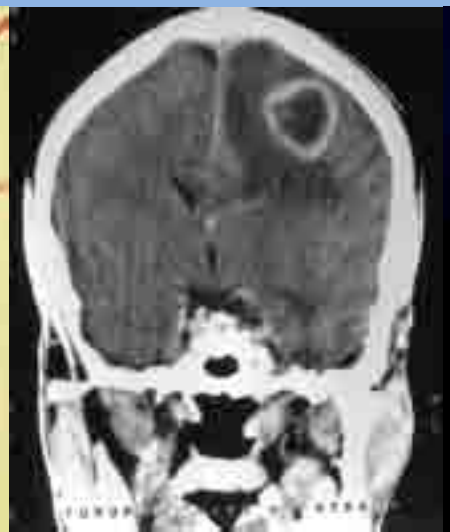
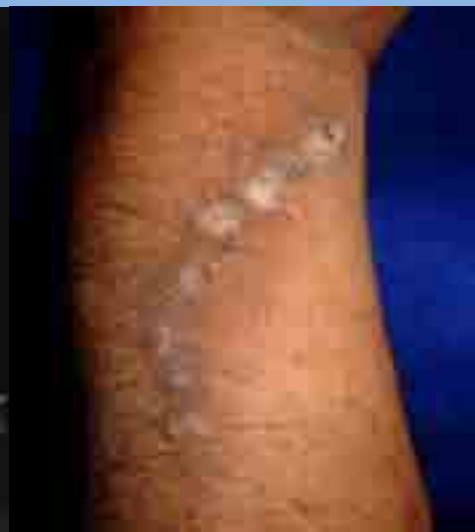
Use of AFLP to identify *Cladophialophora strains related to cerebral phaeohyphomycosis and chromoblastomycosis with in vitro antifungal susceptibility*

H. Badali



General questions

The species-level identification in chaetothyriales based on morphological or physiological characters alone is usually not possible.

Therefore, a reliable molecular approach capable of the unambiguous identification is needed.

- Sequencing
- RFLP
- AFLP
- RAPD
- RCA
- LAMP, etc

General questions

Why AFLP?

- Based on selective amplification of restriction fragments of genomic DNA

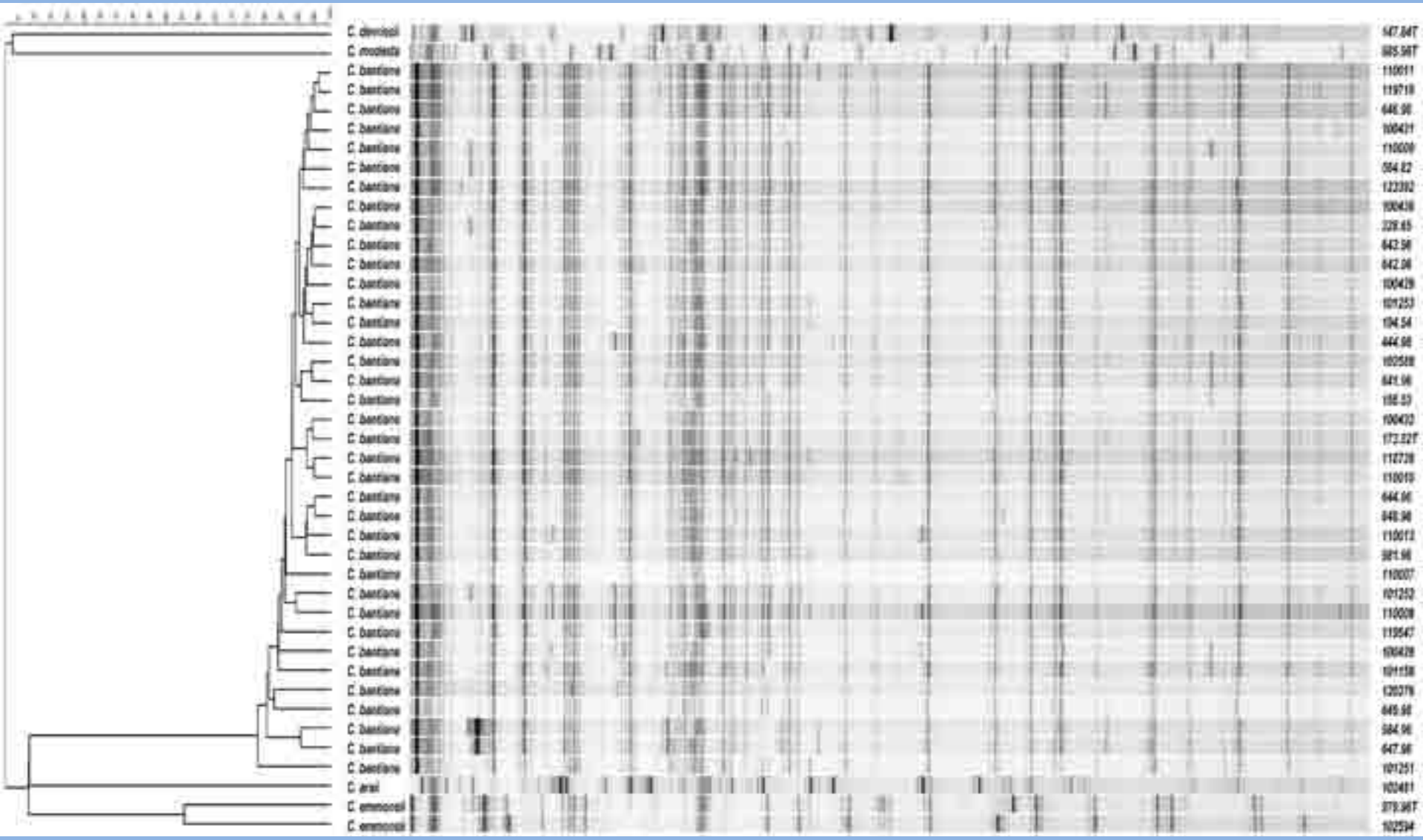
- Higher reproducibility
- Resolution
- Sensitivity
- To amplify 50 and 100 fragments
- No prior sequence is needed

