# Update on Fungal Nomenclature and Antifungal Susceptibility Testing

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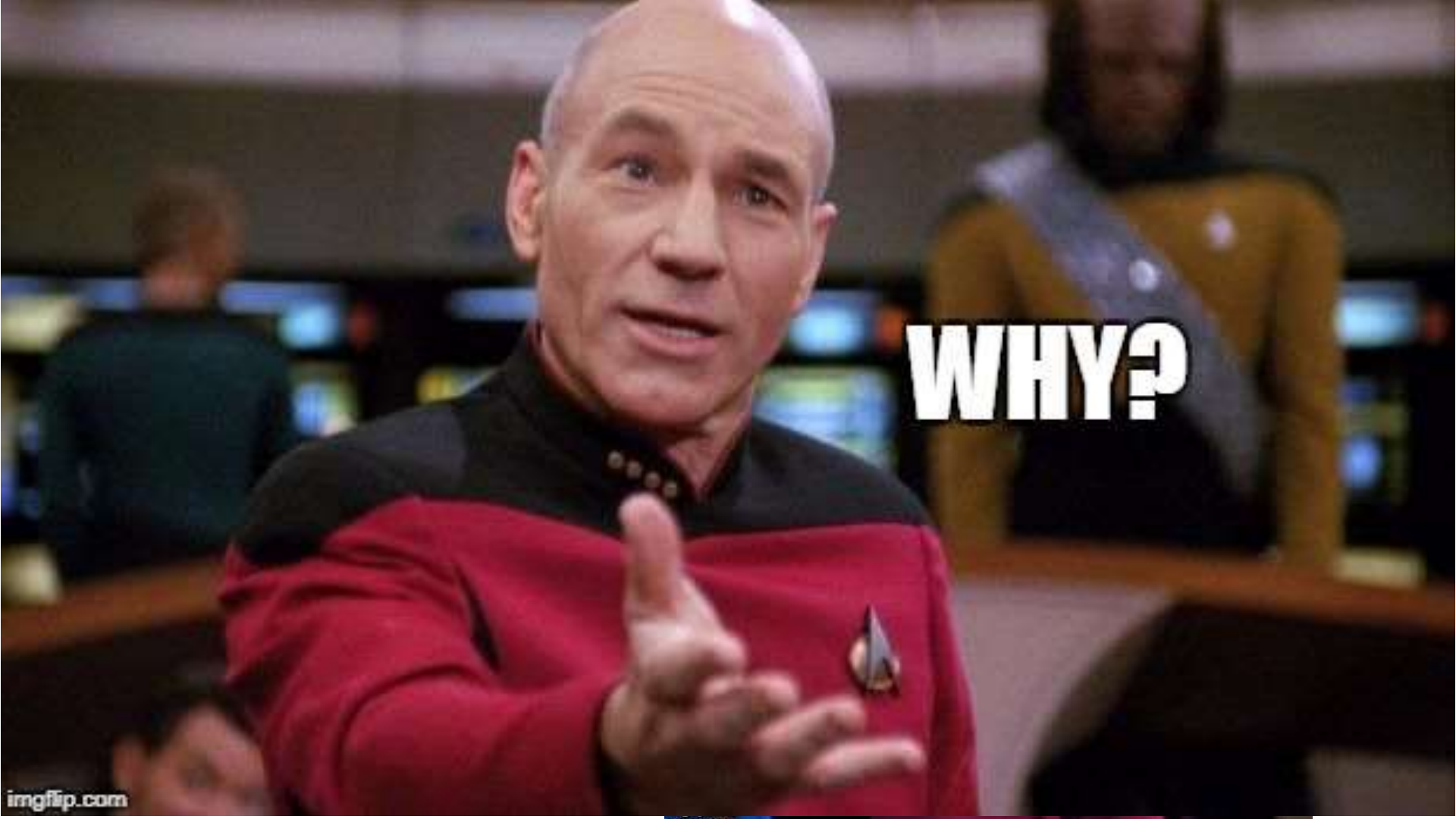
SA PATHOLOGY

# Nomenclature


WHY?
imgflip.com

# Why fungal nomenclature is changing

- Changes to the International Code of Nomenclature (ICN) for algae, fungi and plants (The Melbourne Code, 2012)
  - Fungi can no longer have multiple names for different sexual states
  - Decisions over which names to preserve are highly controversial
- Redefinition of classical species concepts and classification to include phylogenetic data
  - Phylogenetically distinct species are being discovered within morphological defined species (i.e. species complexes)

Hawksworth, *IMA Fungus*. 2011

# *Candida*

- *Candida lusitaniae* → *Clavispora lusitaniae*
- *Candida fabianii* → *Cyberlindnera fabianii*
- *Candida famata* → *Debaryomyces hansenii*
- *Candida kefyr* → *Kluyveromyces marxianus*
- *Candida guilliermondii* → *Meyerozyma guilliermondii*
- *Candida krusei* → *Issatchenckia orientalis* → *Pichia kudriavzevii*
- *Candida norvegensis* → *Pichia norvegensis*
- *Candida pelliculosa* → *Wickerhamomyces anomalus*
- *Candida lipolytica* → *Yarrowia lipolytica*
- *Candida rugosa* → *Diutina rugosa*

