

Les fongémies à levures émergentes dans la région de Sfax : Etude épidémiologique, biologique et moléculaire

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INTRODUCTION

Ampleur des septicémies à levures:

- ❖ Fongémies en  : patients immunodéprimés/Réa
 - ❖ Fongémies: 50,2% des IFI , [RESSIF \(2012-2021\), RAPPORT ANNUEL D'ACTIVITE 2022 CNRMA](#)
 - ❖ Genre *Candida* : 4^{ème} micro-organisme en cause de septicémie d'origine nosocomiale dans le monde [Kotey et al , Infectious Diseases: Research and Treatment,2021,](#)
[P. Koehler et al. Clinical Microbiology and Infection ,2019 .](#)
-  *Candida sp* : > **80%** des levures isolées chez l'homme ,1^{er} rang des infections fongiques+++
[Kotey et al , Infectious Diseases: Research and Treatment,2021](#) [Zeng et al. BMC Infectious Diseases \(2019\)](#)
- ➔ bouleversement de ce spectre: émergence d'autres espèces de *Candida* et d'autres genres de levures rares.

Les Levures émergentes

Selon la littérature: [Megha Sharma, Curr Fungal Infect Rep. 2023](#)

[Sunil Kumar et al, Pathogens. 2022](#)

[KAUR et al , Mycoses. 2021](#)

Candida non albicans sauf :

Candida glabrata, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* (updated nomenclature: *Pichia kudriavzevii*)

considered for analysis. Fungaemia due to rare yeast was considered when the isolate was identified for any species other than *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*. Time to

Levures non Candida

Trichosporon Spp.

Rhodotorula Spp

Malassezia Spp.

Magnusiomyces Spp.

Geotrichum spp.

Exophiala Spp.

Coniochaeta Spp.

Agents récemment renommés hors du genre Candida :

Candida lusitanae (Now Named *Clavispora lusitanae*)

Candida kefyr (New Nomenclature: *Kluyveromyces marxianus* or *Candida pseudotropicalis*)

Candida inconspicua (Now Renamed to *Pichia cactophila*)

Candida lipolytica (Now Renamed to *Yarrowia lipolytica*)

Candida norvegensis (Now Renamed to *Pichia norvegensis*)

Candida bracarensis (New Nomenclature: *Nakaseomyces bracarensis*)

Candida nivariensis (New Nomenclature: *Nakaseomyces nivariensis*)

Candida pararugosa (New Nomenclature: *Wickerhamiella pararugosa*)

Candida rugosa (New Nomenclature: *Diutina rugosa*)

Candida famata (New Nomenclature: *Debaryomyces hansenii*)

Candia pelliculosa (Now Renamed to *Wickerhamomyces anomalus*)

Candida pulcherrima (Updated Nomenclature: *Metschnikowia pulcherrima*)

OBJECTIFS

Mettre en exergue ce
changement du
spectre des levures
isolées

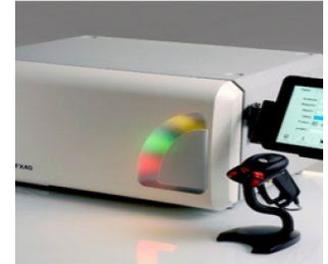
Étudier la sensibilité in
vitro de ces levures
aux différents
antifongiques

Étudier l'apport de la
PCR-séquençage
dans l'identification
de ces levures rares
et émergentes

PATIENTS ET METHODES

- ❖ Prélèvement: stérile sur milieu de Sabouraud liquide (pic fébrile)
- ❖ Examen direct + Incubation à 37°C (Bactec pour les flacons Mycosis BD)
- ❖ Identification phénotypique

- ❖ Étude rétrospective :
 - ➔ Cas de septicémies à levures émergentes
- ❖ Laboratoire de Parasitologie-Mycologie
CHU Habib Bourguiba de Sfax
- ❖ Période : 22 ans (Janvier 2000 –
Décembre 2022)

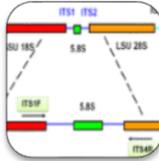


API 20 C AUX- ID 32 C

DIAGNOSTIC MOLECULAIRE: PCR- Séquençage



Extraction d'ADN: Méthode enzymatique:
Protéinase K + Kit DNeasy® (QIAGEN)



PCR classique
(amorces ITS1-ITS4)



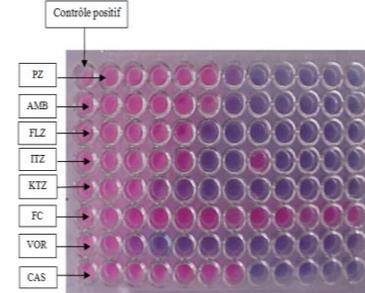
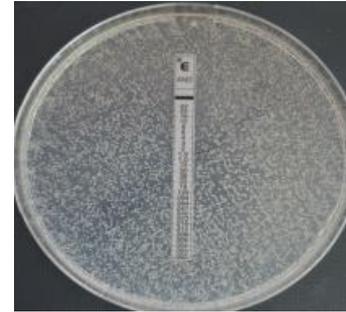
Analyse par PCR – Séquençage:
44 souches de levures



