Aspergillose Invasive: diagnostic biologique et typage moléculaire

INÈS HADRICH[a], FATTOUMA MAKNI[a], SALLEMI HAYET[a], AHLÈM CHROUDA[a], BELLAJ HATEM[b], MOEZ ELLOUMI[b], ALI AYADI[a]

Laboratoire de recherche: biologie moléculaire parasitaire et fongique: Faculté de Médecine, Sfax
Laboratoire de Parasitologie-Mycologie: CHU Habib Bourguiba, Sfax

Objectifs

- Evaluer les trois techniques: GM,PCR-ELISA,

  RT-PCR: diagnostic de l'AI

- Typer les souches (environnement et cliniques) par

  microsatellites

- Étudier la sensibilité des souches: E-test

MATERIEL

Etude prospective (2004-2008): 163 patients

- **Laboratoire de biologie moléculaire parasitaire et fongique, faculté de médecine de Sfax**

- **Service d'onco-hématologie, CHU Hédi Chaker de Sfax**

MATERIEL

Prélèvements	Expectorations :	LBA :	Biopsie :
nasaux : 260	260	14	1

Sérums:

815

≈ 5 prélèvements/ patient

MATERIEL

Prélèvements environnementaux :

1680

METHODES

Examen direct

Culture: ST, Czapek

PCR séquençage:

50 souches:
Environnement et cliniques

Séquenceur ABI 3130
(Applied Biosystems).

METHODES

Antigénémie

ELISA Platellia
Aspergillus, biorad

PCR-ELISA

Roche Diagnostics

PCR temps réel

Sonde fluorescente: FAM (6-carboxy-fluorescein)
Quantification de l'ADN: une série de dilutions de 10^1 à 10^8 / ml

Analyse des microsatellites

- **Répétition en tandem de 2 à 6 nucléotides**

ATGCTTGCGATT-ACACACACACACACACACAC-CTTAGACGCATT

Les répétitions en tandem: polymorphes

Intérêt : génotypage moléculaire des souches

- **PCR séquençage des microsatellites:**

  confirmation de l'unité de répétition

Analyse des microsatellites

Aspergillus flavus | Aflatoxin | Genomics | Research | Protocols | Meetings | Publications | News | Links

Aspergillus flavus Genome Sequencing Project

Project Overview

Gary A. Payne and Ralph A. Dean at North Carolina State University received funding from the Microbial Genome Sequencing Project, USDA National Research Initiative to completely sequence the genome of *A. flavus* strain NRRL 3357 to the level of 5 fold sequence coverage. Whole genome sequencing was done at The Institute for Genomic Research, Rockville, Maryland under the supervision of William Nierman. Jennifer Wortman directed the assembly and automated annotation. The USDA/ARS/SRRC in New Orleans, Louisiana provided its Expressed Sequence Tag (EST) genomic database for *A. flavus* as a matching resource towards the complete genomic characterization of *A. flavus*. They also provided funds for fine closure and finishing of the sequence. Jiujiang Yu at USDA/ARS/SRRC lead the EST sequencing program and has directed the sequencing efforts supported by the USDA/ARS.

The available genome sequence for *A. flavus* provides a powerful resource for research on the biology and evolution of this important plant and animal pathogen. Further, we anticipate that the sequence will reveal critical genetic processes in the fungus that could be interrupted to control aflatoxin contamination, which causes hundreds of millions of dollars in crop losses during years of severe outbreaks.

Sequence Information

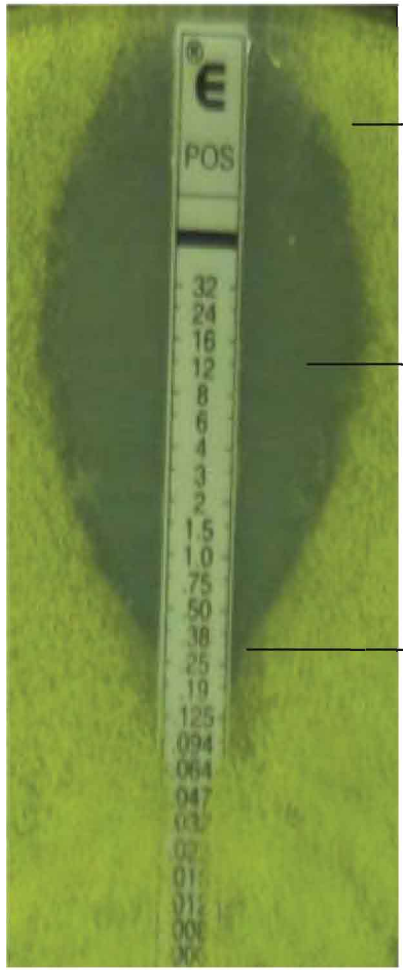
Sequence reads from the 5 X coverage were released to NCBI in July 2005. The annotated genome was released in October 2005 and can be accessed at this site through the genome browser button below. Manual annotation will be coordinated through North Carolina State University.

www.Aspergillusflavus.org

Analyse des microsatellites

http://tandem.bu.edu/trf/trf.html

Étude de la sensibilité: E-test



Zone de croissance

Ellipse d'inhibition

CMI

E-test: 37 souches isolées à partir de différents prélèvements cliniques chez 11 patients