Daniel et al., *Antonie Van Leeuwenhoek* 2014

# *Rhizopus*

*Rhizopus oryzae* → *Rhizopus arrhizus*

*Rhizopus microsporus* var. *chinensis*

*Rhizopus microsporus* var. *microsporus*

*Rhizopus microsporus* var. *oligosporus*

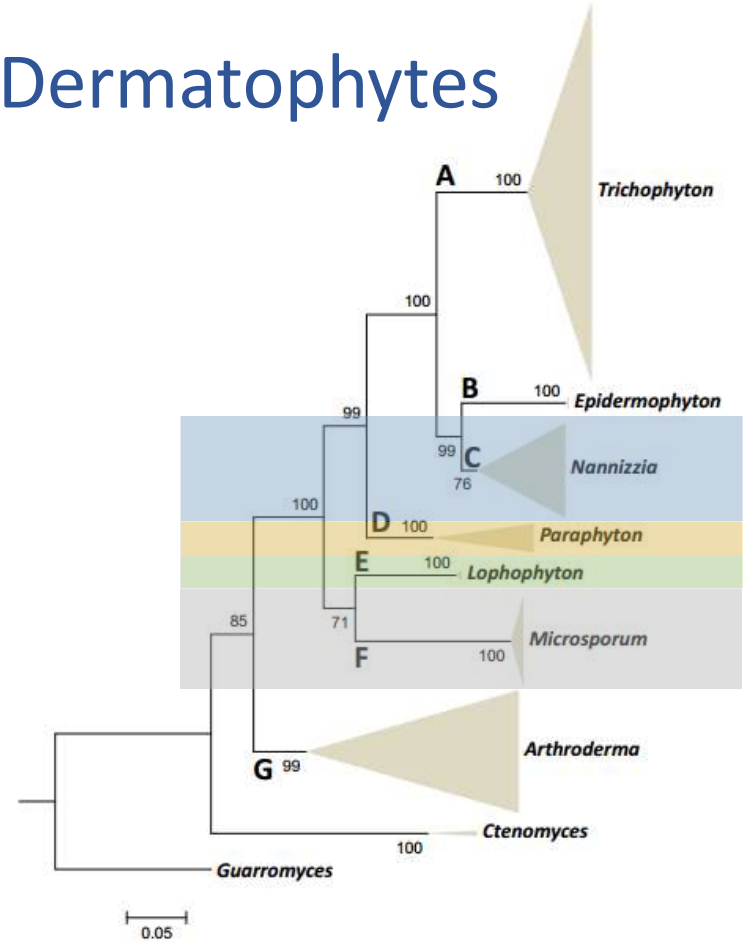
*Rhizopus microsporus* var. *rhizopodiformis*

*Rhizopus azygosporus*

→ *Rhizopus microsporus*

Zheng et al. *Sydowia* 2007; Dolatabadi et al., *Mycoses*. 2014

# Dermatophytes




ML tree based on ITS, partial LSU, *TUB* and 60S L10

*Microsporum*
now 4 genera

*Microsporum*
*Nannizzia*
*Paraphyton*
*Lophophyton*

*Microsporum gypseum* → *Nannizzia gypsea*
*Microsporum fulvum* → *Nannizzia fulva*
*Microsporum persicolor* → *Nannizzia persicolor*
*Microsporum nanum* → *Nannizzia nana*

*Microsporum cookei* → *Paraphyton cookei*

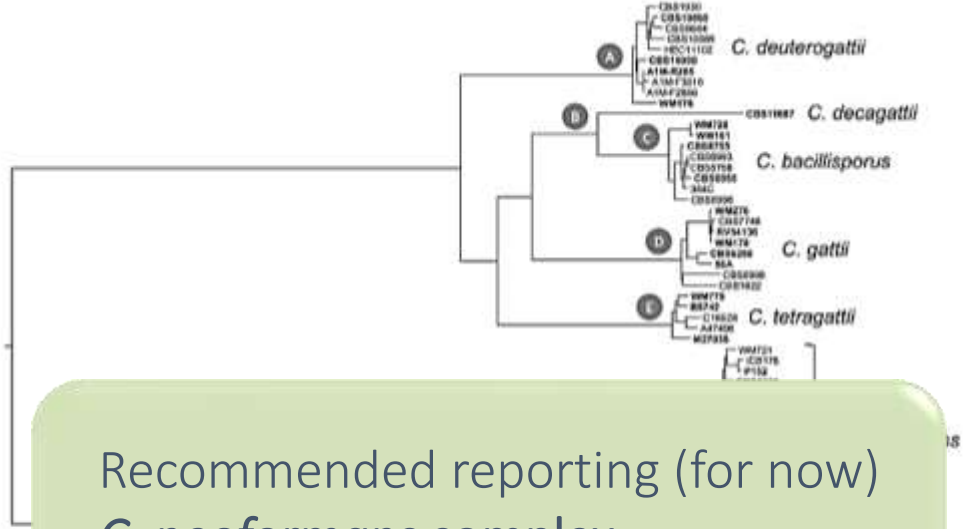
*Microsporum gallinae* → *Lophophyton gallinae*

*Microsporum canis, M. audouinii, M. ferrugineum* unchanged

de Hoog et al., *Mycopathologia*, 2017

# *Cryptococcus neoformans/ C. gattii*

- Proposed changes based on phylogenetic differences:
  - *C. neoformans* → 2 species
  - *C. gattii* → 5 species
- Clinical, pathogenic, and therapeutic relevance of these 7 ‘species’ unclear
- Difficult to ID in routine labs.




Recommended reporting (for now)
*C. neoformans* complex
*C. gattii* complex

Hagen et al., *Fungal Genet Biol* 2015; Kwon-Chung et al., *mSphere* 2017.

# What should labs report?

- No formal guidelines…but labs should adopt to new names as soon as is practical
  - Minimise confusion in literature & DNA sequence databases, build accurate MIC distributions, better understand local epidemiology, etc.
  - But staff education, procedure changes, IT changes take time.
  - **Mycology QAP now deducting marks for use of obsolete names**