Analyse des résultats: logiciel BioEdit
(version 7.0.9), Blastn
(<http://www.ncbi.nlm.nih.gov/>)

Etude de la sensibilité aux antifongiques

- Sensititre YeastOne
- E-test
- Fungitest





RESULTATS DISCUSSION

Etude épidémiologique

- Septicémies à levures: **873** cas (1269 prélèvements positifs):

C.albicans (**26,4%**)

C.tropicalis (**24,05%**)

C.parapsilosis (**13,9 %**)

C.glabrata (**10,88%**)

C.krusei (**1,94%**)



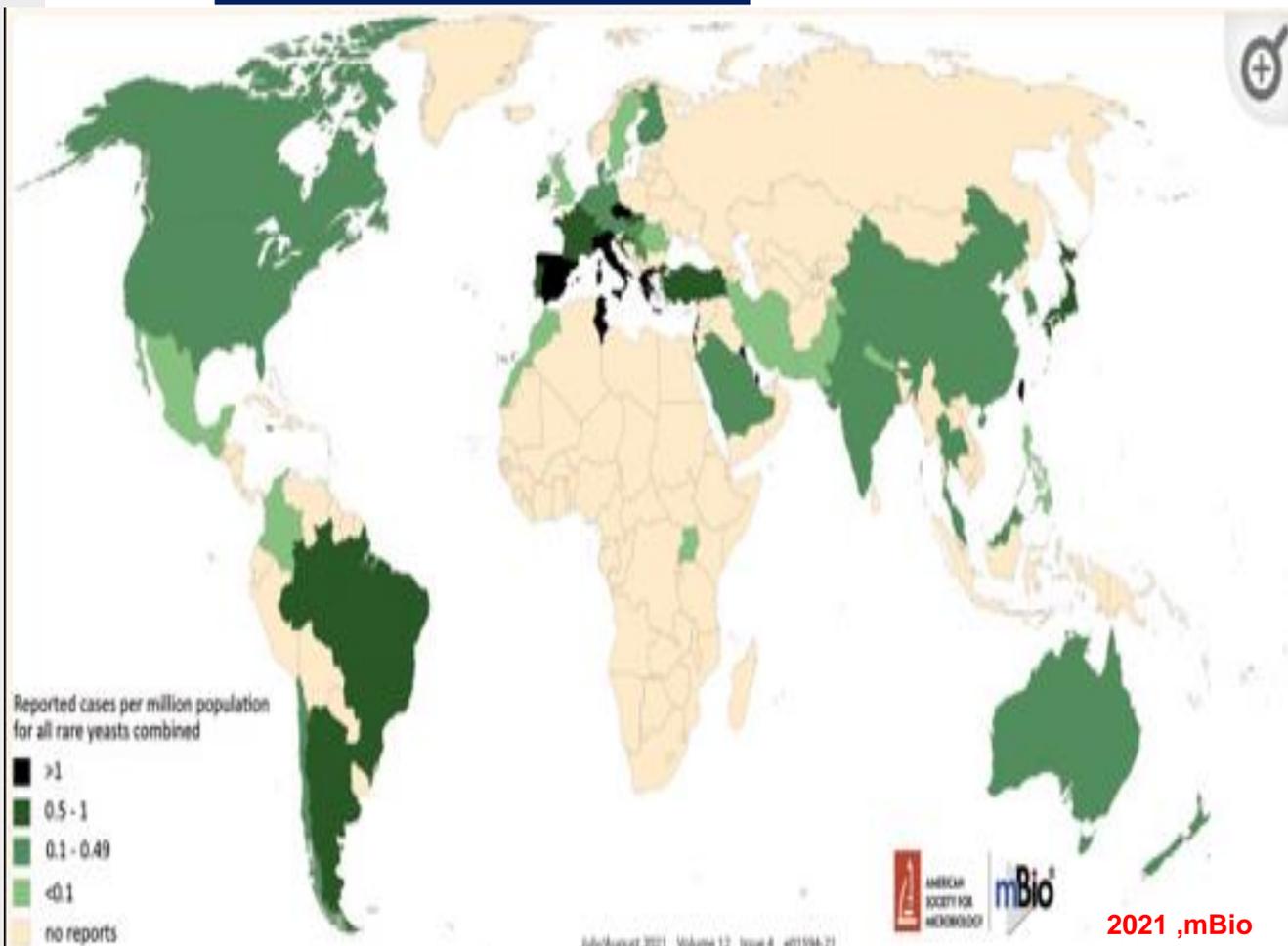
- Septicémies à levures émergentes: **165 cas (20,04%)**