Résultats

⇨ **163 patients neutropéniques:**

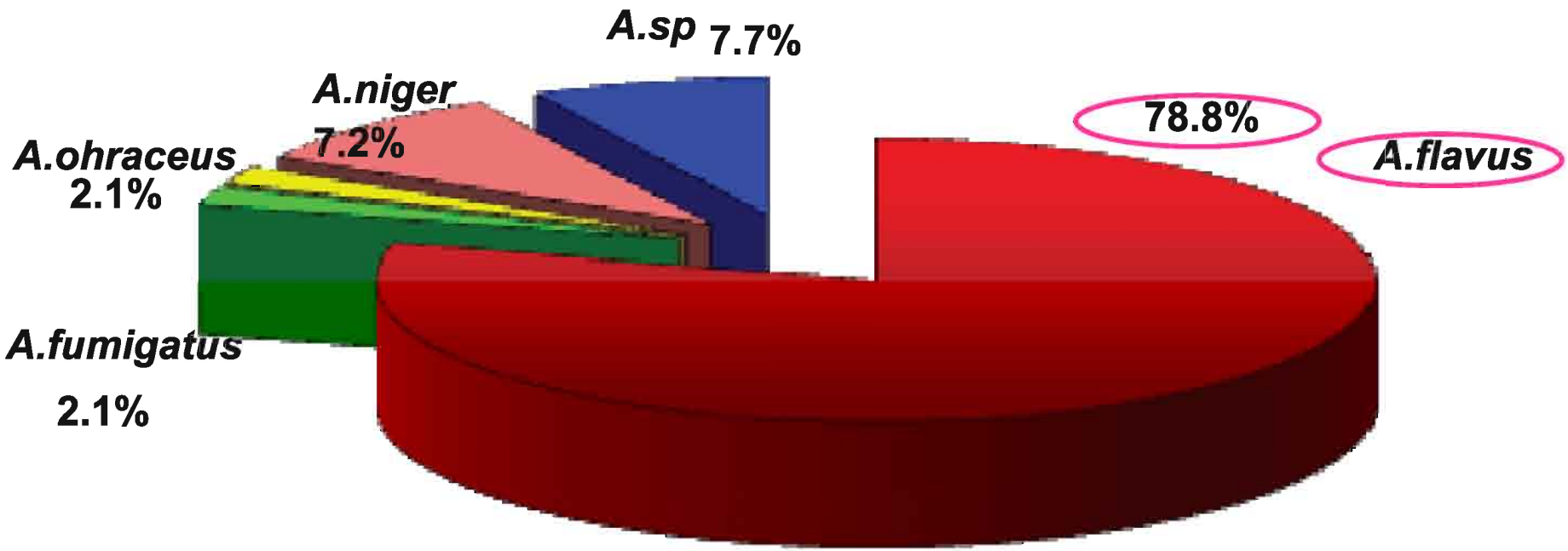
PNN< 500 /µl + T > 38,5°C

Classification selon l'EORTC:

- **1 AI prouvée**
- **31 AI probable**
- **15 AI possible**

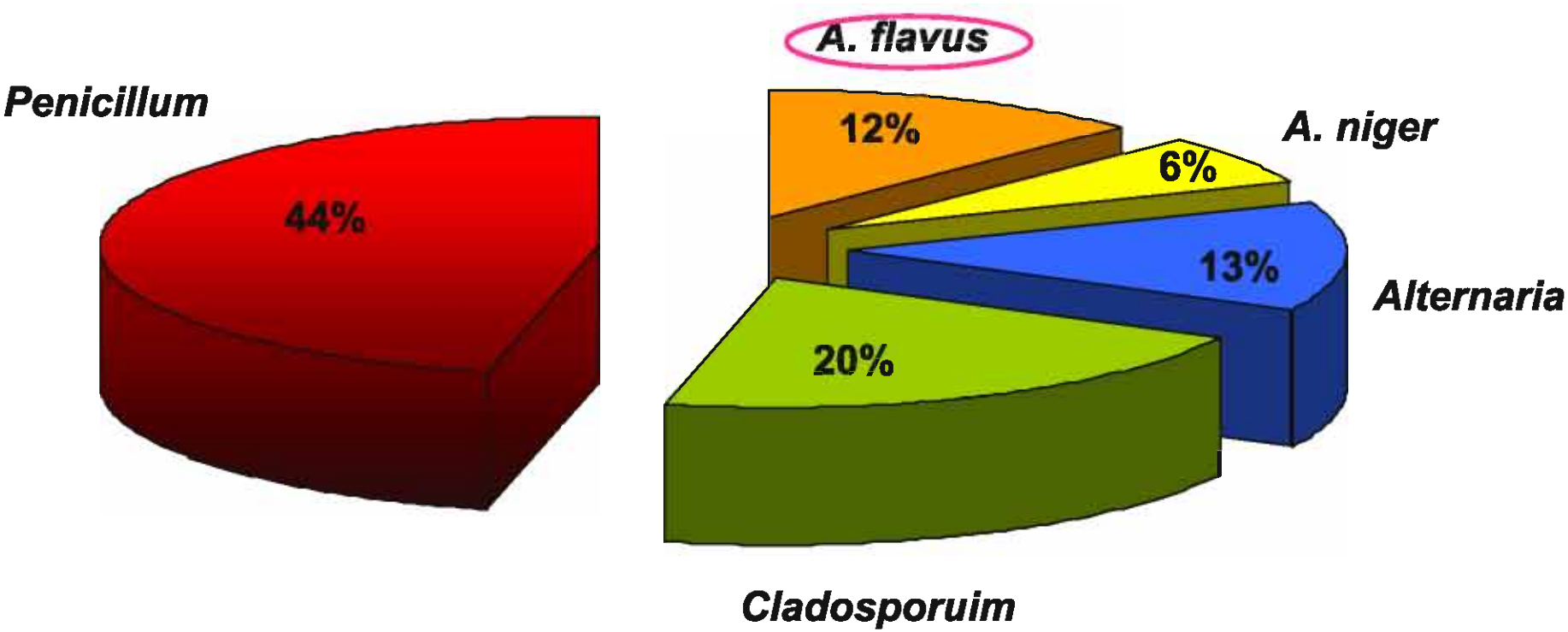
Résultats

Prélèvements cliniques : 824



Résultats

Prélèvements environnementaux: 316

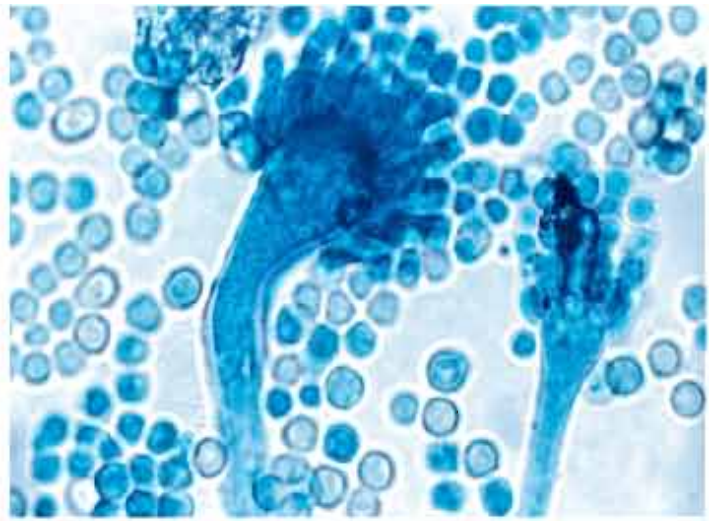


Résultats

A.flavus

Filament mycélien

macroscopie



microscopie

PCR-Séquençage: confirmation de l'identification phénotypique d'*A. flavus*

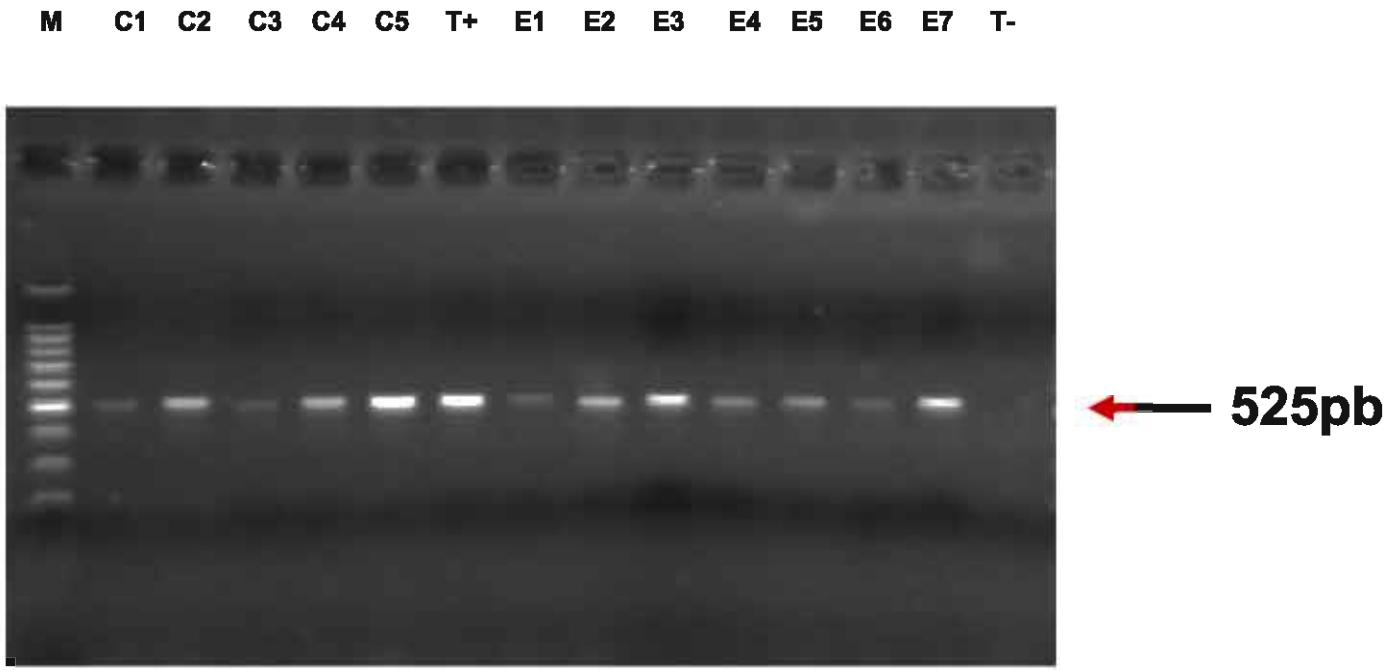
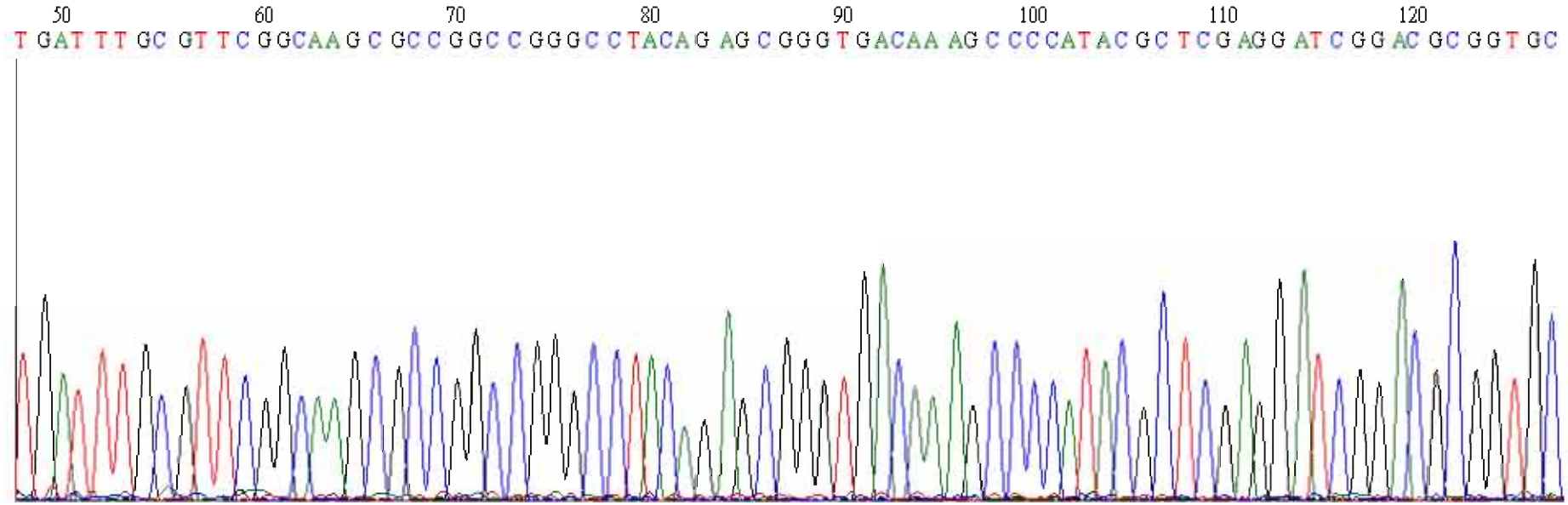