- Include a comment as required, to assist clinician interpreting the report

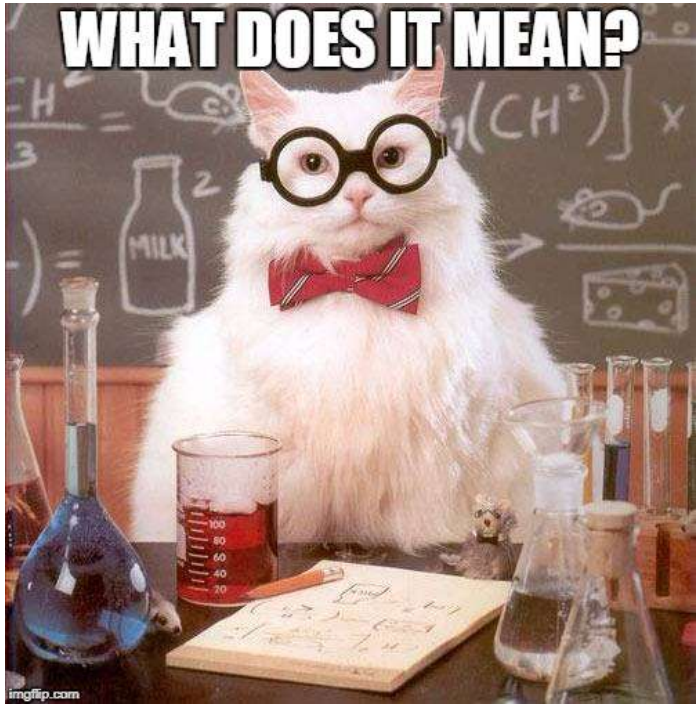
  e.g. Growth of *Pichia kudriavzevii*

  This species was previously know as *Candida krusei*

  e.g. Growth of *Nannizzia gypsea*

  This species was previously know as *Microsporum gypseum*, a geophilic dermatophyte

# UPDATE ON ANTIFUNGAL SUSCEPTIBILITY TESTING



# Current AFST Reference Standards

- **CLSI M27-A4 [2017]** microbroth dilution method for yeasts
- **CLSI M38-A3 [2017]** microbroth dilution method for filamentous fungi
- **CLSI M44-A3 [2018]** disk diffusion method for yeasts
- **CLSI** interp. criteria: **M60 [2017]** (yeast CBPs), **M59-2 [2018]** (ECVs)
- **EUCAST** Definitive Document **E.def 7.3.1** microbroth dilution method for fermentative yeasts
- **EUCAST** Definitive Document **E.def 9.3.1** microbroth dilution method for conidia forming moulds

# Updates to CLSI interpretive criteria

- **CLSI M60 (2017)** a new document for yeast CBPs
  - Replaces M27-S4 (2012) - now obsolete
  - Clinical breakpoints for *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. guilliermondii* vs. echinocandins, fluconazole, voriconazole
  - Voriconazole SDD category replaced by Intermediate
  - Recommends caution if reporting caspofungin MICs and/or interps, esp. **R** (consider another echinocandin as a proxy)

# Updates to CLSI interpretive criteria (cont.)

- **M59 edition 2 (Jan 2018)**
- ECVs for differentiation of WT and NWT MICs, where CBPs are unavailable.
- WT: MICs ≤ ECV
- NWT: MICs > ECV
- Provides guidance on where the MIC sits for that species
- Interpret MICS as WT or NWT on lab reports <u>with appropriate comments</u>

**Table 1. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of Various *Candida* spp. With No Breakpoints**[1-6]

| Antifungal Agent | Species | ECV, µg/mL*,†,‡ |
|---|---|---|
| Amphotericin B | *C. albicans* | 2 |
| | *C. glabrata* | 2 |
| | *C. krusei* | 2 |
| | *C. parapsilosis*§ | 2 |
| | *C. tropicalis* | 2 |
| Anidulafungin | *C. dubliniensis* | 0.12 |
| | *C. lusitaniae* | 1 |
| **Fluconazole**¶ | ***C. dubliniensis*** | **0.5** |
| | ***C. guilliermondii*** | **8** |
| | ***C. lusitaniae*** | **1** |
| Itraconazole | *C. glabrata* | 4 |
| | *C. krusei* | 1 |
| | *C. lusitaniae* | 1 |
| | *C. tropicalis* | 0.5 |
| Micafungin | *C. dubliniensis* | 0.12 |
| | *C. lusitaniae* | 0.5 |
| **Posaconazole**# | ***C. albicans*** | **0.06** |
| | ***C. glabrata*** | **1** |
| | ***C. guilliermondii*** | **0.5** |
| | ***C. krusei*** | **0.5** |
| | ***C. lusitaniae*** | **0.06** |
| | ***C. parapsilosis***§ | **0.25** |
| | ***C. tropicalis*** | **0.12** |
| **Voriconazole**¶ | ***C. glabrata*** | **0.25** |

* The ECVs in M59 were established using broth microdilution as outlined in CLSI document M27.[1] If another methodology is used for susceptibility testing, this method must be validated against broth microdilution before using the ECVs, just as

**Table 2. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of Various *Cryptococcus* spp. With No Breakpoints**[*1-5]

| Antifungal Agent | Species (Genotype) | ECV, µg/mL[†,‡] |
|---|---|---|
| Amphotericin B | *C. neoformans* (VNI) | 0.5 |
| | *C. gattii* (VGI) | 0.5 |
| | *C. gattii* (VGII) | 1 |
| Fluconazole | *C. neoformans* (VNI) | 8 |
| | *C. gattii* (VGI) | 16 |
| | *C. gattii* (VGII) | 32 |
| Flucytosine | *C. neoformans* (VNI) | 8 |
| | *C. gattii* (VGI) | 4 |
| | *C. gattii* (VGII) | 32 |
| Itraconazole | *C. neoformans* (VNI) | 0.25 |
| | *C. gattii* (VGI) | 0.5 |
| | *C. gattii* (VGII) | 1 |
| Posaconazole | *C. neoformans* (VNI) | 0.25 |
| Voriconazole | *C. neoformans* (VNI) | 0.25 |
| | *C. gattii* (VGI) | 0.5 |
| | *C. gattii* (VGII) | 0.5 |

[*] ECVs were adopted by the Subcommittee on Antifungal Susceptibility Tests during a Web conference in May 2016. The ECVs for *Cryptococcus* were established against the distinct molecular types. The phylogeny for *Cryptococcus* is currently