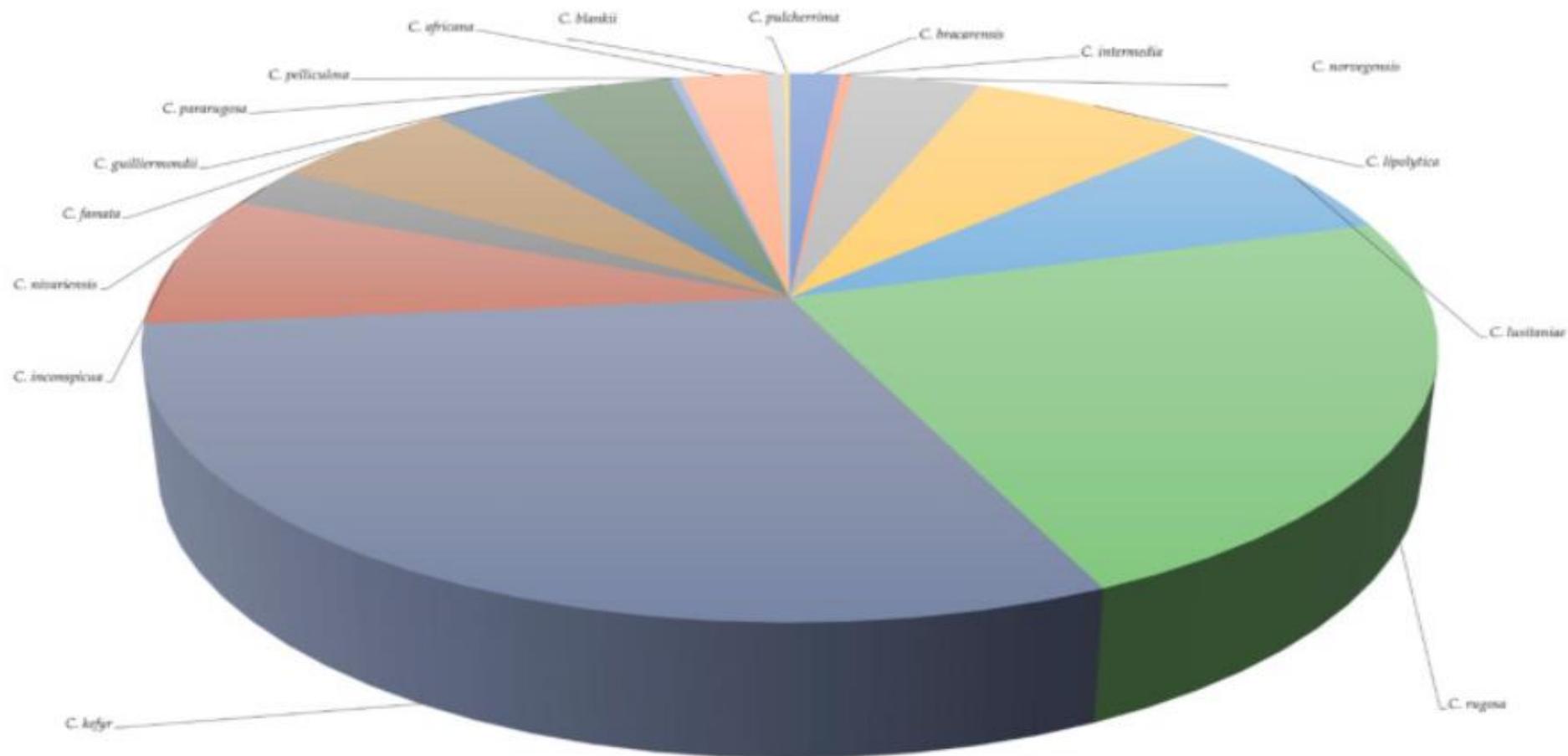
➔ **175** prélèvements positifs :

0 cas en 2000

47 cas en 2013

- sex-ratio :1,75.
- âge moyen :43,6 ans



A

Candida kefir—30.15%, *Candida norvegensis*—3.83%, *Candida inconspicua*—7.84%, *Candida famata*—5.51%, *Candida guilliermondii*—3.23%, *Candida lipolytica*—7.19%, *Candida rugosa*—22.91%, *Candida pararugosa*—4.05%, *Candida lusitaniae*—7.49%, *Candida pelliculosa*—0.30%, *Candida nivariensis*—2.37%, *Candida bracarenis*—1.51%, *Candida intermedia*—0.34%, *Candida africana*—2.58%, *Candida blankii*—0.52%, and *Candida pulcherrima*—0.17%

Notre étude

Espèce	Hémoculture (%)
<i>Y. lipolytica</i>	38,8
<i>C. guilliermondii</i>	7,3
<i>Geotrichum capitatum</i>	12,7
<i>Trichosporon asahii</i>	4,2
<i>Cryptococcus neoformans</i>	4,8
<i>C. lusitaniae</i>	5,5
<i>C. famata</i>	2,4
<i>C. kefyri</i>	1,2
<i>Rhodotorula sp</i>	4,8
<i>Saccharomyces sp</i>	1,8
<i>Trichosporon sp</i>	1,2
<i>Cryptococcus sp</i>	1,2
<i>C.sake</i>	1,2
<i>C.inconspicua</i>	1,8
<i>C.pelliculosa</i>	2,4
<i>C.utilis</i>	0,6
<i>C.silvicola</i>	0,6
<i>C.carsoni</i>	0,6
<i>C.didensiae</i>	0,6
<i>C.menranifaciens</i>	1,2
<i>C.norvegica</i>	0,6
<i>Kloachera apiculata</i>	1,2
<i>C.dublinsiensis</i>	1,2
Levures non identifiés	1,8