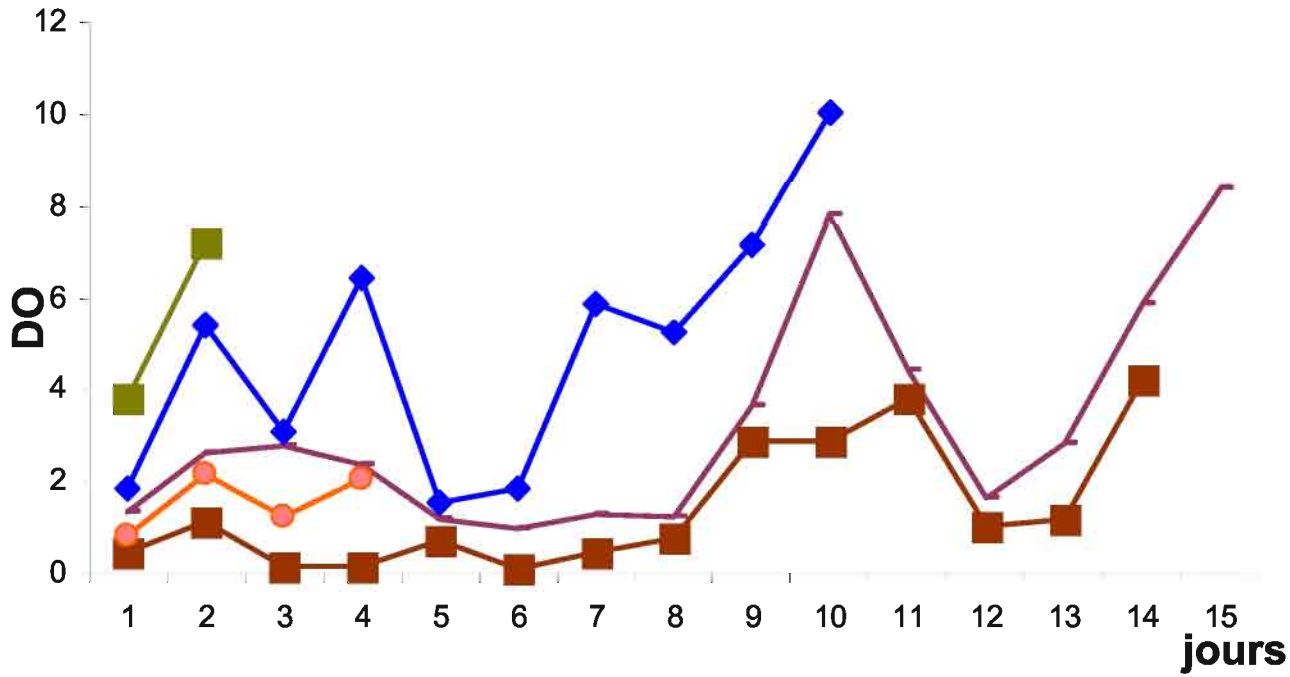
Figure 3: **DNA Amplification extracted from *A.flavus* (Clinical and environmental samples). M: size marker (100 pb); T- : negative control; T+: positive control (*Aspergillus flavus*: type strain control); Clinical samples: C1 (BAL), C2 (nasal), C3 (auricular), C4 (sinusien biopsy), C5 (BAL); Environmental samples: E1 (acclimatizer), E2 (room), E3 (table), E4 (windows), E5 (door), E6 (bed), E7 (hall).**