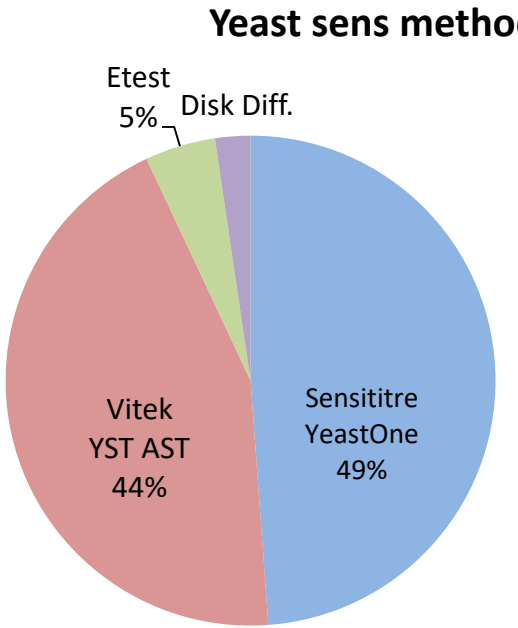
*Aspergillus* ECVs unchanged from previous edition of M59

**Table 3. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of Various *Aspergillus* spp. With No Breakpoints[1-7]**

| Antifungal Agent | Species | ECV, µg/mL[*,†,‡] |
|---|---|---|
| Amphotericin B | *A. flavus* | 4 |
| | *A. fumigatus* | 2 |
| | *A. niger* | 2 |
| | *A. terreus* | 4 |
| | *A. versicolor* | 2 |
| Caspofungin[§] | *A. flavus* | 0.5 |
| | *A. fumigatus* | 0.5 |
| | *A. niger* | 0.25 |
| | *A. terreus* | 0.12 |
| Isavuconazole | *A. flavus* | 1 |
| | *A. fumigatus* | 1 |
| | *A. niger* | 4 |
| | *A. terreus* | 1 |
| Itraconazole | *A. flavus* | 1 |
| | *A. fumigatus* | 1 |
| | *A. niger* | 4 |
| | *A. terreus* | 2 |
| Posaconazole | *A. flavus* | 0.5 |
| | *A. niger* | 2 |
| | *A. terreus* | 1 |
| Voriconazole | *A. flavus* | 2 |
| | *A. fumigatus* | 1 |
| | *A. niger* | 2 |
| | *A. terreus* | 2 |

[*] ECVs capture ≥97.5% of the statistically modeled population. ECVs may overlook potentially resistant isolates (NWT).

# QAP performance (Yeast)




- 43 AU/NZ labs performed Yeast AFST
- 2017 sens item: *Candida tropicalis*
- Overall performance quite good
- MICs broadly similar, even between methods
- BUT:
  - 16 (37%) used obsolete/incorrect/non-existent breakpoints for ≥1 antifungals
  - Despite report commentary advising participants of errors, the same participants made the same errors in 2018!

Kidd et al., *Pathology* 2018; RCPAQAP mycology surveys 2017:4 and 2018:4

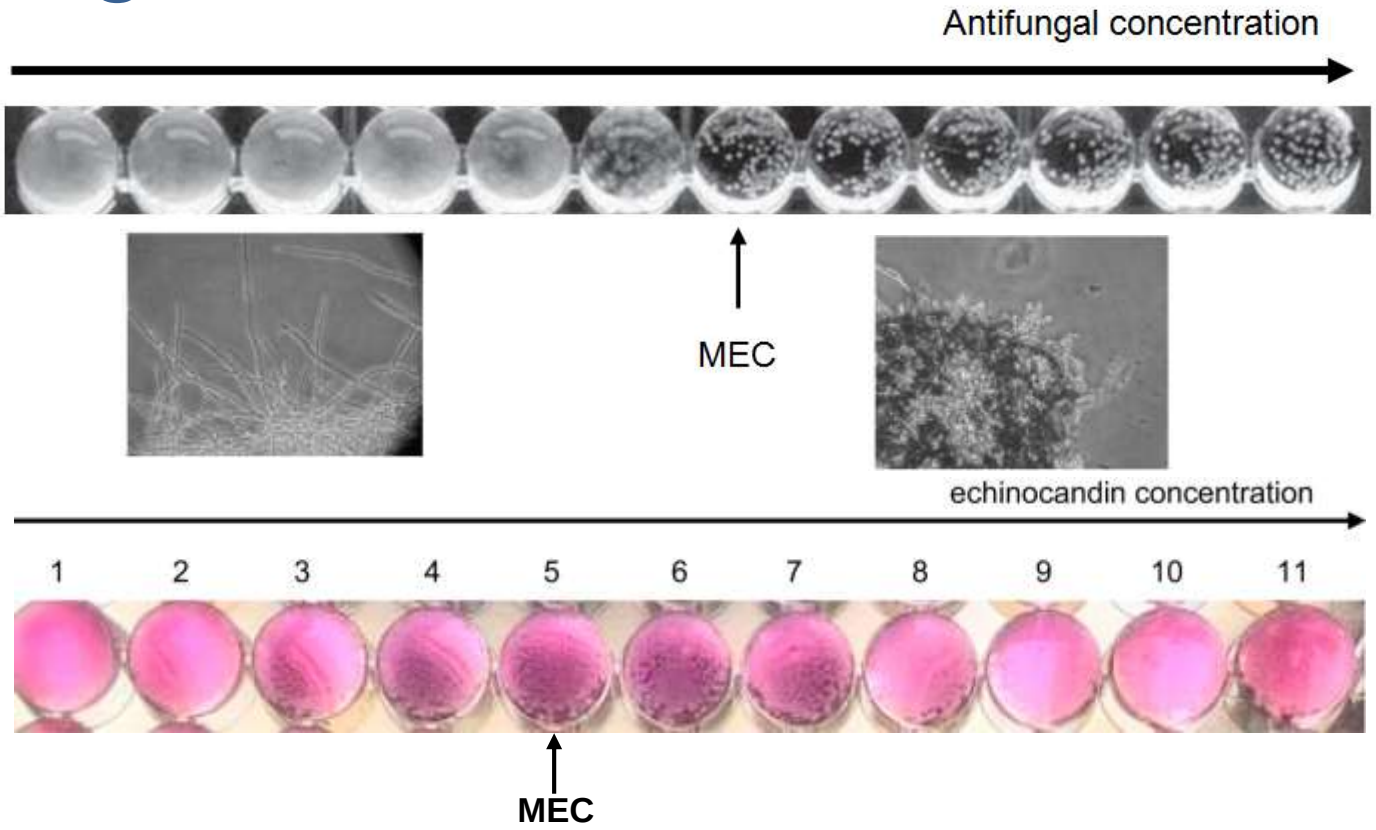
# QAP performance (Moulds)