Fungus	Number of isolates	(%)
Total yeast isolates	2155	100
<i>Candida</i> species	1980	91.9
Non- <i>Candida</i> spp.	175	8.1
<i>Cryptococcus</i> species ^{a,b}	109	5.1
<i>Trichosporon</i> species ^{a,c}	23	1.1
<i>Rhodotorula</i> species ^a	10	0.5
<i>Kodamaea (Pichia) ohmeri</i> ^d	7	0.3
<i>Malassezia</i> species ^{a,d}	4	0.2
<i>Hansenula anomala (Pichia anomala)</i> ^d	4	0.2
<i>Hansenula polymorpha</i> ^d	2	0.1
<i>Yarrowia lipolytica</i> ^d	2	0.1
Other non- <i>Candida</i> yeast ^e	14	0.6

Distribution des différentes espèces de levures isolées des hémocultures en Asie
LIN et al. Mycoses. 2019



RAPPORT ANNUEL D'ACTIVITE 2022
Centre National de Référence
Mycoses Invasives et Antifongiques

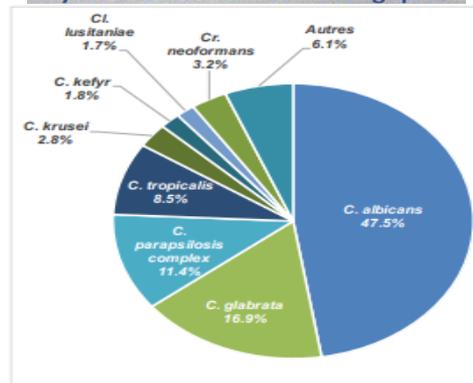


Figure 12 : Distribution des principales espèces responsables de fongémies, ODL 2002-2016

TABLE 3. Summary of rare yeast isolates collected during the national surveillance programme in Denmark 2004-2011 and the surveillance programme in Paris hospitals, France October 2002-May 2012. Only unique isolates are included. For comparison and representing other parts of the world data from a US cancer centre, the Artemis study and a Brazilian study are included

	DK (national) [24,27]	Paris Hosp. ^a	US (cancer centre) [28]	Artemis study 1997-2007 [29]	Sao Paulo Brazil [30]
Fungiemia isolates (total)	3982	3668	3382	NA	1195
Rare yeasts other than <i>Candida</i>	44 (1.1%)	188 (5.1%)	94 (2.8%)	11,240	174 (14.5%)
<i>Cryptococcus neoformans</i>	13 (29.5%)	137 (72.8%)	NA	3,512 (31.2%)	79 (45.4%)
<i>Cryptosporidium</i> spp.	1 (2.3%)	1 (0.5%)	NA	112 (1.0%)	NA
<i>Geotrichum</i> spp.	2 (4.5%)	19 (10.1%)	2 (5%)	NA	NA
<i>Rhodotorula</i> spp.	4 (9.1%)	5 (2.7%)	21 (5.1%)	462 (4.1%)	28 (16.1%)
<i>Saccharomyces</i> spp. ^b	22 (50.0%)	14 (7.4%)	8 (2.0%)	1,321 (11.8%)	NA
<i>Trichosporon</i> spp.	2 (4.5%)	11 (5.9%)	8 (2.0%)	1.9 (0.6%)	NA
<i>Malassezia</i> spp.	0	1 (0.5%)	1 (2.4%)	NA	NA
<i>Pichia anomala</i>	0	NA	1 (2%)	28 (0.2%)	32 (18.4%)
<i>Saprochete capitata</i>	0	NA	NA	109 (1.0%)	NA

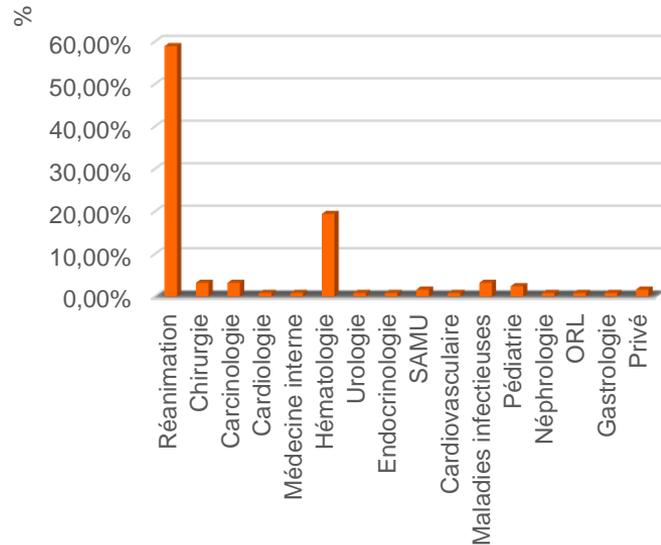
NA, Not available.

