yeasts

Candida species and other yeasts causing infection in neonates should undergo **antifungal susceptibility testing !!**

Summary: diagnostic tests

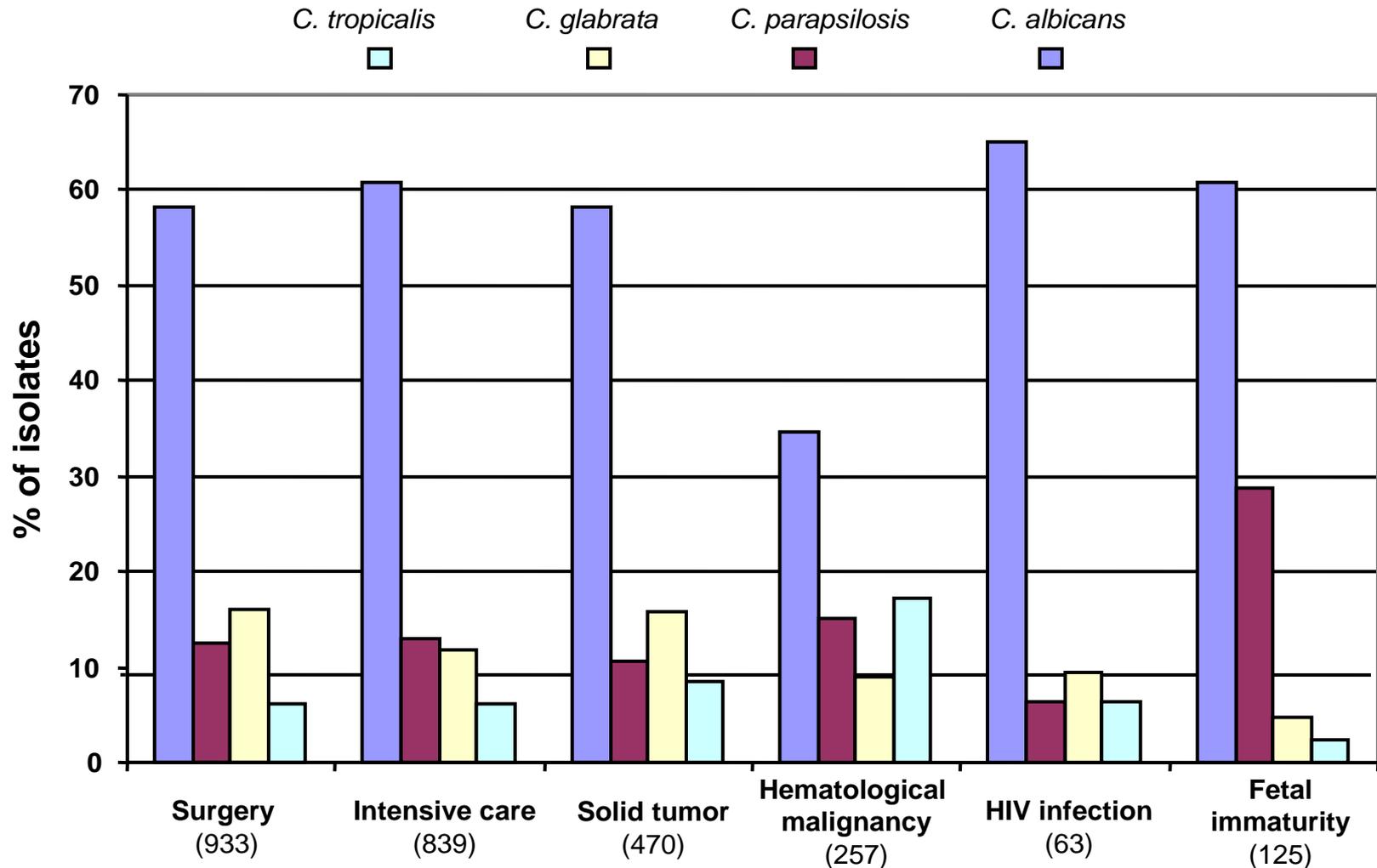
- Blood cultures or cultures from other normally sterile body fluids are still the golden standard
- Rapid and more sensitive diagnostics are needed in neonates for an early diagnoses and early treatment to improve outcome
- Molecular tests such as PCR may provide faster and more sensitive diagnostics but need to be standardized and evaluated in neonates

Thank you for your attention!



ECMM Candidemia study. Tortorano et al. 1998-1999.

Candida species distribution according to underlying conditions



Jinjian Fu et al, 2016

- Forty-eight confirmed cases of candidemia were identified during the study period, indicating an incidence of 106.9 per 1,000 admissions of very-low-birth-weight infants.
- *Candida albicans* was the most common pathogen and was isolated in 39.6% of infants with candidemia
- The mortality rate of the case group was 10.4% versus 2.1% in the control group ($P = .128$)
- The multivariable logistic regression model identified that carbapenem use (odds ratio [OR], 11.39; 95% confidence interval [CI], 3.28-39.54), total parenteral nutrition (OR, 10.16; 95% CI, 2.25-45.94), and prolonged hospitalization (OR, 1.04; 95% CI, 1.01-1.07) were all associated with the risk of developing neonatal candidemia.

The results of 3 studies in which BG testing was evaluated in pediatric patients

- a total of 226 children
 - 38 were diagnosed with proven/probable IFD
 - with ≥ 80 pg/mL as cut off .
 - specificity was 29-82%
 - sensitivity was 50-83%
 - positive predictive value was 17-49%
 - and negative predictive value was 84-96%.
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- * Lehrnbecher T, et al.Clin Infect Dis.2016 Aug 27

Candida PCR

Promising data exists supporting the diagnosis of IC using PCR amplification of Candida DNA from serum or plasma.^{59,60}

- A recent systematic review and meta-analysis of Candida PCR for the detection of IC concluded that these techniques have excellent sensitivity and specificity,⁵⁹
- and a prospective comparative study of Candida PCR, BDG and fungal culture reported that for patients with confirmed deep-seated candidiasis, both PCR and BDG had significantly superior sensitivity than blood culture (88%, 62% and 17% respectively, n=24).⁶⁰
- However, Candida PCR assays need standardization.