First Aim

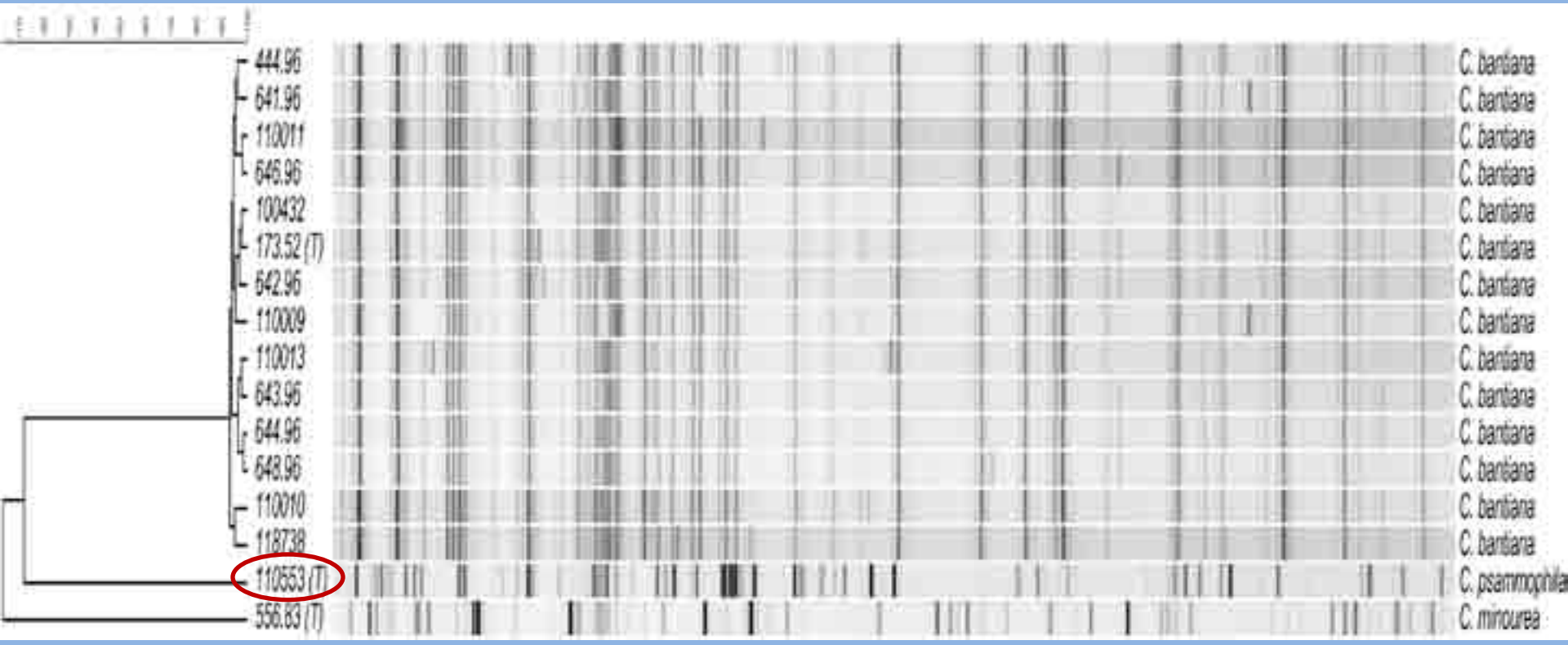
To identified and evaluate the inter- and intraspecific genomic variation of *Cladophialophora* isolates, related to brain infection and chromoblastomycosis