^aEASTS Network, National Reference Centre for Invasive Mycoses and Antifungals, Paris, France (unpublished data).

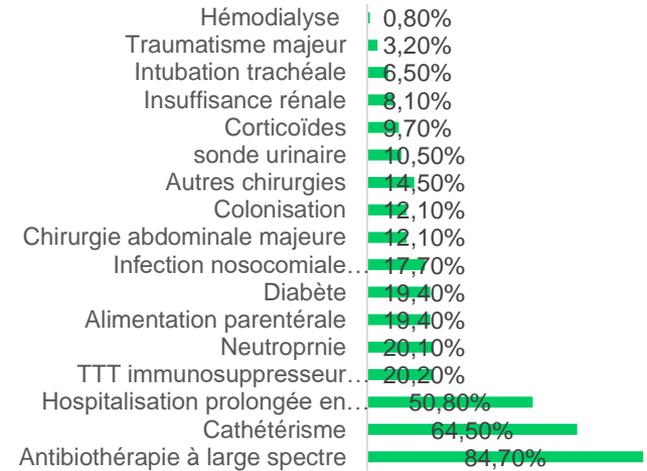
^bTaxonomically also a *Candida* species (*C. robusta*).

Etude épidémiologique

- Répartition des cas de septicémies à levures émergentes selon les services



- Répartition des cas de septicémies à levures émergentes selon les facteurs de risque



Revue générale : S. Ghazi a , R. Rafei Journal De Mycologie Médicale (2019) : ≈50% des épisodes de fongémies à Candida et à levures émergentes surviennent dans les unités de soins intensifs (USI)
 ➔ ce qui indique une relation complexe entre fongémies à LE et les maladies graves chez les patients

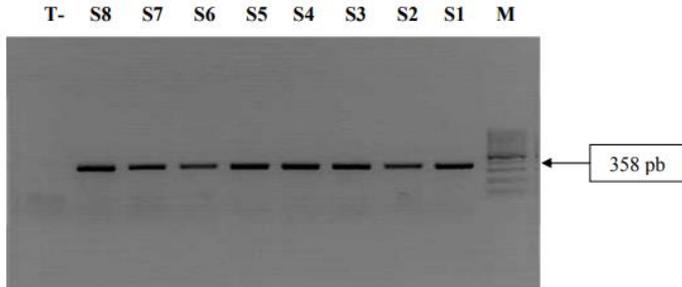
- l'antibiothérapie affaiblit les systèmes immunologiques de défense ➔ colonisation par ces levures
- grandes quantités de biofilm visqueux ➔ coloniser le cathéter

Intérêts de la biologie moléculaire

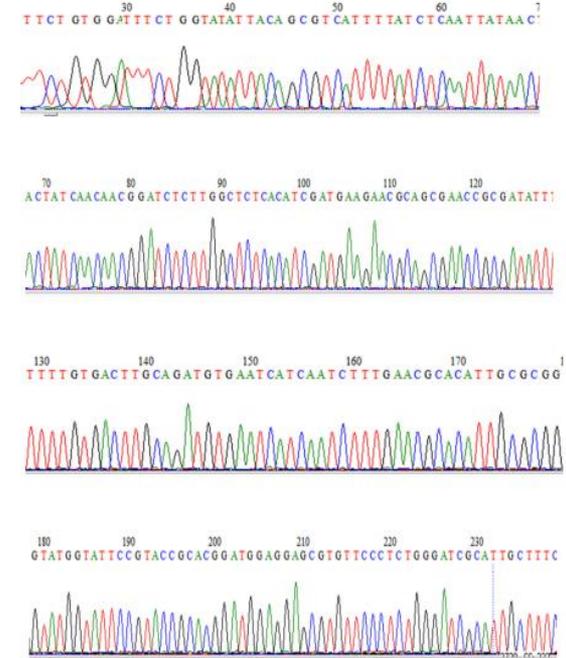
- ❑ Diagnostic précis et plus rapide d'espèces
 - ➔ Adapter le traitement antifongique
- ❑ Détecter les associations de levures
- ❑ Typage des souches isolées

Etude moléculaire de *Y.lipolytica*

14 souches de *Y. lipolytica*



Amplification par PCR des souches de *Y. lipolytica*

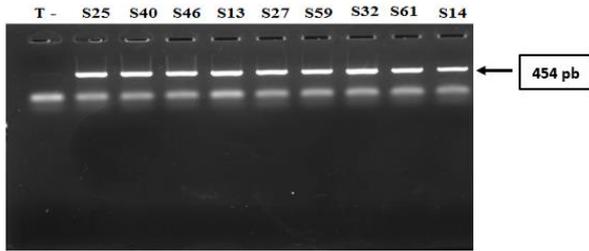


Courbe du séquençage fluorescent de *Y.lipolytica*

Résultat de l'alignement de la séquence de *Y. lipolytica*

	Description	Score (bits)	E value	id (%)	Length	Diff.
40 HC (S9)	KY105975.1 <i>Yarrowia lipolytica</i> culture-collection CBS:5699 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	641	0.0	99	358/359	1