- 10 AU/NZ labs perform Mould AFST
- 2017 sens item: *Aspergillus flavus*
  - All 10 labs use Sensititre YeastOne
  - Triazole MICs broadly consistent between labs; some variability in MICs for Amphotericin B [0.5 - 8 mg/L]

- BUT:
  - 1 (10%) used invalid (?EUCAST) breakpoints to interpret itraconazole as **S**
  - 4 (40%) incorrectly read echinocandin-*Aspergillus* endpoints as MICs rather than MECs
  - Despite report commentary advising participants of errors, same participants made the same errors in 2018!

Kidd et al., *Pathology* 2018; RCPAQAP mycology surveys 2017:6 and 2018:6

# *Aspergillus*-Echinocandin MECs



# Summary

- Names are changing. Keep up with science, report using current accepted nomenclature
- Several CLSI methodology and interpretation updates
  - Voriconazole **SDD** category replaced by **I [M60]**
  - Caution recommended if reporting/interpreting caspofungin MICs **[M60]**
  - New ECVs for *Candida* and *Cryptococcus* spp. **[M59-2]**
- ~37 % of AU/NZ mycology labs that perform AFST are making serious errors – we must improve our performance!

# Acknowledgements


- Deb Walker


# Disclosures

- Scientific Advisory Boards: Merck, Pfizer, Mayne Pharma
- Research funds: Gilead, Merck
- Conference/Travel funds: Merck, Pfizer
- Honoraria: Gilead, Mayne Pharma, Pfizer
- **No benefits received from ‘Big Nomenclature’**

# SPARE SLIDES

# CLSI vs. EUCAST (Yeasts)

| Characteristics | CLSI M27A-4 | EUCAST Edef 7.3 |
|---|---|---|
| Format | U shaped wells | Flat bottom wells |
| Inoculum (CFU/mL) | 0.5 - 2.5 x $10^3$ | 0.5 - 2.5 x $10^5$ |
| Test medium | RPMI 1640<br>with 0.2% Glucose | RPMI 1640<br>with 2% Glucose |
| Growth Temperature | 35°C | 35°C |
| Incubation time | 24-48 h for yeasts<br>72 h for Cryptococcus | 24 h<br>24-48 h for *Cryptococcus* |
| Endpoint | 100% inhibition AmB<br>50% azole/candin inhibition | 90% AmB<br>50% azole/candin inhibition |
| Reading | Visually | Plate reader |

# CLSI vs. EUCAST (Moulds)

| Characteristics | CLSI M38A-3 | EUCAST Edef 9.3 |
|---|---|---|
| Format | U shaped wells | Flat bottom wells |
| Inoculum (CFU/mL) | 0.4 - 5 x $10^4$ (spec adjusted) | 0.5 - 1.25 x $10^5$ (counted) |
| Test medium | RPMI 1640 with 0.2% Glucose | RPMI 1640 with 2% Glucose |
| Growth Temperature | 35°C | 35°C |
| Incubation time | 48 h (24-72 h) | 48 h (24-72 h) |
| Endpoint | 100% inhibition (echinocandins MEC) | 100% inhibition (echinocandins MEC) |
| Reading | Visually | Visually |

# Factors influencing susceptibility testing

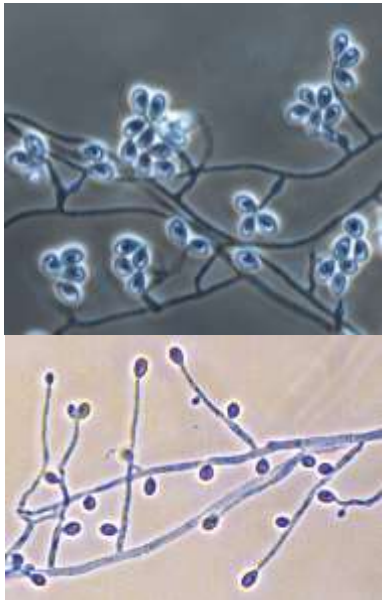
- Inoculum size: More inoculum = ↑ MIC
- Inoculum growth phase: Shorter lag phase = ↑ MIC
- Incubation temp: Affects growth rate and expression of resistance mechanisms
- Incubation duration: More growth = ↑ MIC
- Media: Type, brand/batch, glucose concentration
- Drug purity/conc./activity Variations = ↓ reproducibility of MICs
- Endpoint definition: 50%, 80%, 100% inhibition. ↑ stringency = ↑ MIC
- Reading factors: Visual vs. spectrophotometric, trailing, etc.

Rex et al., *Clin Microbiol. Rev.* 1993; Shawn Lockhart, ISHAM 2015.

# *Scedosporium*

- *Scedosporium prolificans* → *Lomentospora prolificans*

- ***Scedosporium apiospermum* complex (8 species)**
  - *Scedosporium apiospermum*
  - *Pseudallescheria boydii* → *Scedosporium boydii*
  - *Scedosporium aurantiacum*
  - *Scedosporium desertorum*
  - *Pseudallescheria minutispora* → *Scedosporium minutisporum*
  - *Scedosporium dehoogii*
  - *Pseudallescheria ellipsoidea*
  - *Pseudallescheria angusta*



Lackner et al., *Fungal Diversity* 2014; Lackner et al., *AAC* 2014; Harun et al., *Med Mycol* 2010a; 2010b; Gilgado et al., *JCM* 2005; 2008.