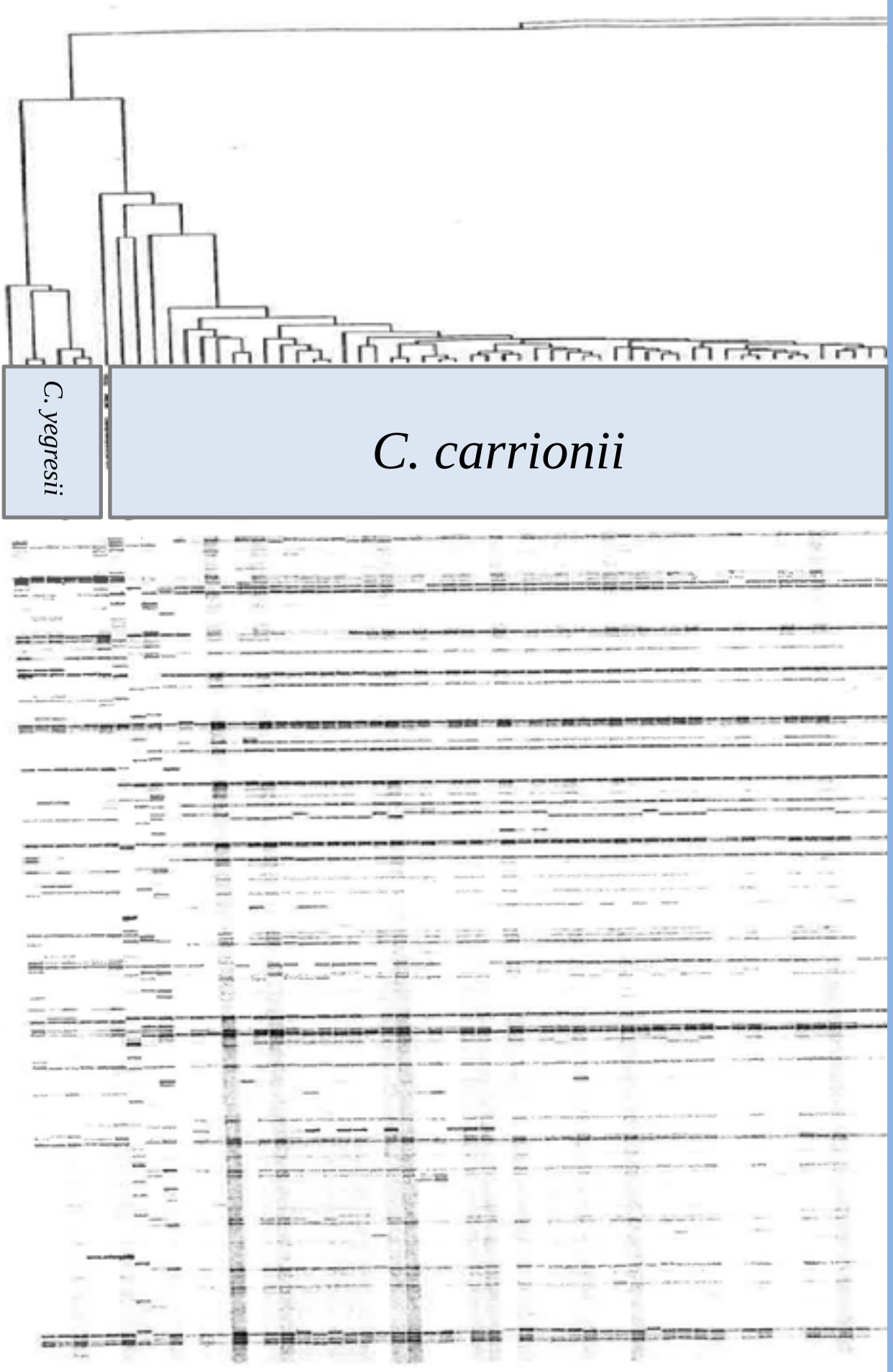
Although cerebral infections are rare, increasingly recognized in human disease with high mortality by *C. bantiana*

Chromoblastomycosis is a chronic, cutaneous and subcutaneous infection with muriform cells in tissue.