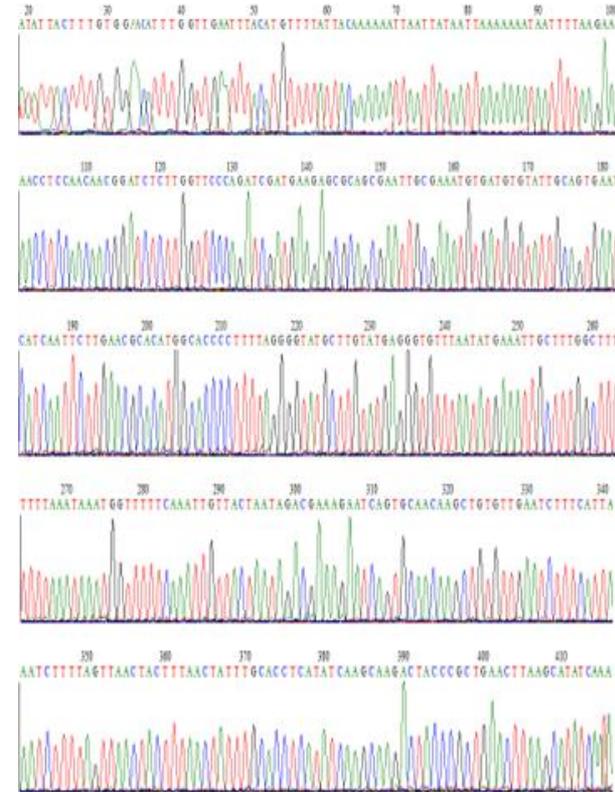
Etude moléculaire de *Geotrichum*



8 souches de *Geotrichum capitatum*

Concordance identification
phénotypique et PCR
séquençage : **100%**.

Visualisation sur gel d'agarose des produits de PCR de différentes souches de *Geotrichum capitatum*

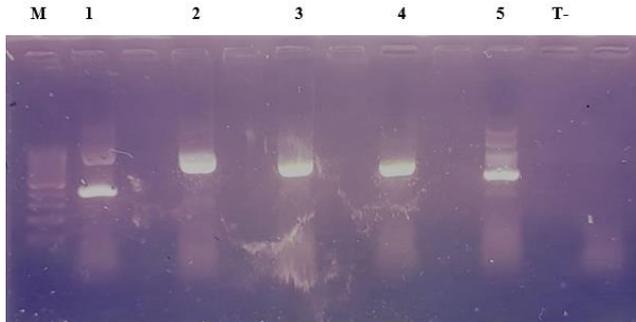


Courbe du séquençage fluorescent du produit d'amplification des régions ITS de *Geotrichum capitatum*.

Résultat de l'alignement de la séquence de *Geotrichum capitatum*

Accession	Description	Score (bits)	E value	Id (%)	length	Diff
NR_164086	<i>Magnusiomyces capitatus</i> CBS 162.80 ITS region; from TYPE material	839	0.0	100	454/454	
MH_999810.1	<i>Magnusiomyces capitatus</i> strain SGX1001 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	839	0.0	100	454/454	

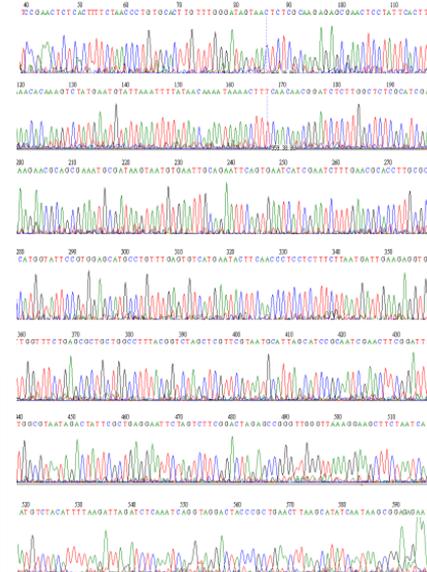
Etude moléculaire d'autres levures rares



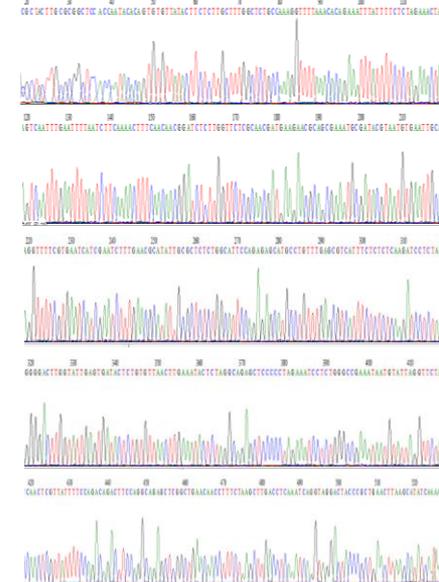
22 souches

M : marqueur de taille 100pb,

1 : *C.lusitaniae*, 2 : *C.kefyr*, 3 : *C.guillermondii*, 4 : *C.blankii*, 5 : *C.lusitaniae*



courbe de séquençage
fluorescent: une souche de
Rhodotorula



courbe de séquençage
fluorescent:
souche 136 HC/19(*C.utilis*).