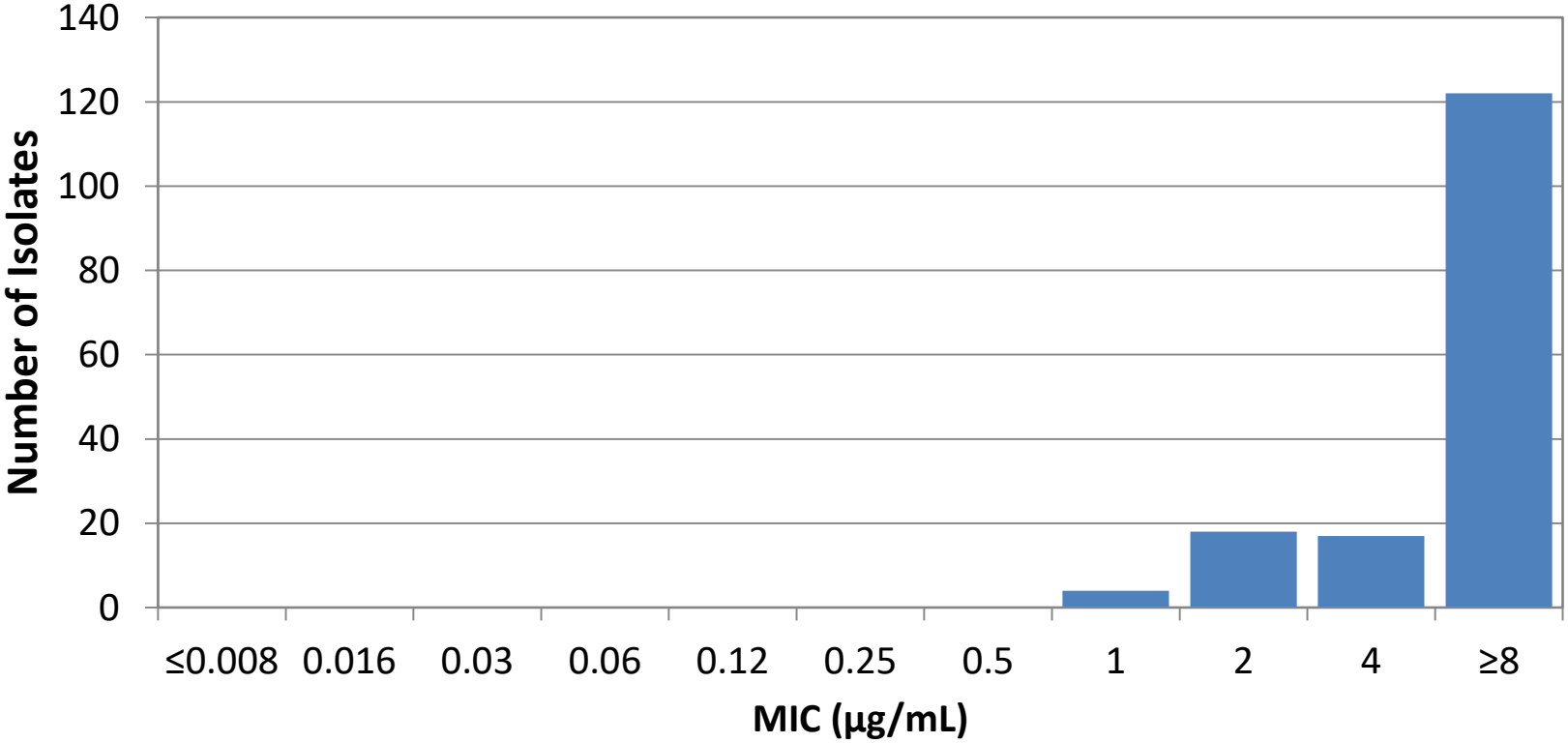
# *Scedosporium/Lomentospora*

- *Scedosporium apiospermum*
  - Study using immunosuppressed mice suggests isolates with Voriconazole
    MIC ≤2 mg/L → 92% response rate, while
    MIC ≥4 mg/L → 33% response rate
    But mice are not humans!

- *Lomentospora prolificans* (previously *Scedosporium prolificans*)
  - aren't they always completely resistant?
  - Is there any point?

Slide borrowed from Sharon Chen; Martin-Vicente et al. *JAC* 2017

## *Lomentospora prolificans:* 2010 to 2018
## Voriconazole, N=161




National Mycology Reference Centre, SA Pathology, unpublished data

# Synergy Testing

- Sporadic requests for Vori/Terb synergy testing on *Lomentospora prolificans* isolates.
- Very labour intensive (no longer viable, even in a reference lab)

**109 *L. prolificans* isolates tested between 2001 and 2011**

| Drug combination | ∑FIC < 0.5 (S) | ∑FIC > 0.5-4 (NS) | ∑FIC > 4 (A) |
|---|---|---|---|
| Voriconazole/Terbinafine (n=109) | 94 (86.2%) | 15 (13.8%) | 0 |

*The summation of the fractional inhibitory concentration (ΣFIC) was calculated as follows: (MIC agent A in combination/MIC agent A alone) + (MIC agent B in combination/MIC agent B alone). Synergy was defined as a ΣFIC of ≤ 0.5; No synergy >0.5 - 4;Antagonism > 4.*