AFLP pattern


444.96
641.96
110011
646.96
100432
173.52 (T)
642.96
110009
110013
643.96
644.96
648.96
110010
118738
110553 (T)
556.83 (T)
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. bantiana
C. psammophila
C. mirrourea


C. yegresii
C. carrionii

Second Aim

Why AFST?

- changing epidemiology of isolated agents
- increased of drug resistance
- newer drugs-more choice
- drug discovery
- optimizing therapy
- predict patients outcome

Second Aim

- No standard therapy, unfavourable results, very limited data

- This is the first evaluation of new generation of triazoles and echinocandins.

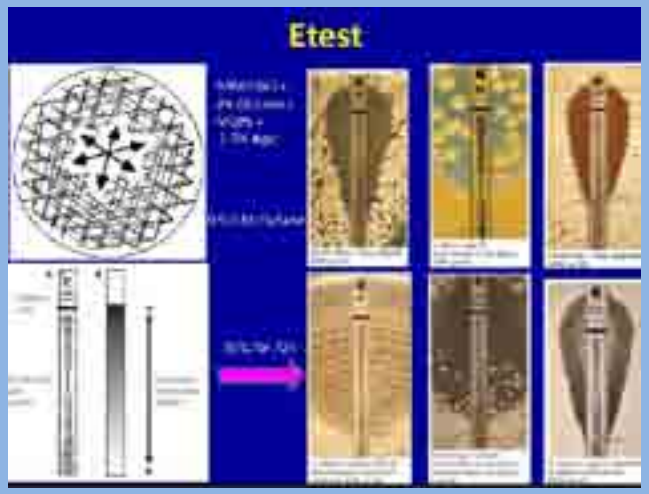
- Sufficient data on in vitro activity can be performed to improve the managements of infections

Methods for susceptibility testing

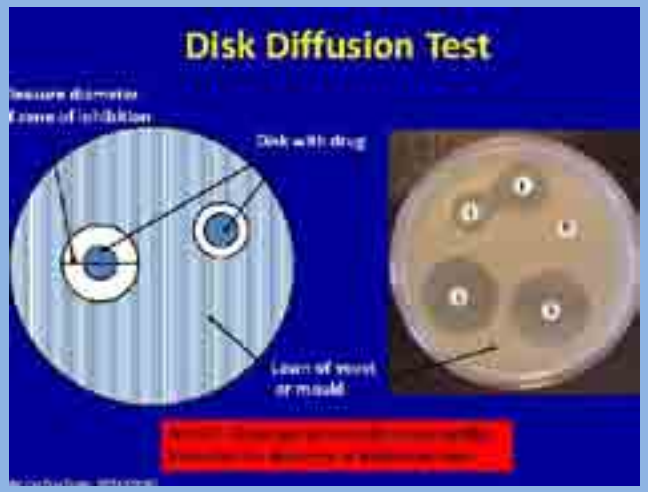
Broth dilution

- Macrodilution and Microdilution

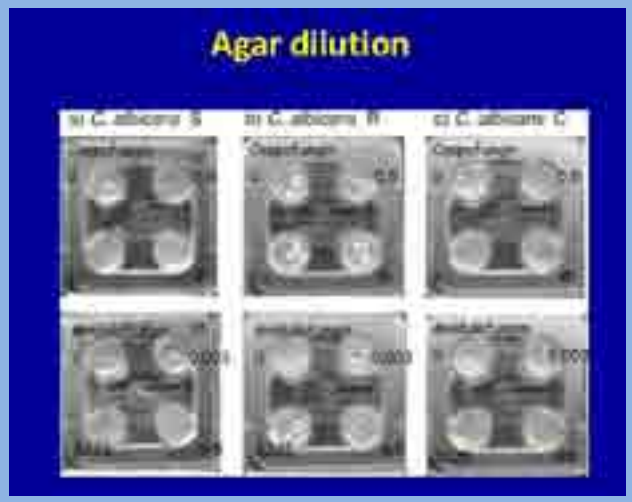
E-test determination



Disk diffusion test



Agar diffusion test



Microdilution methods (CLSI M38-A2 Guidelines)