Séquençage



Blast TN: 100 % identité avec *A.flavus*

Antigénémie GM



AI probable: suivi quantitatif du GM: ↗ 61.3 % → létalité 89.5%

AI possible: suivi quantitatif du GM: ↘ 40 % → survie 100%

Performance diagnostique: Sang

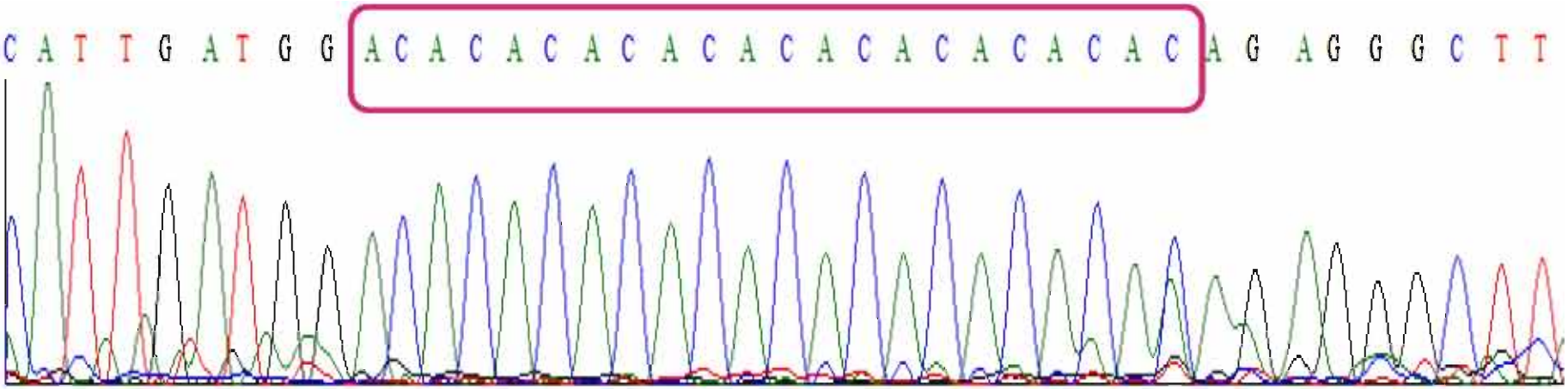
	PCR-ELISA	Real-time PCR
Sensibilité	96.9	93.8
[95% CI]	[90.2-96.9]	[86.6-93.8]
Specificité	**100**	**100**
[95% CI]	[95.5-100]	[95.1-100]
LR+	Inf	Inf
[95% CI]	[19.890-inf]	[17.681-inf]
LR-	0.031	0.063
[95% CI]	[0.031-0.103]	[0.063-0.141]
Yule Q[a]	1.0	1.0

Performance diagnostique: LBA

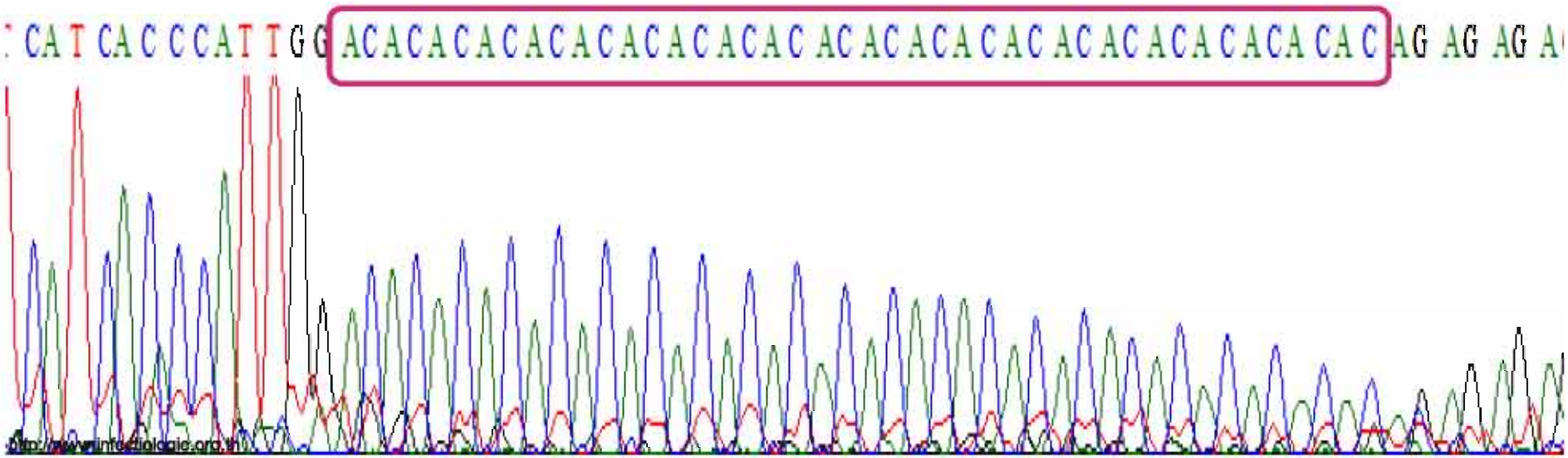
	GM Ag	PCR-ELISA	Real-time PCR	Culture
Sensibilité	85.7	71.4	64.3	40
[95% CI]	[67.4-94.3]	[53.6-77.2]	[46.4-70.1]	[21.4-35.7]
Spécificité	92.9	96.4	96.4	100
[95% CI]	[83.7-97.2]	[87.5-99.3]	[87.5-99.3]	[60.1-100]
LR+	12	20	18	Inf
[95% CI]	[4.144-33.322]	[4.282-115.970]	[3.711-106.123]	[3.007-Inf]
LR-	0.154	0.296	0.370	0.643
[95% CI]	[0.058-0.389]	[0.229-0.531]	[0.301-0.612]	[0.643-0.846]
Yule Q[a]	0.975	0.971	0.96	1.0