- Concordance identification phénotypique et PCR-séquençage: **14 cas**
- Rectification du diagnostic morphologique: **4 cas**
- Identification exacte de l'espèce : **4 cas** → **1 association** :
Hanseniaspora opuntiae et *Candida parapsilosis*
→ supériorité de cette technique par rapport aux techniques phénotypiques

Notre étude : identification de levures jamais identifiés auparavant dans notre laboratoire ou même dans notre pays (*C.utilis*, *C.diddensiae*, *C.blankii*)

N° souche	Identification phénotypique	Identification moléculaire (PCR-séquençage)	% de similarité
512 HC/12	<i>C.guilliermondii</i>	<i>Meyerozyma guilliermondii</i>	100%
268 HC/14	<i>C.inconspicua</i>	<i>Pichia kudriavzevii</i>	99,8%
35 HC/14	<i>C.pelliculosa</i>	<i>Wickerhamomyces anomalus</i>	99,5%
213 HC/19	<i>C.silvicola</i>	<i>Candida blankii</i>	97,8%
384 HC/19	<i>Levure non identifiable</i>	<i>C.parapsilosis</i> + <i>Hanseniaspora opuntiae</i>	100% 98 %
458 HC/12	<i>Levure non identifiable</i>	<i>Candida diddensiae</i>	100%
160 HC/19	<i>Levure non identifiable</i>	<i>C.parapsilosis</i>	100%
204 HC/07	<i>Levure non identifiable</i>	<i>C.tropicalis</i>	99,4%
213 HC/07	<i>C.carsoni</i>	<i>C.parapsilosis</i>	100%
478 HC/07	<i>C.kefyr</i>	<i>Kluyveromyces marxianus</i>	100%
593 HC/09	<i>C.lusitaniae</i>	<i>Clavispora lusitaniae</i>	100%
11 HC/14	<i>C.guilliermondii</i>	<i>Meyerozyma guilliermondii</i>	97,8%
617 HC/15	<i>C.lusitaniae</i>	<i>Clavispora lusitaniae</i>	97,4%
288 HC/15	<i>C.lusitaniae</i>	<i>Clavispora lusitaniae</i>	99,6%
388 HC/19	<i>C.guilliermondii</i>	<i>Meyerozyma guilliermondii</i>	100%
259 HC/19	<i>C.lusitaniae</i>	<i>Clavispora lusitaniae</i>	100%
136 HC/19	<i>C.utilis</i>	<i>Cyberlindnera jadinii</i>	99,8%
183 HC/20	<i>C.lusitaniae</i>	<i>Clavispora lusitaniae</i>	99%
172 HC/13	<i>Rhodotorula mucilaginosa</i>	<i>Rhodotorula mucilaginosa</i>	100%
357 HC/12	<i>Rhodotorula glutinis</i>	<i>Rhodotorula mucilaginosa</i>	98%
272 HC/18	<i>Kloeckera apiculata</i>	<i>Hanseniaspora guilliermondii</i>	99%
94 HC/21	<i>Rhodotorula mucilaginosa</i>	<i>Rhodotorula mucilaginosa</i>	100%

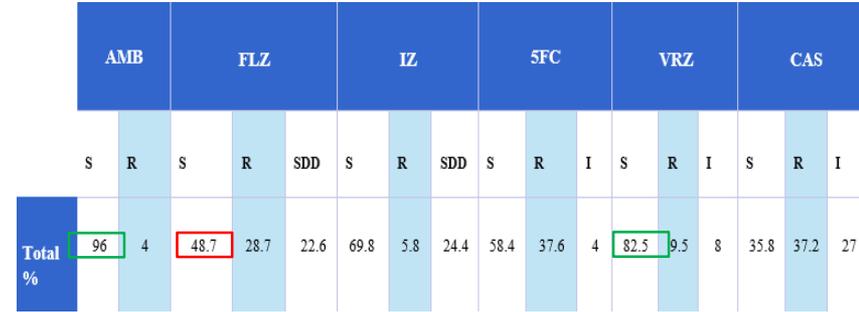
Etude de la sensibilité aux antifongiques

Table 1 Species distribution of noncandidal yeast isolates causing invasive infections and azole susceptibility of each species