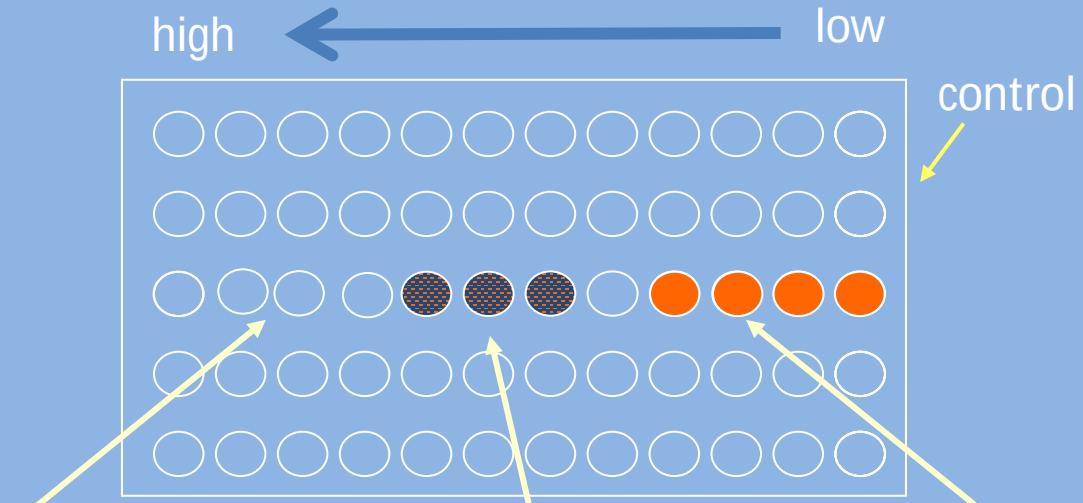
Characteristics	CLSI M38A2
• Suitable	• Spore forming fungi
• Inoculum	• $2x10^4$-$5x10^4$ CFU/ml
• Inoculum Standardization	• Spectrophotometrically (in %T): (ranged 68 to 71 T%)
• Test medium	• RPMI 1640
• Format	• Microdilution
• Temperature	• 35 °C
• Duration of incubation	• 96h
• Endpoint	• No growth

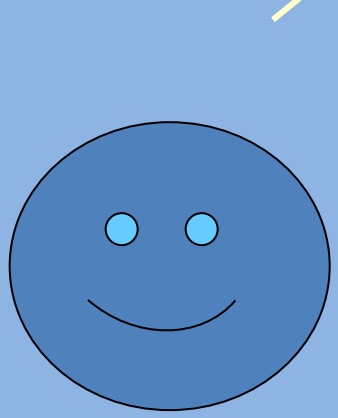
• No breakpoints for new generation agents

Interpretative Guidelines for In Vitro Susceptibility Testing of filamentous fungi

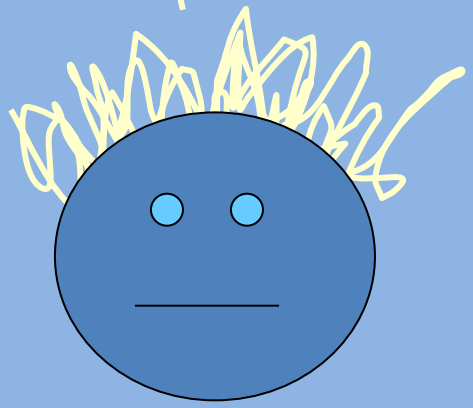
Antifungal	MIC and MEC breakpoint (µg/ml)		
	R [3]	I	S £
Amphotericin B (100%)	2	-	0.5
Fluconazole (50%)	64		
Itraconazole (100%)	4	2	1
Voriconazole (100%)	4	2	1
Posaconazole (100%)	4	2	1
Isavuconazole (100%)	-	-	1
Caspofungin (MEC)	>1		1
Anidulafungin (MEC)	>1		1

MIC: The lowest concentration of an antifungal agent that substantially inhibits growth of the organisms, as detected visually.