Kidd et al., *ASA Newsletter* 38, 2011

# Other antifungals vs. *L. prolificans*

- Isavuconazole: new-ish triazole antifungal, approved for treatment of aspergillosis and mucormycosis currently available by compassionate access.
  - *L. prolificans* n=30 MICs 8 – 32 µg/mL ($MIC_{90}$ 16 µg/mL) [1]
  - *L. prolificans* n=6 MICs 16 – >16 µg/mL [2]
    - compare to Vori: n=30 MICs 0.5 – 16 µg/mL, GM 5.66 µg/mL) [1]
      n=7 MICs 2 – 8 µg/mL [3]
      n= 4 MICs 1 – 8 µg/mL [4]
- Olorofim (F901318): novel orotomide antifungal, disrupts fungal pyrimidine synthesis by inhibiting dihydroorotate dehydrogenase.
  - Previously shown potent *in vitro* activity against *Aspergillus* spp.
  - Potent *in vitro* activity against all *Scedosporium*/*Lomentospora* species [1]
  - *L. prolificans* n=30 (MICs 0.12 – 0.5 µg/mL, GM=0.26 µg/mL) [1] (20x lower than other triazoles)

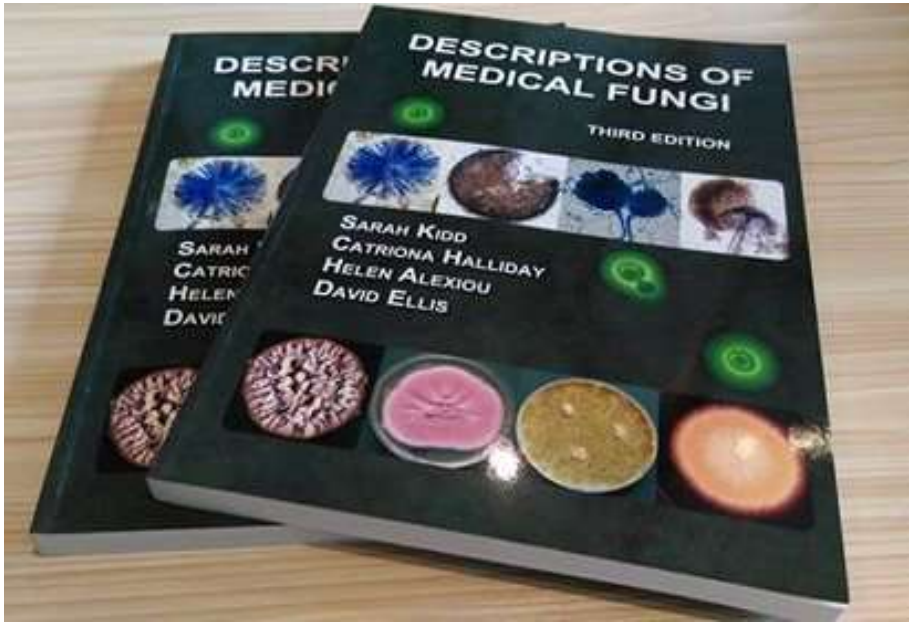
[1] Biswas et al., *Med Mycol* 2018; [2] Guinea et al., *AAC* 2008; [3] Cooley et al., *EID* 2007; [4] Halliday et al., *IJAA* 2016

# Miltefosine

- ‘Good’ activity against *Scedosporium* spp.
  - *L. prolificans* n=10, MIC = 4.0 µg/mL [1]
- Some published instances of synergism when combined with azoles
  - *L. prolificans*: 1/2 isolates showed Vori/Milt synergy and 0/2 isolates showed Posa/Milt synergy *in vitro* [2]
  - Miltefosine successfully used as tri-therapy with Vori and Terb in two published cases of *L. prolificans* osteomyelitis [3,4]
- Not a routine susceptibility test!

[1] Widmer et al., *AAC* 2006; [2] Biswas et al., *JAC* 2013; [3] Quaesaet et al., *Med Mal Infect* 2018; [4] Kesson et al., *CID* 2009

# How do labs find out about name changes?

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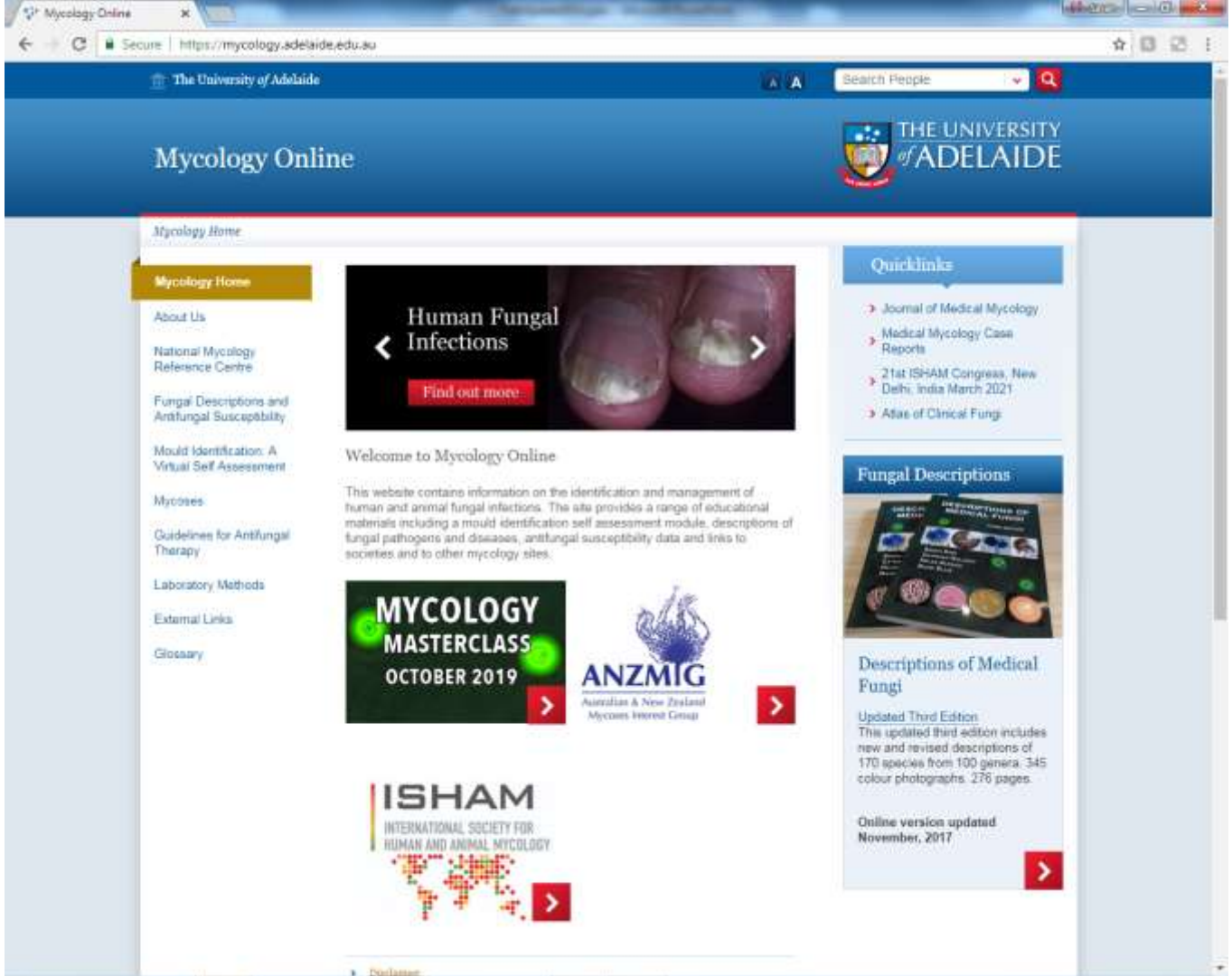
**List of fungi that may be despatched in the QAP program.** Please note the currently accepted genus and species names that will be used for scoring purposes, the expected level of identification, and the risk group rating.