Species	Total	%	Antifungal susceptibility (%)					
			Fluconazole			Voriconazole		
			S	SDD	R	S	SDD	R
Cryptococcus spp.	654	77.5	73.7	16.4	9.9	99.4	0.5	0.2
<i>Cryptococcus neoformans</i>	638	76.4	74.1	16.1	9.7	99.5	0.5	
<i>Cryptococcus gattii</i>	7	0.8	57.1	28.6	14.3	100		
<i>Cryptococcus laurentii</i>	4	0.5	50.0	25.0	25.0	100		
<i>Cryptococcus curvatus</i>	3	0.4	66.7	33.3		100		
<i>Cryptococcus arboriformis</i>	1	0.1	100			100		
<i>Cryptococcus humicola</i>	1	0.1			100			100
Trichosporon spp.	74	8.8	77.0	16.2	6.8	97.3		2.7
<i>Trichosporon asahii</i>	58	6.9	81.0	13.8	5.2	98.3		1.7
<i>Trichosporon mucoides</i>	3	0.4	66.7	33.3		100		
<i>Trichosporon japonicum</i>	3	0.4	33.3	33.3	33.3	66.7		33.3
<i>Trichosporon asteroides</i>	3	0.4	100			100		
<i>Trichosporon inkin</i>	3	0.4	33.3	33.3	33.3	100		
<i>Trichosporon dermatis</i>	1	0.1	100			100		
<i>Trichosporon interdigitale</i>	1	0.1		100		100		
<i>Trichosporon jirovecii</i>	1	0.1	100			100		
<i>Trichosporon montevideense</i>	1	0.1	100			100		
Rhodotorula spp.	44	5.2			100			100
<i>Rhodotorula mucilaginosa</i>	43	5.1			100			100
<i>Rhodotorula diobovatum</i>	1	0.1			100			100
Other yeast spp.	72	8.5	51.4	23.6	25.0	95.8	2.8	1.4

Xiao et al., Infection and Drug Resistance 2018

- *C.lusitaniae*: sensible aux triazolés et aux échinocandines, bien qu'une résistance acquise à ce dernier groupe d'agents ait été rapportée (Pfaller MA et al, 2021, Desnos-Ollivier M et al, 2011)
- *C.guilliermondii* : taux élevés de résistance aux azolés (Jung DS et al, 2015; Savini V et al, 2011)
- *Trichosporon* spp, *Rhodotorula* spp et *Magnusiomyces capitatus*: intrinsèquement résistants aux échinocandines, et *Rhodotorula* spp présente une résistance à certains azolés (Sprute R, Cornely OA, 2021)



Profil de sensibilité de certaines souches de levures émergentes aux antifongiques

Species	Sensible (%)					
	AMB	FCZ	IZ	FC	VOR	CAS
<i>C.lipolytica</i>	95.3	46.9	78.1	18.8	90.6	21.9
<i>G.capitatum</i>	100	63.2	88.2	94.7	94.1	23.5
<i>C.guilliermondii</i>	100	36.4	20	100	77.8	100
<i>C.lusitaniae</i>	100	100	100	75	100	85.7
<i>Cryptococcus neoformans</i>	85.7	14.3	71.4	57.1	57.1	-
<i>Rhodotorula</i>	100	12.5	37.5	100	12.5	12.6
<i>T.asahii</i>	100	42.9	66.7	100	100	16.7

CONCLUSION



All You Need To Know and More about the Diagnosis and Management of Rare Yeast Infections

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ABSTRACT Invasive infections with emerging yeasts such as *Gastrophysalis*, *Saprochroa*, *Hoplostium*, *Trochophora*, and other species are associated with high morbidity and mortality rates. Due to the rarity and heterogeneity of these yeasts, medical mycology has lacked guidance in critical areas affecting patient management. Now, physicians and life scientists from multiple disciplines and all world regions have united their expertise to create the “Global guideline for the diagnosis and management of rare yeast infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology.” Recommendations are stratified for high- and low-resource settings and are therefore applicable worldwide. The advantages and disadvantages of various diagnostic methods and treatment options are outlined. This guideline reflects the current best practice management for invasive rare yeast infections in a range of settings, with the intent of establishing a global standard of care for laboratories and clinicians alike.

KEYWORDS Moleculare, Parasitosen, Rhodospirale, antifungale Therapie, invasive fungale Infektion, invasive Mikroorganismen, yeast, ECMM, ISHAM, ASM

The German surgeon Bernhard von Langenbeck was probably the first to directly link yeasts as an etiological agent of oropharyngo-esophageal thrush, publishing the first case of esophageal candidiasis in a patient who died of typhoid fever in 1839 (1, 2). It took another 22 years, however, to discover that yeasts also lead to disseminated disease, when the pathologist Friedrich Albert Zenker described a case of disseminated yeast infection as metastatic brain lesions in 1861 (3). Today, *Candida* species are major pathogens in hospitalized and immunocompromised patients and the third most dominant cause of nosocomial bloodstream infections (4). However, uncommon yeasts other than *Candida* and *Cryptosporidium* spp. have emerged as significant pathogens during the last 2 decades (5, 6). These fungi are commonly encountered in the environment and frequent colonizers of human skin and mucosal surfaces (7–9). As such, they may be inadvertently dismissed as innocent bystanders or as contaminants. Particularly in the setting of immunosuppression or other immune compromise, they are increasingly reported to cause life-threatening invasive infections (5, 10). This may

Citation: Sprute R, Cornely OA, Chen SCA, Seidel D, Scharz AN, Zhang SX (2021) All you need to know and more about the diagnosis and management of rare yeast infections. *mSystems* 16:e00000-21, 2021. <https://doi.org/10.1128/mSystems.00000-21>

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Published: 24 August 2021

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