Séquençage des microsatellites

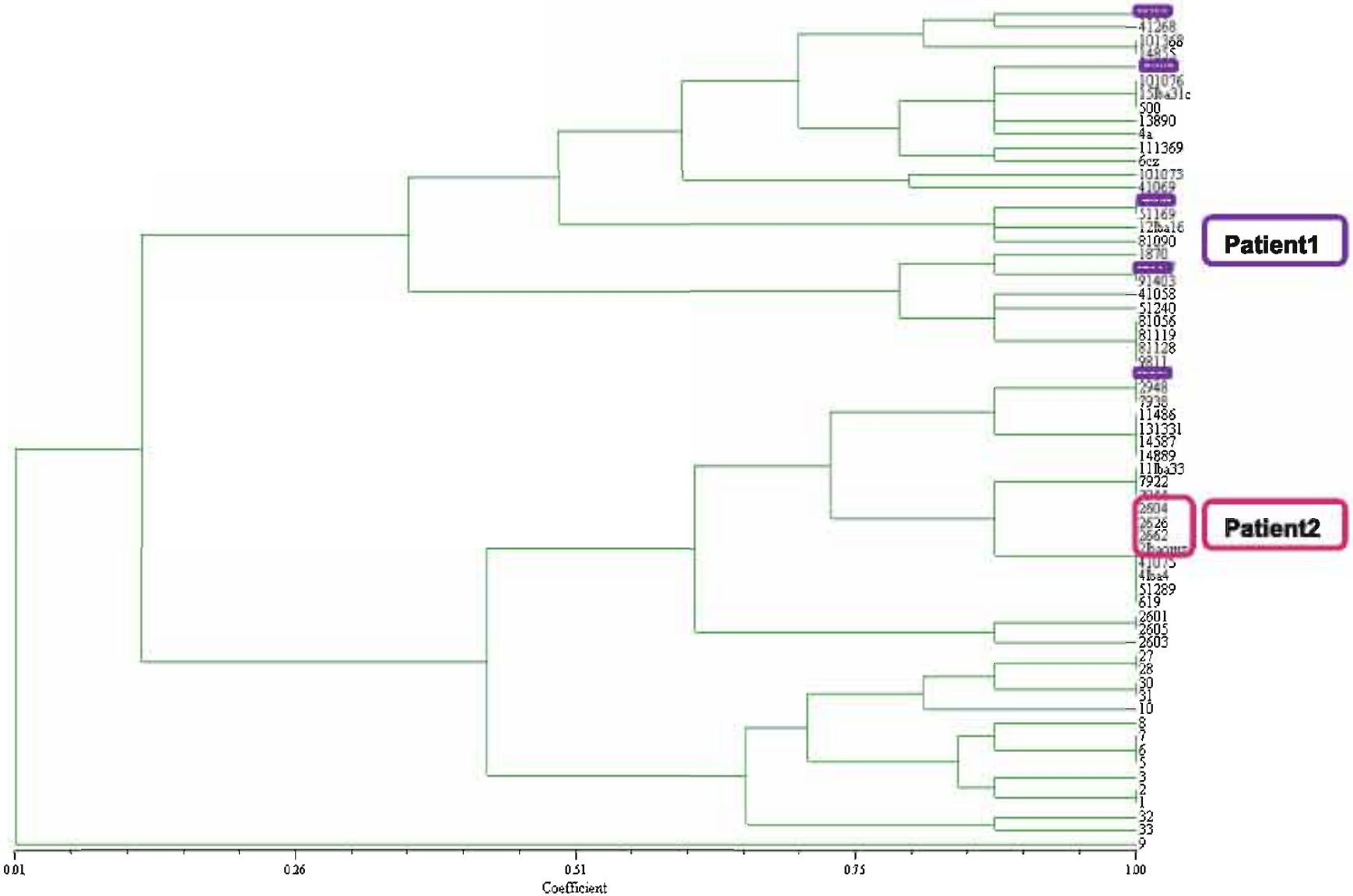
AC11 fois



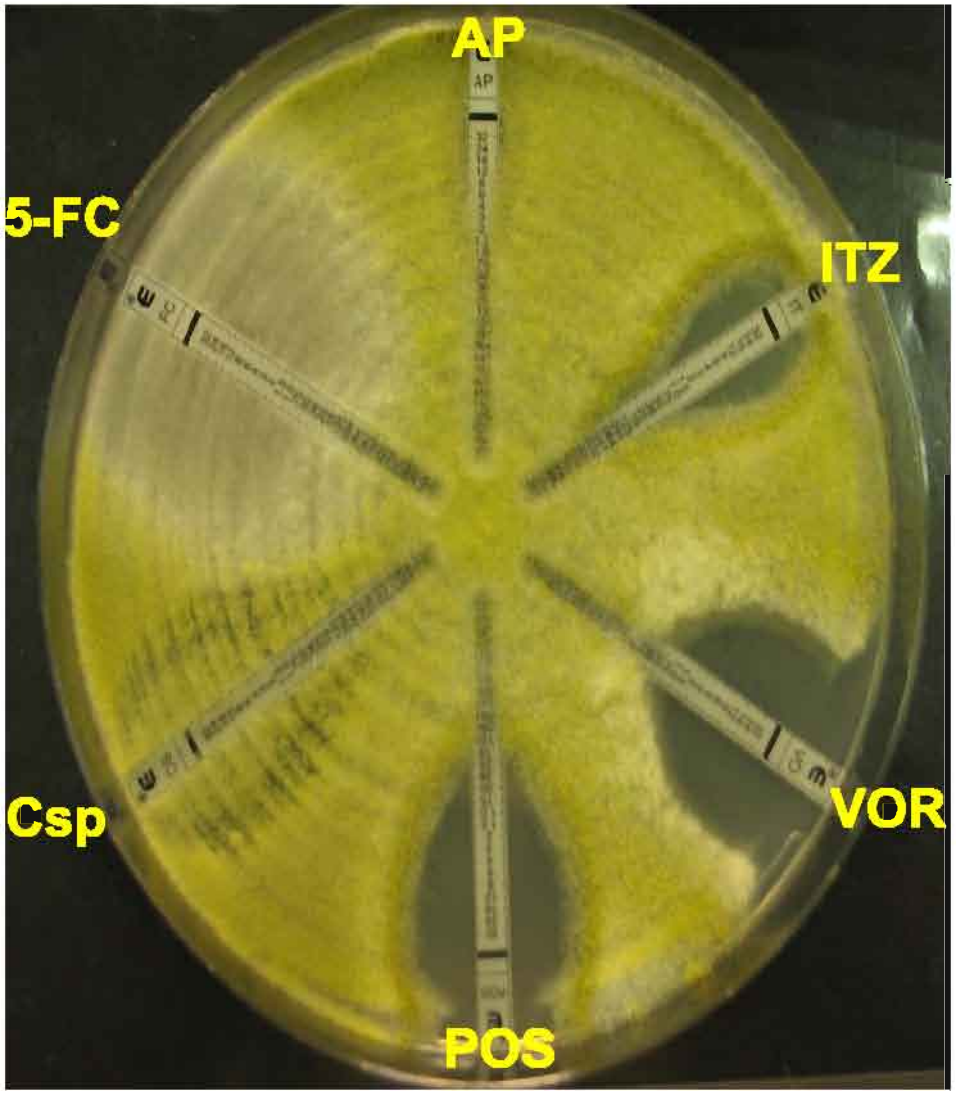
AC 22 fois

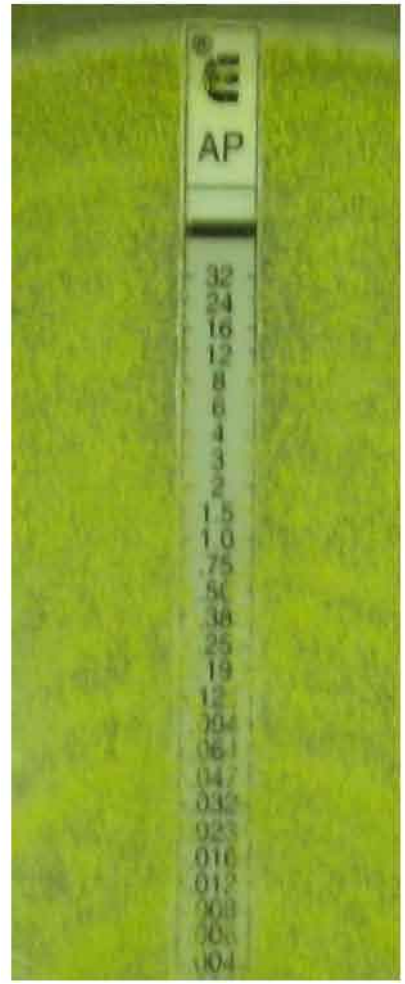


Arbre de phylogénie



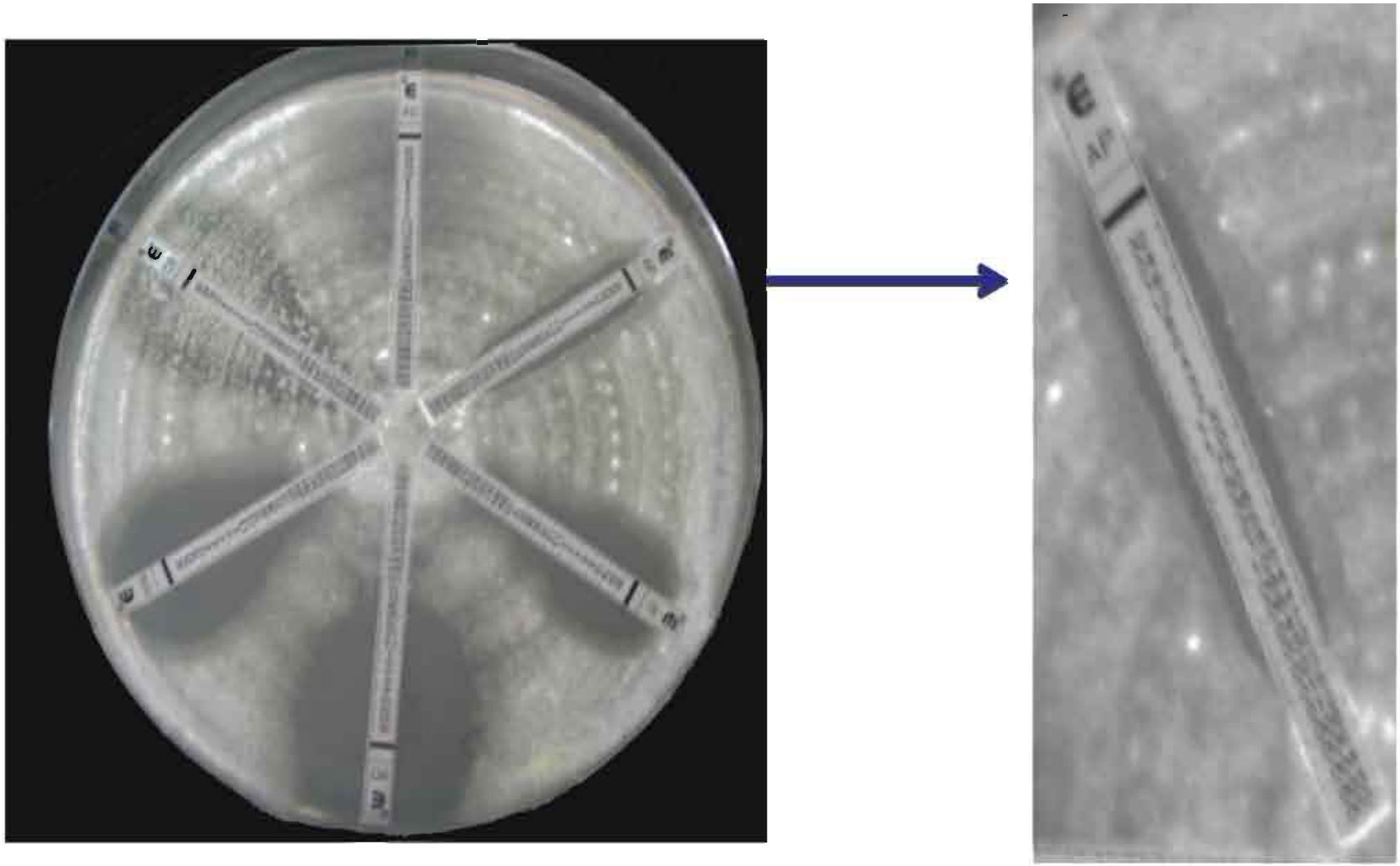
Etude de sensibilité





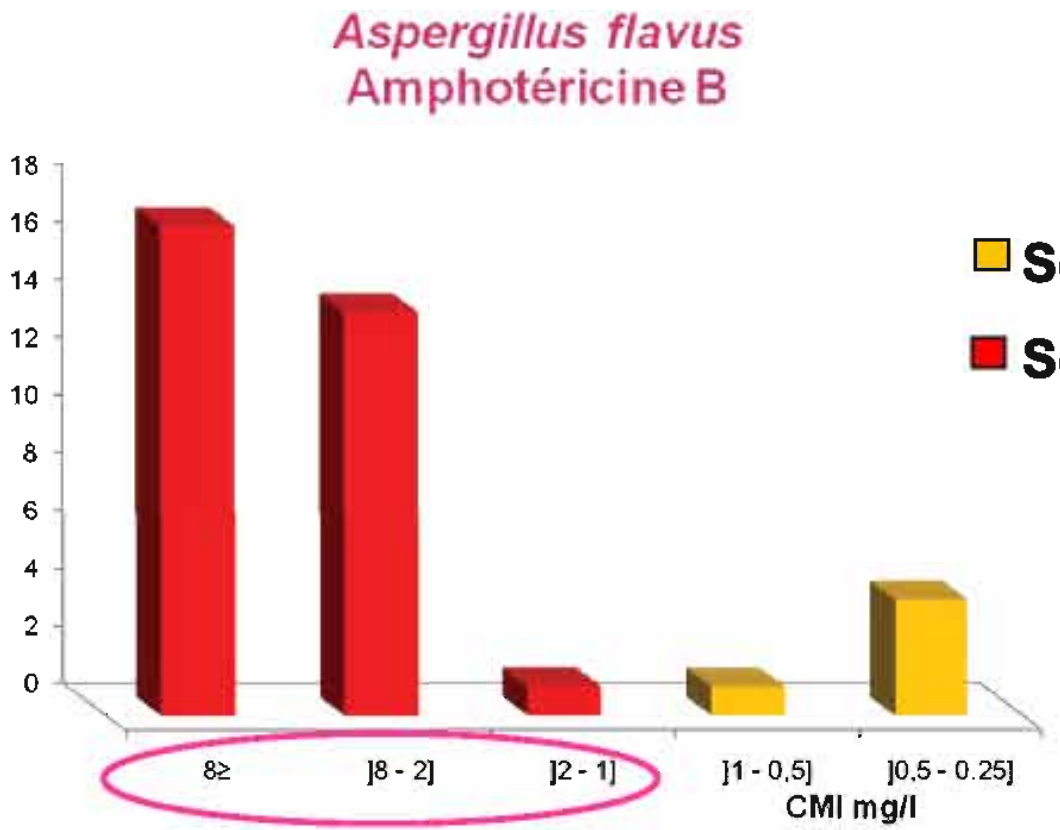
Amph résistant
CMI > 32 µg/ml

Etude de sensibilité



Souche sensible à l'amphotéricine B (CMI=0.23 µg/ml)

Etude de sensibilité



Souche sensible: 16.2%

Souche résistante: 83.8%

Etude de sensibilité

		5-FC	AMB	IT	Vor	Posa	Casp
A. flavus *n* = 37	MIC 90 min	> 32	0.25	0.025	0,025	0,064	0,125
	MIC 90 max	> 32	> 32	1,5	0.75	1.5	> 32
Sensibles (%)			16.2	81..2	100	83.8	