| Accepted genus and species names | RG level | Expected ID level |
|---|---|---|
| *Acrophialophora fusispora* | 1 | genus |
| *Alternaria sp.* | 1 | genus |
| *Aphanoascus fulvescens* | 1 | genus |
| *Apophysomyces elegans* | 2 | species |
| Note: The main clinical *Aspergillus* species are now known to contain many species that are only identified reliably by DNA sequence (β-tubulin gene). If identification is based upon morphology or ITS sequencing, it should be reported to species complex level. | | |
| *Aspergillus flavus* complex | 2 | species complex |
| *Aspergillus fumigatus* complex | 2 | |
| – *Aspergillus fumigatus sensu stricto* | | |
| – *Aspergillus lentulus* | | |
| – *Aspergillus fischeri* (syn. *Neosartorya fischeri*) | | |
| – *Aspergillus thermomutatus* (syn *Neosartorya pseudofischeri*) | | |
| *Aspergillus nidulans* complex | 1 | |
| *Aspergillus niger* complex | 1 | |
| *Aspergillus terreus* complex | 2 | |
| *Aureobasidium pullulans* | 1 | species |
| *Basidiobolus ranarum* | 2 | genus |
| *Beauveria sp.* | 1 | genus |
| *Bipolaris sp.* | 1 | genus |
| Note: *Candida glabrata* and *C. parapsilosis* are known to comprise multiple species of clinical importance that can only be reliably identified by ITS sequencing. If identification is based upon phenotypic methods, it should be reported to species complex level. | | |
| *Candida albicans* | 2 | species or species complex |
| *Candida dubliniensis* | 2 | |
| *Candida glabrata* complex | 2 | |
| – *C. glabrata sensu stricto* | | |
| – *C. nivariensis* | | |
| – *C. bracarensis* | | |
| *Candida parapsilosis* complex | 1 | |
| – *C. parapsilosis sensu stricto* | | |
| – *C. orthopsilosis* | | |
| – *C. metapsilosis* | | |
| – *Lodderomyces elongisporus* | | |
| *Candida rugosa* | 1 | |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21st ISHAM Congress, New Delhi, India March 2021
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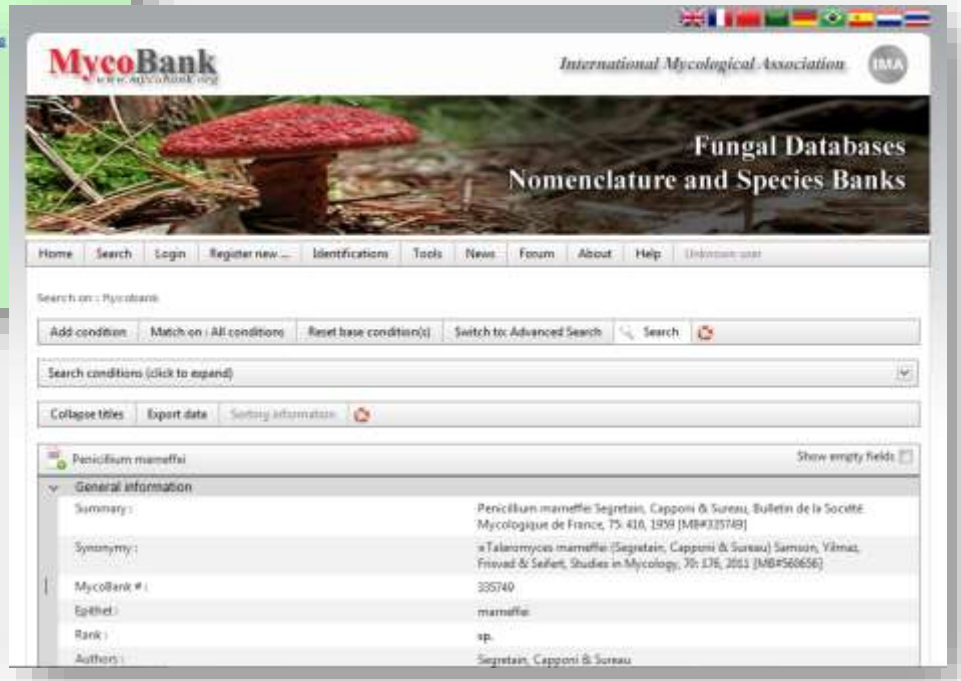
Online version updated November, 2017


www.speciesfungorum.org




www.mycobank.org

http://nomenclature.clinicalfungi.org/