Complete inhibition MIC 100

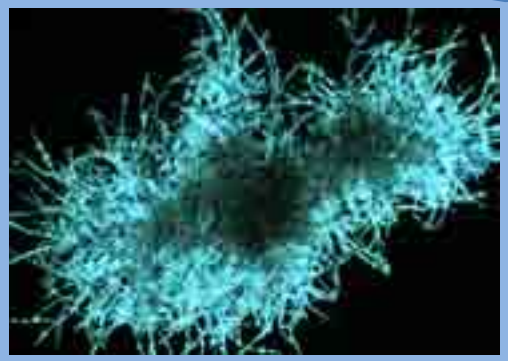


Prominent inhibition MIC 50

No inhibition

Test Drug Concentration (µg/mL)

MEC: The lowest concentration of drug that leads to growth of small, rounded, compact hyphal forms as compared to the growth control well.



CG

Echinocandins — prominent inhibition (>50%) at 72 hours

AmB — 100% Inhibition at 72 hours

In vitro activity of 8 antifungal agents against clinical isolates of *C. bantiana*

Name/Number	*Cladophialophora bantiana* (n=37)			*C. modesta* (n=1)	*C. arxii* (n=1)	*C. devriesii* (n=1)	*C. emmonsii* (n=2)	
Drugs	Range	MIC_{50}	MIC_{90}	MICs	MICs	MICs	MICs	MICs
Amphotericin B	0.125 - 2	1	1	1	1	2	0.5	1
Fluconazole	16 - 64	32	64	32	8	16	32	32
Itraconazole	< 0.016 - 0.25	0.063	0.125	0.5	0.016	0.031	0.125	0.125
Voriconazole	0.125 - 4	1	2	2	0.125	0.25	2	0.5
Posaconazole	< 0.016 - 0.25	0.031	0.125	0.25	0.016	0.031	0.063	0.063
Isavuconazole	0.008 - 1	0.25	0.5	2	0.063	0.031	1	1
Caspofungin	1 - 8	2	4	4	2	2	2	4
Anidulafungin	0.016 - 4	0.063	2	0.5	1	1	1	1

0.5–2 µg/ml

>64 µg/ml

S £ 1 µg/ml

R < 1

In vitro activity of 9 antifungal agents against clinical isolates of *C. carrionii*

Name/Number	*Cladophialophora carrionii* (n=31) (Clinical)			*Cladophialophora yegresii* (n=3) (environmental)		
Drugs	Range	MIC_{50}	MIC_{90}	MICs	MICs	MICs
Amphotericin B	0.5 - 16	4	8	0.5	0.5	0.25
Fluconazole	8 - 32	16	32	32	32	16
Itraconazole	0.016 - 0.25	0.063	0.125	0.25	0.25	0.5
Voriconazole	0.125 - 1	0.25	1	2	2	2
Posaconazole	0.016 - 0.063	0.031	0.063	0.125	0.125	0.125
Isavuconazole	0.063 – 0.5	0.125	0.25	0.5	0.5	0.125
Caspofungin	1 - 16	4	8	1	1	1
Anidulafungin	0.125 - 4	1	2	0.25	0.25	0.25
Terbinafine	0.016 – 0.063	0.031	0.063	0.063	0.063	0.063

0.5–2 µg/ml

>64 µg/ml

S £ 1 µg/ml

R < 1

Conclusion

- AFLP results are in line with previous sequencing data and also show obvious differences among clinically important *Cladophialophora* species.

- While sequence-based identification is a powerful tool, sequence data should be interpreted carefully, because of missing or incomplete GenBank data.

- Whenever possible, should be evaluated by other technique.

Conclusions

- Our results are in line with animal data, demonstrating that ITC and POS had the highest *in vitro* antifungal *activity*

- ISA seems to have also significant *in vitro* activity

- Based on this *in vitro* study, ITC, POS and ISA are superior drugs in the initial treatment of central nervous system phaeohyphomycosis.

Conclusions

- AmB has been accepted as the gold standard but has more side effect and poor outcome in patients with immunodeficiency, although AmB had very low efficacy.

- ITC and POS are the broad spectrum azole and has high efficacy comparable to others, and exhibited potent in vitro and in vivo activity.

- Concerning echinocandins, demonstrated less activity against all isolates.

Suggestion:

- Low MIC in vitro does not uniformly predict clinical success in vivo

- High MIC in vitro will often correlates with treatment failure

- Results need to be correlated with clinical outcome.

Acknowledgments

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