Résistants (%)		100	83.8	18.8		16.2	100

Discussion

***A. flavus*: agent étiologique de l'aspergillose invasive dans notre région**

⇨ **confirmé par PCR séquençage**

Arabie Saoudite, Soudan: *A. flavus*

Espagne, France, Italie: *A. fumigatus* (80%)

Falvey D.G. et al *J Hosp Infect 2007*

Diagnostic moléculaire: PCR

D'après une Méta-analyse: 16 études incluses/7059 publications

Intérêt dans le dépistage d'AI et le diagnostic

Un seul test négatif exclue AI

Deux tests positifs: confirment le diagnostic

Carlo et al, *lancet* 2009

Diagnostic moléculaire: LBA

Auteur	Année	Technique	Sensibilité	Spécificité
Raad	2002	PCR	69	90
Sanguinetti	2003	RT-PCR	100	100
Rantakoko-J	2003	RT-PCR	73	93
Musher	2004	RT-PCR	67	100
Tuon	2007	PCR	79	94
E. Fréalle	2008	RT-PCR	88	94
Prasanna D.K	2008	RT-PCR	76.9	87.8
Notre étude		RT-PCR PCR-ELISA	64.3 71.4	96.4 96.4

Etude de la sensibilité

	MICs µg ml^{-1}							
	A. fumigatus (*n* = 27)		*A. terreus* (*n* = 20)		*A. flavus* (*n* = 17)		*A. niger* (*n* = 12)	
Drug	Range	MIC 90	Range	MIC 90	Range	MIC 90	Range	MIC 90
Ampho B	0.12–1	1	0.25 to >2	>2	0.12 to >2	2	0.12–0.5	0.5
Itraconazol	0.25–1	1	0.12–0.5	0.12	0.12–0.5	0.12	1 to >2	>2
Posaconazol	0.06–2	0.5	0.12–1	1	0.5–1	0.5	1	1
Voriconazol	0.12–1	0.25	0.25–2	1	0.5–1	0.5	0.5–2	2
Caspofungin[1]	0.5–1	1	0.25–0.5	0.25	0.25–0.5	0.5	0.25–1	0.25

MIC 90 (MIC causing inhibition of 90% of isolates).

C. Lass-Floerl, *mycoses* 2008


Merci